

NEUTROPHIL PROGENITORS AND RELATED METHODS AND USES

TECHNICAL FIELD

The present disclosure relates broadly to a granulocyte-monocyte progenitor (GMP) subpopulation, such as neutrophil progenitors, and related methods and uses.

BACKGROUND

Hematopoietic Stem Cell Transplantation (HSCT) is a common treatment for hematological disorders and neoplastic diseases. However, the generation of neutrophils, which are important immune cells that provide protection against bacterial and fungal infections, from stem cells is a long process that takes an estimated 3-4 weeks in human. Thus, neutropenia after transplantation often leads to morbidity and mortality caused by infections. Current clinical management involves administration of prophylactic antimicrobial drugs, recombinant growth factors (e.g., G-CSF) or repeated Donor Granulocyte Infusion (DGI) in isolation wards to provide immune protection for patients during the neutropenic period, which can be ineffective and associated with high healthcare costs.

Thus, there is a need to address or at least ameliorate one or more of the above-mentioned problems. In particular, there is a need to provide a method of identifying a neutrophil progenitor, a method of sorting and/or separating neutrophil progenitors from a cell population, a composition that is enriched in neutrophil progenitors and related uses and methods that address or at least ameliorate one or more of the above-mentioned problems.

SUMMARY

In one aspect, there is provided a method of identifying a neutrophil progenitor, the method comprising: determining an expression of at least one biomarker selected from the group consisting of: CD71, LOX-1, CD164, CD112, CD181, TACSTD2, CD11b and CD49d in a cell; and identifying the cell as a neutrophil progenitor when it is determined to have at least one of the following expression profiles: CD71^{hi/+}, LOX-1^{int/lo/-}, CD164^{hi/+}, CD112^{hi/+}, CD181^{int/lo/-}, TACSTD2^{hi/+}, CD11b^{lo/-} and/or CD49d^{int/hi/+}.

In one embodiment, the biomarker comprises CD71 and the cell is identified to be a neutrophil progenitor when it is determined to have CD71^{hi/+} expression.

In one embodiment, where the cell is identified as a neutrophil progenitor, the method further comprises: determining an expression of a further biomarker selected from CD49d and/or a side-scatter (SSC) property of the neutrophil progenitor; and identifying a subtype of the neutrophil progenitor based on the expression of the further biomarker and/or the side-scatter property.

In one embodiment, where the neutrophil progenitor is determined to be CD49d^{hi/+} and/or SSC^{lo}, the neutrophil progenitor is identified as an early committed neutrophil progenitor, and wherein where the neutrophil progenitor is determined to be CD49d^{int/lo/-} and/or SSC^{hi}, the neutrophil progenitor is identified as an intermediate neutrophil progenitor that is downstream in neutrophil lineage to the early committed neutrophil progenitor.

In one embodiment, determination of the expression of the at least one biomarker and/or the further biomarker comprises contacting the cell with one or more antibodies against the biomarker and/or the further biomarker.

In one aspect, there is provided a method of sorting and/or separating neutrophil progenitors from a cell population, the method comprising: selecting for cells having at least one of the following expression profiles: CD71^{hi/+}, LOX-1^{int/lo/-}, CD164^{hi/+}, CD112^{hi/+}, CD181^{int/lo/-}, TACSTD2^{hi/+}, CD11b^{lo/-} and/or CD49d^{int/hi/+}.

In one embodiment, the method comprises selecting for CD71^{hi/+} cells.

In one embodiment, the cell population is derived from cord blood and/or bone marrow.

In one embodiment, the method further comprises culturing the neutrophil progenitors to obtain proliferation and/or differentiation of the neutrophil progenitors to obtain progenies thereof.

In one embodiment, the method further comprises administering the neutrophil progenitors and/or the progenies thereof to a subject in need thereof.

In one embodiment, the subject has neutropenia.

In one embodiment, the selecting step comprises contacting the cells with one or more antibodies against one or more of CD71, LOX-1, CD164, CD112, CD181, TACSTD2, CD11b and CD49d.

In one aspect, there is provided a composition that is enriched in neutrophil progenitors having at least one of the following expression profiles: CD71^{hi/+}, LOX-1^{int/lo/-}, CD164^{hi/+}, CD112^{hi/+}, CD181^{int/lo/-}, TACSTD2^{hi/+}, CD11b^{lo/-} and/or CD49d^{int/hi/+}.

In one embodiment, the composition is enriched in CD71^{hi/+} neutrophil progenitors.

In one aspect, there is provided the composition for use in therapy.

In one aspect, there is provided the composition for use in treating neutropenia.

In one aspect, there is provided use of the composition in the manufacture of a medicament for treating neutropenia.

In one aspect, there is provided a method of preparing a transfusion composition, the method comprising: enriching a composition for neutrophil progenitors having at least one of the following expression profiles: CD71^{hi/+}, LOX-1^{int/lo/-}, CD164^{hi/+}, CD112^{hi/+}, CD181^{int/lo/-}, TACSTD2^{hi/+}, CD11b^{lo/-} and/or CD49d^{int/hi/+}.

In one embodiment, the method comprises enriching the composition for CD71^{hi/+} neutrophil progenitors.

In one embodiment, the enriching step comprises contacting the composition with one or more antibodies against one or more of CD71, LOX-1, CD164, CD112, CD181, TACSTD2, CD11b and CD49d.

DEFINITIONS

The term "treatment", "treat" and "therapy", and synonyms thereof as used herein refer to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) a medical condition, which includes but is not limited to diseases, symptoms and disorders. A medical condition also includes a body's response to a disease or disorder, e.g., inflammation. Those in need of such treatment include those already with a medical condition as well as those prone to getting the medical condition or those in whom a medical condition is to be prevented.

As used herein, the term "therapeutically effective amount" of a compound will be an amount of an active agent that is capable of preventing or at least slowing down (lessening) a medical condition, such as neutropenia. Dosages and administration of compounds, compositions and formulations of the present disclosure may be determined by one of ordinary skill in the art of clinical pharmacology or

pharmacokinetics. See, for example, Mordenti and Rescigno, (1992) *Pharmaceutical Research*. 9:17-25; Morenti et al., (1991) *Pharmaceutical Research*. 8:1351-1359; and Mordenti and Chappell, "The use of interspecies scaling in toxicokinetics" in *Toxicokinetics and New Drug Development*, Yacobi et al. (eds) (Pergamon Press: NY, 1989), pp. 42-96. An effective amount of the active agent of the present disclosure to be employed therapeutically will depend, for example, upon the therapeutic objectives, the route of administration, and the condition of the patient. Accordingly, it may be necessary for the therapist to titer the dosage and modify the route of administration as required to obtain the optimal therapeutic effect.

The term "subject" as used herein includes patients and non-patients. The term "patient" refers to individuals suffering or are likely to suffer from a medical condition such as cancer, while "non-patients" refer to individuals not suffering and are likely to not suffer from the medical condition. "Non-patients" include healthy individuals, non-diseased individuals and/or an individual free from the medical condition. The term "subject" includes humans and animals. Animals include murine and the like. "Murine" refers to any mammal from the family Muridae, such as mouse, rat, and the like.

As used herein, an "expression" refers to both genotypic as well as phenotypic expression of biomarkers in the present disclosure.

A "biomarker" refers to a molecule, for example, a protein, carbohydrate structure, glycolipid, glycoprotein (including cell surface glycoprotein), receptor (including cell surface receptor) or gene (or nucleic acid encoding the gene), the expression of which in or on a cell (or sample) derived from a subject (such as a mammalian tissue) can be detected by standard methods in the art (as well as those disclosed herein). In some examples, a biomarker may be any molecule that may serve as an identifier (i.e., marker) of a target of interest. In some examples, a biomarker may be a cell surface receptor, a cell surface glycoprotein, a transcription factor, and the like. In some examples, the biomarker may be used to classify, identify, detect, isolate, purify, enrich, select, sort, and/or separate a cell/ cell population, and/or determine a stage of differentiation, a stage of development and/or activity state of a cell.

In some examples, where the biomarker comprises a cell surface marker, the expression of the marker may be denoted in accordance to the acceptable denotation known in common general knowledge. For example, for a cell surface marker CD71, a CD71⁺ refers to the cell positively expressing CD71, a CD71⁻ refers to the cell not

expressing detectable CD71, CD71^{lo} refers to the cell expressing low CD71, CD71^{int} refers to the cell expressing intermediate CD71, and CD71^{hi} refers to the cell expressing high CD71.

As used in the specification herein, agents for detecting biomarkers in the present disclosure refer to any compound, molecule and/or system that functions to detect the presence/absence and/or expression or level thereof of biomarkers in the present disclosure. Such agents are capable of detecting and/or binding directly or indirectly to a biomarker. In the present disclosure, additional moieties may be required to enhance the detection of the biomarkers, for example, by/through amplifying optical diffraction. Examples of agents and the additional moieties include but are not limited to proteins (for example antigen binding proteins such as antibodies or fragments thereof, enzymes such as horseradish peroxidases and alkaline phosphatase, and the like), polynucleotides (for example aptamers), and small molecules (for example metallic nanoparticles).

The term "micro" as used herein is to be interpreted broadly to include dimensions from about 1 micron to about 1000 microns.

The term "nano" as used herein is to be interpreted broadly to include dimensions less than about 1000 nm.

The term "particle" as used herein broadly refers to a discrete entity or a discrete body. The particle described herein can include an organic, an inorganic or a biological particle. The particle used described herein may also be a macro-particle that is formed by an aggregate of a plurality of sub-particles or a fragment of a small object. The particle of the present disclosure may be spherical, substantially spherical, or non-spherical, such as irregularly shaped particles or ellipsoidally shaped particles. The term "size" when used to refer to the particle broadly refers to the largest dimension of the particle. For example, when the particle is substantially spherical, the term "size" can refer to the diameter of the particle; or when the particle is substantially non-spherical, the term "size" can refer to the largest length of the particle.

The terms "coupled" or "connected" as used in this description are intended to cover both directly connected or connected through one or more intermediate means, unless otherwise stated.

The term "associated with", used herein when referring to two elements refers to a broad relationship between the two elements. The relationship includes, but is not limited to a physical, a chemical or a biological relationship. For example, when

element A is associated with element B, elements A and B may be directly or indirectly attached to each other or element A may contain element B or vice versa.

The term "adjacent" used herein when referring to two elements refers to one element being in close proximity to another element and may be but is not limited to the elements contacting each other or may further include the elements being separated by one or more further elements disposed therebetween.

The term "and/or", e.g., "X and/or Y" is understood to mean either "X and Y" or "X or Y" and should be taken to provide explicit support for both meanings or for either meaning.

Further, in the description herein, the word "substantially" whenever used is understood to include, but not restricted to, "entirely" or "completely" and the like. In addition, terms such as "comprising", "comprise", and the like whenever used, are intended to be non-restricting descriptive language in that they broadly include elements/components recited after such terms, in addition to other components not explicitly recited. For example, when "comprising" is used, reference to a "one" feature is also intended to be a reference to "at least one" of that feature. Terms such as "consisting", "consist", and the like, may in the appropriate context, be considered as a subset of terms such as "comprising", "comprise", and the like. Therefore, in embodiments disclosed herein using the terms such as "comprising", "comprise", and the like, it will be appreciated that these embodiments provide teaching for corresponding embodiments using terms such as "consisting", "consist", and the like. Further, terms such as "about", "approximately" and the like whenever used, typically means a reasonable variation, for example a variation of $\pm 5\%$ of the disclosed value, or a variance of 4% of the disclosed value, or a variance of 3% of the disclosed value, a variance of 2% of the disclosed value or a variance of 1% of the disclosed value.

Furthermore, in the description herein, certain values may be disclosed in a range. The values showing the end points of a range are intended to illustrate a preferred range. Whenever a range has been described, it is intended that the range covers and teaches all possible sub-ranges as well as individual numerical values within that range. That is, the end points of a range should not be interpreted as inflexible limitations. For example, a description of a range of 1% to 5% is intended to have specifically disclosed sub-ranges 1% to 2%, 1% to 3%, 1% to 4%, 2% to 3% etc., as well as individually, values within that range such as 1%, 2%, 3%, 4% and 5%. It is to be appreciated that the individual numerical values within the range also include

integers, fractions and decimals. Furthermore, whenever a range has been described, it is also intended that the range covers and teaches values of up to 2 additional decimal places or significant figures (where appropriate) from the shown numerical end points. For example, a description of a range of 1% to 5% is intended to have specifically disclosed the ranges 1.00% to 5.00% and also 1.0% to 5.0% and all their intermediate values (such as 1.01%, 1.02% ... 4.98%, 4.99%, 5.00% and 1.1%, 1.2% ... 4.8%, 4.9%, 5.0% etc.,) spanning the ranges. The intention of the above specific disclosure is applicable to any depth/breadth of a range.

Additionally, when describing some embodiments, the disclosure may have disclosed a method and/or process as a particular sequence of steps. However, unless otherwise required, it will be appreciated that the method or process should not be limited to the particular sequence of steps disclosed. Other sequences of steps may be possible. The particular order of the steps disclosed herein should not be construed as undue limitations. Unless otherwise required, a method and/or process disclosed herein should not be limited to the steps being carried out in the order written. The sequence of steps may be varied and still remain within the scope of the disclosure.

Furthermore, it will be appreciated that while the present disclosure provides embodiments having one or more of the features/characteristics discussed herein, one or more of these features/characteristics may also be disclaimed in other alternative embodiments and the present disclosure provides support for such disclaimers and these associated alternative embodiments.

DESCRIPTION OF EMBODIMENTS

Exemplary, non-limiting embodiments of a method of characterising/identifying a granulocyte-monocyte progenitor (GMP) population, such as a neutrophil progenitor subpopulation, and related compositions, methods and kits are disclosed hereinafter.

GMPs are known to give rise to granulocytes (such as neutrophils, eosinophils, and basophils) and monocytes. In some examples, GMPs are found to contain a mixed population of monocytic, neutrophilic, eosinophilic and basophilic progenitor subsets. In various embodiments, there is provided a method of classifying, identifying, detecting, isolating, purifying, enriching, selecting, sorting and/or separating a GMP cell/population/subpopulation/subset and/or determining a stage of differentiation, a

stage of development and/or activity state of a cell/population/subpopulation/subset. GMP subpopulations (or subsets) may include a monocytic progenitor subpopulation, a neutrophilic progenitor subpopulation, an eosinophilic progenitor subpopulation and a basophilic progenitor subpopulation. Further distinct subpopulations (or subsets) may exist within these progenitor subpopulations.

In various embodiments, the method comprises determining a transcriptomic profile and/or proteomic profile of the cell/population/subpopulation/subset. In various embodiments, the method comprises determining and/or measuring the presence/absence/amount/level/proportion of one or more markers/biomarkers/signatures in the cell/population/subpopulation/subset. In various embodiments, the method comprises determining and/or measuring one or more physical properties of the cell/population/subpopulation/subset. The determination or measurement may be quantitative, semi-quantitative or qualitative.

In various embodiments, the method comprises determining or measuring the presence/absence/amount/level/proportion of at least about one, at least about two, at least about three, at least about four, at least about five, at least about six, at least about seven, at least about eight, at least about nine or at least about ten markers/biomarkers and/or physical properties associated with the cell/population/subpopulation/subset. In various embodiments, the method comprises determining or measuring the presence/absence/amount/level/proportion of no more than about ten, no more than about nine, no more than about eight, no more than about seven, no more than about six, no more than about five, no more than about four, no more than about three, no more than two or not more than about one marker/biomarker and/or physical property associated with the cell/population/subpopulation/subset.

Markers/biomarkers/signature or a component thereof include, but are not limited to, polypeptides (e.g., cell surface proteins) and polynucleotides (e.g., DNA and RNA). The markers/biomarkers/signature may be transcriptomic and/or proteomic markers/biomarkers/signatures. In some embodiments, the marker comprises an RNA marker/biomarker/signature. In some embodiments, the marker comprises a protein marker/biomarker/signature. In some embodiments, the marker comprises a surface/cell surface marker/biomarker/signature. In some embodiments, the marker comprises a transcription factor.

In various embodiments, the marker/biomarker/signature is selected from the group consisting of: 33D1, 4-1BB Ligand, APCDD1, B220, B7H4, B7-H4, C3aR, Cadherin 11, CCR10, CCRL2, CCX-CKR (CCRL1), CD10, CD100, CD102, CD103, CD104, CD105 (Endoglin), CD106, CD107a, CD107b, CD109, CD111, CD112, CD114, CD115, CD116, CD117, CD119, CD11a, CD11b, CD11c, CD120a, CD120b, CD121a, CD122, CD123, CD124, CD126, CD127, CD129, CD13, CD130, CD131, CD132, CD133, CD134, CD135, CD137, CD138, CD14, CD140a, CD140b, CD141, CD142, CD143, CD144, CD146, CD147, CD148, CD15, CD150, CD151, CD152, CD152 (CTLA-4), CD153, CD154, CD155, CD156c, CD157, CD158, CD158b, CD158e1, CD159a, CD16, CD16.2, CD160, CD161, CD162, CD163, CD164, CD165, CD166, CD169, CD170, CD172a (SIRP α), CD172b (SIRP β), CD172g (SIRP γ), CD178, CD179a, CD179b, CD18, CD180, CD181, CD182, CD183, CD184, CD185, CD186, CD186 (CXCR6), CD19, CD191, CD192, CD193, CD194, CD195, CD196, CD197, CD198, CD199, CD1a, CD1b, CD1c, CD1d, CD2, CD20, CD200, CD200R, CD200R3, CD201, CD202b, CD203c, CD205, CD206, CD207, CD209, CD21, CD210, CD213 α 1, CD213 α 2, CD215, CD217, CD218a, CD22, CD220, CD220R, CD221, CD223 (LAG-3), CD226, CD227, CD229, CD23, CD230 (Prion), CD235ab, CD24, CD243, CD244 (2B4), CD245, CD25, CD252, CD253, CD254, CD255, CD258, CD26, CD261, CD262, CD263, CD265, CD266, CD267, CD268, CD269, CD27, CD272, CD273, CD274, CD275, CD276, CD277, CD278, CD279, CD28, CD282, CD284, CD29, CD290, CD294, CD298, CD3, CD30, CD300c, CD300d, CD300LG, CD301, CD301b, CD303, CD304, CD307e, CD309, CD31, CD314, CD317, CD318, CD319, CD32, CD323, CD324, CD325, CD326, CD328, CD33, CD334, CD335, CD336, CD337, CD338, CD339, CD34, CD34_MEC14.7, CD34_SA376A4, CD340, CD344, CD35, CD351, CD352, CD354, CD355, CD357, CD36, CD360, CD365, CD366, CD368, CD369, CD36L1, CD37, CD370, CD371, CD38, CD39, CD3e, CD4, CD40, CD41, CD42b, CD43, CD44, CD45, CD45.1, CD45.2, CD45RA, CD45RB, CD45RO, CD46, CD47, CD48, CD49a, CD49b, CD49c, CD49d, CD49e, CD49f, CD5, CD50, CD51, CD52, CD54, CD55, CD56 (NCAM), CD57, CD58, CD59, CD59a, CD6, CD61, CD62L, CD62P, CD63, CD64, CD66a, CD66b, CD66c, CD66e, CD69, CD7, CD70, CD71, CD73, CD74, CD79b, CD80, CD81, CD82, CD83, CD84, CD85, CD85d, CD85h, CD85k, CD86, CD87, CD88, CD89, CD8a, CD8b, CD9, CD90, CD90.1, CD90.2, CD92, CD93, CD94, CD95, CD96, CD97, CD98, CD99, cKit, CLEC4A, CX3CR1, CXCL16, CXCR7, DcTRAIL R1, Delta Opioid Receptor, Delta-like 1 (DLL1),

Delta-like 4 (DLL4), Dopamine Receptor D1 (DRD1), DR3, E-Cadherin, EGFR, EphA2, erbB3, ESAM, F4/80, FcεRIα, FLT3, FPR3, FR4, Galectin9, Ganglioside GD2, GARP, GATR Ligand, GL7, GPR19, GPR56, GPR83, Gr1, H2, HLA-A, HLA-B, HLA-C, HLA-DR, HLA-E, HVEM, IA/IE, IFNAR1, IFNγ R b chain, IFN-γ R b chain, Ig light chain κ, Ig light chain λ, IgD, IgG Fc, IgM, IL21R, IL23R, IL-28RA, IL33R, IL33Ra, Integrin α9β1, integrin β5, Integrin β7, Isotype AHlgG, Isotype mlgG1, Isotype mlgG2a, Isotype mlgG2b, Isotype mlgM, Isotype rlgG1, Isotype rlgG2a, Isotype rlgG2b, Isotype rlgG2c, Isotype rlgM, Isotype SHlgG, Jagged 2, JAML, KLRG1, Ksp37, LAP, LOX-1, LPAM_1, Ly-49A, Ly108, Ly49CFIH, Ly49D, Ly49H, Ly51, Ly6C, Ly6D, Ly6G, LY6G6D, Ly6K, Lymphotoxin β Receptor, Mac2, Mac3, MAdCAM1, MAIR-IV, MAIRV, MD1, MERTK, MICA, MICB, MRGX2, MSC, MUC-13, NK1.1, NKG2D, NKp80, Notch 1, Notch 2, Notch 3, Notch 4, Notch3, NPC, PD1H, PDC-TREM, PIR A, PIR B, Plexin B2, Podoplanin, PSMA, RAE1y, Sca-1, Sialyl Lewis X (dimeric), Siglec H, Siglec-10, Siglec-8, Siglec-9, SiglecF, SiglecH, SSC, SSEA-1, SSEA-3, SSEA-4, SSEA-5, SUSD2, TACSTD2, TCR gd, TCR Vd1.1_1.2, TCR α, TCR β, TCRb chain, TER119, TIGIT (VSTM3), Tim-2, Tim-4, TLR4, TLT-2, TM4SF20, TMEM8A, TNAP, TRA-1-60-R, TRA-1-81, TRA-2-49, TRA-2-54, Trem-like 4, TSLPR, VEGFR-3, VISTA and XCR1. In various embodiments, the markers/biomarkers/signature comprises one discussed in the Examples section of the present disclosure.

In some examples, a population of early neutrophil progenitors is found to exist within GMPs, the population extending along the developmental trajectory towards mature neutrophils. In various embodiments therefore, there is provided a method of classifying, identifying, detecting, isolating, purifying, enriching, selecting, sorting and/or separating a neutrophil progenitor / neutrophil progenitor population and/or determining a stage of differentiation, a stage of development and/or activity state of a neutrophil progenitor / neutrophil progenitor population. In various embodiments, the neutrophil progenitor comprises a committed neutrophil progenitor. For example, the neutrophil progenitor may be incapable of differentiating into a cell of a non-neutrophil lineage, such as a monocyte, under physiological conditions. In some examples, neutrophil progenitors give rise to Ly6G⁺ neutrophils but not macrophages when cultured with a factor (e.g., CSF-1) that promotes differentiation towards the monocytic lineage. Thus, while a neutrophil progenitor may differentiate (exclusively) along the neutrophil lineage (for example, to give rise to a pre-neutrophil (preNeu), an immature neutrophil or a mature neutrophil), a neutrophil progenitor may not differentiate into a

cell of a non-neutrophil lineage. In various embodiments, the neutrophil progenitor is capable of generating mature neutrophils, for example, CD16⁺CD10⁺ mature neutrophils. In various embodiments, the neutrophil progenitor comprises an early neutrophil progenitor (e.g., a neutrophil progenitor that is upstream of preNeu and other neutrophil precursors in neutrophil lineage). In various embodiments, the neutrophil progenitor has a high proliferative capacity (e.g., higher proliferative capacity than preNeu and other neutrophil precursors).

The neutrophil progenitor may be that of an animal or a human. In some embodiments, the neutrophil progenitor comprises the neutrophil progenitor of a mammal (e.g., humans, non-human primates, canine, murine (e.g., mouse, rat, rabbit etc.) and the like). In some embodiments, the neutrophil progenitor comprises a mouse neutrophil progenitor. In some embodiments, the neutrophil progenitor comprises a human neutrophil progenitor.

In various embodiments, there is provided a method of identifying a neutrophil progenitor, the method comprising: determining an expression of at least about one, at least about two, at least about three, at least about four, at least about five or at least about six, at least about seven, at least about eight, at least about nine, at least about ten, at least about 11, at least about 12, at least about 13, at least about 14 or at least about 15 biomarker(s) selected from the group consisting of: CD71, LOX-1, CD164, CD112, CD181, TACSTD2, Ly6C, CD115, CD11b, CD34, CD81, CD49a, CD49d, CD106 and CD63 in a cell. In various embodiments, there is provided a method of identifying a neutrophil progenitor, the method comprising: determining an expression of at least about one, at least about two, at least about three, at least about four, at least about five, at least about six, at least about seven or at least about eight biomarker(s) selected from the group consisting of: CD71 (transferrin receptor protein 1), LOX-1 (lectin-type oxidized low-density lipoprotein (LDL) receptor-1), CD164 (sialomucin core protein 24 or endolyn or cluster of differentiation 164), CD112 (poliovirus receptor-related 2 (PVRL2) or nectin-2), CD181 (Interleukin 8 receptor alpha or cluster of differentiation 181), TACSTD2 (Tumor Associated Calcium Signal Transducer 2), CD11b (Integrin alpha M or cluster of differentiation molecule 11b) and CD49d (Integrin α 4) in a cell. The cell may be a GMP cell (e.g., a cell having one or more of the following expression profiles: Lin⁻, cKit⁺, Sca-1⁻, CD34^{hi/+}, and CD16/32^{hi}), a bone marrow cell (e.g., a fetal bone marrow cell) or a cord blood cell. The cell may be an animal cell or a human cell. In various embodiments, where the cell is

determined to be $CD71^{hi/+}$, $LOX-1^{int/lo/-}$, $CD164^{hi/+}$, $CD112^{hi/+}$, $CD181^{int/lo/-}$, $TACSTD2^{hi/+}$, $CD11b^{lo/-}$ and/or $CD49d^{int/hi/+}$, the cell is identified to be a neutrophil progenitor. In various embodiments therefore, the method comprises: determining an expression of at least one biomarker selected from the group consisting of: $CD71$, $LOX-1$, $CD164$, $CD112$, $CD181$, $TACSTD2$, $CD11b$ and $CD49d$ in a cell; and identifying the cell as a neutrophil progenitor when it is determined to have at least about one, at least about two, at least about three, at least about four, at least about five, at least about six, at least about seven or at least about eight of the following expression profiles: $CD71^{hi/+}$, $LOX-1^{int/lo/-}$, $CD164^{hi/+}$, $CD112^{hi/+}$, $CD181^{int/lo/-}$, $TACSTD2^{hi/+}$, $CD11b^{lo/-}$ and/or $CD49d^{int/hi/+}$. Advantageously, the biomarkers disclosed herein are found to be capable to distinguishing a neutrophil progenitor from cells of other types or from a heterogenous cell population (e.g., a GMP population). In other words, a neutrophil progenitor may be characterized or identified by the expression of one or more of these biomarkers.

In one embodiment, the biomarker comprises $CD71$. In one embodiment therefore, the cell is identified to be a neutrophil progenitor when it is determined to express $CD71$ or when it is determined to be $CD71^{hi/+}$. In various embodiments therefore, there is provided a method of identifying a neutrophil progenitor, the method comprising: determining an expression of $CD71$ in a cell; and identifying the cell as a neutrophil progenitor when it is determined express $CD71$. In some examples, $CD71$ is found to be exclusively expressed by neutrophil progenitors among total cord blood cells.

In some embodiments, the method may comprise determining an expression of a transcription factor in the cell. In some embodiments, the method may comprise determining an expression of at least about one, at least about two, at least about three, at least about four, at least about five, at least about six, or at least about seven transcription factor(s) selected from the group consisting of: $Jag1$, $Sox13$, $Gfi1$, $Per3$, $Cebpe$, $Ets1$ and $Gata1$ in the cell.

Among neutrophil progenitors (termed as proNeus), the inventors found two phenotypically distinct neutrophil progenitors (termed as proNeu1 and proNeu2). In various examples, proNeu1 was found to possess higher self-renewing potential/properties and/or enhanced colony forming potential than proNeu2. In various examples, transcriptomic pathway analysis supported the decrease in progenitor function of proNeu2, showing an exclusive enrichment in neutrophil effector

functions, while proNeu1 was enriched in cellular components and cell survival. In various examples, proNeu1 increased in numbers while proNeu2 remained largely unchanged during the course of an infection in a sepsis/inflammation model. In various examples, proNeu2 is shown to be a bridging point between proNeu1 and preNeu. In various examples, proNeu1 is shown to give rise to proNeu2 and subsequent neutrophil subsets and proNeu2 is shown to specifically give rise to preNeus and immature Neus. In various examples, proNeu1 is shown to exclusively give rise to downstream neutrophil subsets through a C/EBP ϵ -dependent proNeu2 development. In various examples, proNeu1 is shown to be an early committed neutrophil and proNeu2 is shown to be an intermediate neutrophil progenitor that is downstream in neutrophil lineage to proNeu1.

In various embodiments therefore, where the cell is identified as a neutrophil progenitor, the method further comprises determining a subtype of the neutrophil progenitor. Determining a subtype of the neutrophil progenitor may comprise determining/measuring an expression of further biomarker(s) in the cell and/or one or more physical property associated with the cell.

In various embodiments, determining/measuring an expression of further biomarker(s) in the cell comprises determining at least about one, at least about two, at least about three or at least about four biomarker(s) selected from the group consisting of: CD49d, CD34, CD106, CD11b in the cell. In various embodiments, the cell is identified as an early committed neutrophil when it is determined to have one or more of the following expression profiles: CD49d^{hi/+}, CD34^{hi/+}, CD106^{lo/-} and CD11b^{lo/-}. In various embodiments, the cell is identified as an intermediate neutrophil progenitor that is downstream in neutrophil lineage to the early committed neutrophil progenitor when it is determined to have one or more of the following expression profiles: CD49d^{int/lo/-}, CD34^{lo/-}, CD106^{hi/+} and CD11b^{hi/+}.

In various embodiments, determining/measuring one or more physical property associated with the cell comprises determining/measuring a granularity or complexity of the cell and/or a nucleus morphology of the cell. In various embodiments, the cell is identified as an early committed neutrophil when it is determined to have no or minimal hollowing of the nucleus that falls below a threshold diameter. In various embodiments, the cell is identified as an intermediate neutrophil progenitor that is downstream in neutrophil lineage to the early committed neutrophil progenitor when it is determined to have a hollowing of the nucleus that corresponds to or exceeds the threshold

diameter. In various embodiments, the cell is identified as an early committed neutrophil when it is determined to have low granularity or complexity. In various embodiments, the cell is identified as an intermediate neutrophil progenitor that is downstream in neutrophil lineage to the early committed neutrophil progenitor when it is determined to have high granularity or complexity. Granularity or complexity may be determined/measured by, for example, by evaluating a side-scatter property in flow cytometry. In various embodiments therefore, the cell is identified as an early committed neutrophil when it is determined to have low side scatter. In various embodiments therefore, the cell is identified as an intermediate neutrophil progenitor that is downstream in neutrophil lineage to the early committed neutrophil progenitor when it is determined to have high side scatter.

In some embodiments, where the cell is identified as a neutrophil progenitor, the method further comprises determining an expression of a further biomarker selected from CD49d and/or a side-scatter property of the neutrophil progenitor; and identifying a subtype of the neutrophil progenitor based on the expression of the further biomarker and/or the side-scatter property. In various embodiments, where the cell is determined to have CD49d^{hi/+} expression, the cell is identified as an early committed neutrophil progenitor. In various embodiments, where the cell is determined to have CD49d^{int/lo/-} expression, the cell is identified as an intermediate neutrophil progenitor that is downstream in neutrophil lineage to the early committed neutrophil progenitor. In various embodiments, where the cell is determined to have low side scatter, the cell is identified as an early committed neutrophil progenitor. In various embodiments, where the cell is determined to have high side scatter, the cell is identified as an intermediate neutrophil progenitor that is downstream in neutrophil lineage to the early committed neutrophil progenitor. In various embodiments therefore, where the neutrophil progenitor is determined to be CD49d^{hi/+} and/or SSC^{lo}, the neutrophil progenitor is identified as an early committed neutrophil progenitor, and wherein where the neutrophil progenitor is determined to be CD49d^{int/lo/-} and/or SSC^{hi}, the neutrophil progenitor is identified as an intermediate neutrophil progenitor that is downstream in neutrophil lineage to the early committed neutrophil progenitor. Embodiments of the method may also be used to determine a stage of differentiation and/or development of a neutrophil progenitor. For example, a neutrophil progenitor that is determined to be CD49d^{hi/+} and/or SSC^{lo} may be identified as being in an earlier stage of

differentiation and/or development than a neutrophil progenitor that is determined to be CD49d^{int/lo/-} and/or SSC^{hi}.

The expression of the biomarker may be determined or measured by methods known in the art. In some embodiments, determining/measuring an expression of a biomarker in a cell comprises performing RNA sequencing such as single-cell RNA sequencing. In some embodiments, determining/measuring an expression of a biomarker in a cell comprises contacting/incubating the cell with an agent for detecting the biomarker, for example, a molecule capable of binding to the biomarker or having affinity for the biomarker. Examples of agents/molecules that may be employed include, but are not limited to, small molecules, peptides, proteins, antigen-binding proteins, antibodies, polynucleotides, aptamers, fragments thereof and the like. In some embodiments, determining/measuring an expression of a biomarker in a cell comprises contacting/incubating the cell with at least about one, at least about two, at least about three, at least about four, at least about five, at least about six, at least about seven, at least about eight, at least about nine or at least about ten agents/molecules. In some embodiments, determining/measuring an expression of a biomarker in a cell comprises contacting/incubating the cell with no more than about ten, no more than about nine, no more than about eight, no more than about seven, no more than about six, no more than about five, no more than about four, no more than about three, no more than two or not more than about one agent/molecule.

In various embodiments, the molecule comprises an antigen-binding molecule. Examples of antigen-binding molecules include, but are not limited to, antibodies and fragments thereof such as antigen binding fragments. Non-limiting examples of antigen binding fragments include one or more fragments or portions of an antibody that retain the ability to specifically bind to an antigen (e.g., CD71), or synthetic modifications of an antibody fragments that retain the desired binding ability to the antigen. In various embodiments, antigen binding fragments include single domain antibodies, further engineered molecules (such as, but is not limited to diabodies, triabodies, tetrabodies, minibodies, and the like), Fab fragments, Fab' fragments, F(ab')₂ fragments, Fd fragments, Fv fragments, single-chain Fv (scFv) molecules, seFv molecules, scFv dimer, BsFv molecules, dsFv molecules, (dsFv)₂ molecules, dsFv-dsFv' molecules, Fv fragments, dAb fragments, bispecific antibodies, ds diabodies, nanobodies, domain antibodies, bivalent domain antibodies, and minimal

recognition units consisting of the amino acid residues that mimic the hypervariable region of an antibody (e.g. an isolated complementarity determining region (CDR)).

In various embodiments, determination of the expression of the biomarker comprises contacting the cell with one or more antibodies against the biomarker. In various embodiments, the antibody is coupled or conjugated to a label, for example, a fluorescent label such as a fluorescent dye. In various embodiments, flow cytometry is performed to determine the expression of the biomarker.

In various embodiments, there is provided a method of sorting and/or separating neutrophil progenitors from a cell population or a method of enriching neutrophil progenitors in a cell population, the method comprising: selecting for cells having/expressing one or more of the biomarkers as described herein. In various embodiments, there is provided a method of sorting and/or separating neutrophil progenitors from a cell population, the method comprising: selecting for cells having at least about one, at least about two, at least about three, at least about four, at least about five, at least about six, at least about seven or at least about eight of the following expression profiles: CD71^{hi/+}, LOX-1^{int/lo/-}, CD164^{hi/+}, CD112^{hi/+}, CD181^{int/lo/-}, TACSTD2^{hi/+}, CD11b^{lo/-} and/or CD49d^{int/hi/+}. In some embodiments, the method comprises selecting for CD71-expressing cells or CD71^{hi/+} cells.

In various embodiments, the cell population is derived from cord blood and/or bone marrow. In various embodiments, the cell population is derived from fetal bone marrow. In various embodiments therefore, the cell population comprises or consists of cord blood cells, bone marrow cells and/or fetal bone marrow cells.

In various embodiments, the cord blood and/or bone marrow is collected from a mammal, such as, but not limited to, humans, non-human primates, canine, murine (e.g., mouse, rat, rabbit, and the like) and the like. In some embodiments, the cord blood and/or bone marrow is collected from a mouse or a human. In some embodiments therefore, the cell population comprises at least one selected from the group consisting of: mouse cord blood cells, mouse bone marrow cells, mouse fetal bone marrow cells, human cord blood cells, human bone marrow cells and human fetal bone marrow cells. In some embodiments, the cell population consists of mouse cord blood cells, mouse bone marrow cells, mouse fetal bone marrow cells, human cord blood cells, human bone marrow cells and/or human fetal bone marrow cells. In various examples, there is provided a method isolating a subset of neutrophil

progenitors (proNeus) from human biological samples such as human cord blood and fetal bone marrow comprising the use of CD71 as a selection marker.

In various embodiments, the method further comprises culturing the neutrophil progenitors to obtain proliferation and/or differentiation of the neutrophil progenitors, for example, to mature neutrophils. In various embodiments, the mature neutrophils comprise CD16⁺CD10⁺ mature neutrophils.

In various embodiments, the method further comprises administering the neutrophil progenitors and/or progenies thereof (e.g., progenies resulting from the proliferation of the neutrophil progenitors and/or differentiation of the neutrophil progenitors) to a subject in need thereof. Examples of a subject in need thereof include, but is not limited to, a neutropenic subject, a subject having cancer, a subject having an infection (e.g., bacterial and/or fungal infections), a subject undergoing chemotherapy and/or radiation therapy, a subject having disorder of the blood, a subject having disorder of the bone marrow, a subject having disorder of the immune system, a subject who is a candidate for hematopoietic stem cell transplantation, a subject who is a candidate for myeloablative therapy and a subject having one or more of the following conditions: multiple myeloma, leukemia, lymphomas, aplastic anemia, thalassemia, sickle cell disease, severe combined immune deficiency syndrome and the like. In one embodiment, the subject has neutropenia.

In some embodiments, the cell population (i.e., the cell population which neutrophil progenitors is being separated from or in which neutrophil progenitors is being enriched in) is obtained/derived from the subject. In some embodiments therefore, the method further comprises a step of obtaining the cell population from the subject, for example, prior to a procedure such as hematopoietic stem cell transplantation or myeloablative therapy. In some embodiments, the cell population is obtained/derived from a donor.

In various embodiments, the selecting step comprises contacting/incubating the cell with agents for detecting the biomarker(s), for example, molecule(s) capable of binding to the biomarker(s) or having affinity for the biomarker(s) as described herein. In some embodiments, the selecting step comprises contacting/incubating the cell with at least about one, at least about two, at least about three, at least about four, at least about five, at least about six, at least about seven, at least about eight, at least about nine or at least about ten agents/molecules. In some embodiments, the selecting step comprises contacting/incubating the cell with no more than about ten, no more than

about nine, no more than about eight, no more than about seven, no more than about six, no more than about five, no more than about four, no more than about three, no more than two or not more than about one agent/molecule. In some embodiments, the molecule comprises an antibody. In various embodiments, the selecting step comprises contacting the cells with one or more antibodies against one or more of CD71, LOX-1, CD164, CD112, CD181, TACSTD2, CD11b and/or CD49d. The antibody may be coupled or conjugated to a label, for example, a fluorescent label such as a fluorescent dye. In various embodiments, flow cytometry is performed to select for neutrophil progenitors. In various embodiments, fluorescence-activated cell sorting (FACS) is used to select for neutrophil progenitors.

In various embodiments, the method further comprises washing the cells. In various embodiments, the method further comprises removing the antibodies bound to the biomarkers. For example, the REAlease Releasable Antibody technology (Miltenyl Biotech) may be utilized. A REAlease Release Reagent may be added to allow the spontaneous dissociation of the antibodies bound. In various embodiments, the method further comprises expanding the cells (e.g., *in vitro*) to dilute the bound antibodies. In various embodiments, the method further comprises expanding the cells, and subsequently removing those cells with antibodies bound thereto. Advantageously, dilution and/or removal of antibodies minimises or eliminates the problem of the antibodies interacting with the host immune system (e.g., the human immune system) when the cells (e.g., neutrophil progenitors and progenies thereof) are infused into the host, particularly when the antibodies are from a different species than the host (for example, when mouse-derived monoclonal antibodies are used to isolate neutrophil progenitors for infusion into a human).

As compared to using hematopoietic stem and progenitors (HSPCs) as the starting material and expanding neutrophils from these cells, embodiments of the method provide a more efficient way of producing neutrophils as the HSPCs may differentiate and expand into various cell lineages, resulting in lower yield and a longer processing time. The robust supply of neutrophils that may be produced by embodiments of the method may be advantageously harnessed for therapy or research (e.g., *in vitro* research).

Neutrophil progenitors may be used for large scale production of neutrophils for therapy (e.g., cell therapy) in, for example, myelo-ablated and neutropenic patients and for research. In various embodiments therefore, there is provided a composition

that is enriched in neutrophil progenitors and/or progenies thereof, for example, neutrophil progenitors having at least about one, at least about two, at least about three, at least about four, at least about five, at least about six, at least about seven or at least about eight of the following expression profiles: CD71^{hi/+}, LOX-1^{int/lo/-}, CD164^{hi/+}, CD112^{hi/+}, CD181^{int/lo/-}, TACSTD2^{hi/+}, CD11b^{lo/-} and/or CD49d^{int/hi/+}. In one embodiment, the composition is enriched in CD71-expressing or CD71^{hi/+} neutrophil progenitors and/or progenies thereof. In various embodiments, the amount/concentration of neutrophil progenitors and/or progenies thereof contained in the enriched composition is higher than the amount/concentration of neutrophil progenitors and/or progenies thereof naturally found in an animal or a human biological sample such as blood and bone marrow. In various embodiments, the composition further comprises one or more components of blood such as red blood cells, white blood cells of various types and platelets. In various embodiments, the composition comprises one derived/obtained from the bone marrow and/or blood (e.g., cord blood, peripheral blood etc.) which is subsequently enriched. In various embodiments, the composition comprises one derived/obtained from human bone marrow and/or human blood.

In various embodiments, there is provided a composition consisting of neutrophil progenitors and/or progenies thereof, for example, neutrophil progenitors having at least about one, at least about two, at least about three, at least about four, at least about five, at least about six, at least about seven or at least about eight of the following expression profiles: CD71^{hi/+}, LOX-1^{int/lo/-}, CD164^{hi/+}, CD112^{hi/+}, CD181^{int/lo/-}, TACSTD2^{hi/+}, CD11b^{lo/-} and/or CD49d^{int/hi/+}. In various embodiments, the composition consists of CD71-expressing neutrophil progenitors and/or CD71^{hi/+} neutrophil progenitors and/or progenies thereof. In various embodiments, the composition consists of neutrophils derived from neutrophil progenitors, for example, neutrophil progenitors having at least about one, at least about two, at least about three, at least about four, at least about five, at least about six, at least about seven or at least about eight of the following expression profiles: CD71^{hi/+}, LOX-1^{int/lo/-}, CD164^{hi/+}, CD112^{hi/+}, CD181^{int/lo/-}, TACSTD2^{hi/+}, CD11b^{lo/-} and/or CD49d^{int/hi/+}, or CD71-expressing neutrophil progenitors or CD71^{hi/+} neutrophil progenitors.

In various embodiments, the composition comprises a therapeutic composition. In various embodiments, there is provided embodiments of the composition for use in therapy. In various embodiments, there is provided embodiments of the composition

for use in cell therapy. In various embodiments, there is provided embodiments of the composition for use in treating a condition selected from the group consisting of: a blood disorder, a bone marrow disorder, an immune system disorder, cancer, blood cancer, an infection (e.g., bacterial and/or fungal infections), neutropenia, multiple myeloma, leukemia, lymphomas, aplastic anemia, thalassemia, sickle cell disease, severe combined immune deficiency syndrome and the like. In one embodiment, there is provided embodiments of the composition for use in treating neutropenia.

In various embodiments, there is provided use of embodiments of the composition in the manufacture of a medicament for treating a condition selected from the group consisting of: a blood disorder, a bone marrow disorder, an immune system disorder, cancer, blood cancer, an infection (e.g., bacterial and/or fungal infections), neutropenia, multiple myeloma, leukemia, lymphomas, aplastic anemia, thalassemia, sickle cell disease, severe combined immune deficiency syndrome and the like. In one embodiment, there is provided use of embodiments of the compositions in the manufacture of a medicament for treating neutropenia.

In various embodiments, there is provided a method of treating a condition selected from the group consisting of: a blood disorder, a bone marrow disorder, an immune system disorder, cancer, blood cancer, an infection (e.g., bacterial and/or fungal infections), neutropenia, multiple myeloma, leukemia, lymphomas, aplastic anemia, thalassemia, sickle cell disease, severe combined immune deficiency syndrome and the like in a subject, the method comprising administering to the subject embodiments of the composition. In some embodiments, the composition is derived from the subject (for example, prior to enrichment). In some embodiments, the composition is not derived from the subject. In some embodiments, the composition is derived from a donor. In some embodiments, the method comprises a method of treating neutropenia comprising the administration of CD71^{hi/+} or CD71-expressing neutrophil progenitor (proNeus) to neutropenic patients.

In various embodiments, there is provided use of embodiments of the compositions in the manufacture of a transfusion composition, for example, for treating a condition selected from the group consisting of: a blood disorder, a bone marrow disorder, an immune system disorder, cancer, blood cancer, an infection (e.g., bacterial and/or fungal infections), neutropenia, multiple myeloma, leukemia, lymphomas, aplastic anemia, thalassemia, sickle cell disease, severe combined immune deficiency syndrome and the like. In one embodiment, there is provided use

of embodiments of the compositions in the manufacture of a transfusion composition for treating neutropenia.

In various embodiments, there is provided a method of preparing a transfusion composition or a composition for transplantation e.g., for a subject such as a neutropenic subject, the method comprising: enriching a composition (or a starting composition) for neutrophil progenitors and/or progenies thereof, for example, neutrophil progenitors having at least one of the following expression profiles: CD71^{hi/+}, LOX-1^{int/lo/-}, CD164^{hi/+}, CD112^{hi/+}, CD181^{int/lo/-}, TACSTD2^{hi/+}, CD11b^{lo/-} and/or CD49d^{int/hi/+}. In various embodiments, the method comprises enriching the composition (or the starting composition) for CD71-expressing or CD71^{hi/+} neutrophil progenitors and/or progenies thereof. In various embodiments, the composition (or the starting composition) is derived/obtained from the bone marrow and/or blood (e.g., cord blood, peripheral blood etc.). In various embodiments, the composition (or the starting composition) is derived/obtained from human bone marrow and/or blood. The composition (or the starting composition) may be derived/obtained from the subject or a donor. In some embodiments thereof, the method comprises obtaining the composition (or the starting composition) from the subject prior to enrichment.

In various embodiments, the enriching step comprises contacting the composition (or the starting composition) with one or more agents for detecting the biomarkers, e.g., molecules capable of binding to one or more of the biomarkers as described herein. In various embodiments, the enriching step comprises contacting the composition (or the starting composition) with one or more antibodies against one or more of CD71, LOX-1, CD164, CD112, CD181, TACSTD2, CD11b and/or CD49d.

In various embodiments, there is provided a method of producing mature neutrophils for use in transplantation comprising of the steps: isolating a subset of neutrophil progenitor (proNeus) from human biological samples such as human cord blood and fetal bone marrow comprising the use of CD71 as a selection marker; culturing the isolated neutrophil progenitor population, for example, for three days in serum-free media containing myeloid expansion supplement for expansion.

In various embodiments, there is provided a method of identifying an agent that is capable of increasing the amount/concentration of neutrophils and/or neutrophil progenitors in a composition or in a subject, the method comprising: determining the amount/concentration of neutrophils and/or neutrophil progenitors in a first sample obtained from the composition or the subject at a first timepoint before the

administration/treatment of a candidate agent to the composition or the subject, determining the amount/concentration of neutrophils and/or neutrophil progenitors in a second sample obtained from the composition or the subject at a second timepoint after the administration/treatment of the candidate agent to the composition or the subject, and comparing the amount/concentration of neutrophils and/or neutrophil progenitors in the first sample and second sample. In various embodiments, the method further comprises concluding that the candidate agent is an agent capable of increasing the amount/concentration of neutrophils and/or neutrophil progenitors when the amount/concentration of neutrophils and/or neutrophil progenitors in the second sample is increased relative to the first sample and concluding that the candidate agent is not an agent capable of increasing the amount/concentration of neutrophils and/or neutrophil progenitors when the amount/concentration of neutrophil progenitors in the second sample is not increased relative to the first sample. In various embodiments, determining the amount/concentration of neutrophils and/or neutrophil progenitors in the first and/or second sample comprising determining the amount/concentration of cells having at least one of the following expression profiles: CD71^{hi/+}, LOX-1^{int/lo/-}, CD164^{hi/+}, CD112^{hi/+}, CD181^{int/lo/-}, TACSTD2^{hi/+}, CD11b^{lo/-} and/or CD49d^{int/hi/+}. In various embodiments, determining the amount/concentration of neutrophils and/or neutrophil progenitors in the first and/or second sample comprising determining the amount/concentration of CD71-expressing or CD71^{hi/+} cells.

In various embodiments, there is provided a kit, optionally a kit for use in a method as described herein, the kit comprising an agent for detecting biomarker, for example, a molecule capable of binding to the biomarker or having affinity for the biomarker. In various embodiments, the kit comprises at least about one, at least about two, at least about three, at least about four, at least about five, at least about six, at least about seven, or at least about eight molecules capable of binding to at least about one, at least about two, at least about three, at least about four, at least about five, at least about six, at least about seven or at least about eight of the biomarkers as described herein. In various embodiments, the kit comprises molecule(s) for binding to at least about one, at least about two, at least about three, at least about four, at least about five, at least about six, at least about seven or at least about eight biomarker(s) selected from the group consisting of: CD71, LOX-1, CD164, CD112, CD18, TACSTD2, CD11b and CD49d. In various embodiments, the kit comprises antibody or a plurality of antibodies for binding to at least about one, at

least about two, at least about three, at least about four, at least about five, at least about six, at least about seven or at least about eight biomarker(s) selected from the group consisting of: CD71, LOX-1, CD164, CD112, CD181, TACSTD2, CD11b and CD49d.

In various embodiments, there is provided a method or a product as described herein.

BRIEF DESCRIPTION OF FIGURES

FIG. 1. Single-cell RNA sequencing analysis reveal GMPs as a heterogeneous group of progenitors. (A) Re-analysis of available and annotated single-cell datasets containing mouse BM GMPs. Normalised counts from each dataset were downloaded and analysed using Seurat package. Louvain clustering was performed and annotations were assigned based on key lineage-related genes. (B) Gating strategy containing mouse BM CMPs, Ly6C⁻GMPs and Ly6C⁺GMPs. Colored dots represent index-sorted cells used for Smart-Seq2 scRNA-Seq. (C) (top) Sequenced cells in (B) are subjected to Monocle2 trajectory prediction. Cells are aligned according to pseudotime from left to right. Branching points are indicated and (bottom) the description of each lineage is shown. (D) Branchpoint #1 analysis of lineage-related genes and their expression across pseudotime between granulocytes (black line) and monocytes (grey line) (See also FIG. 8D). (E) Branchpoint #2 analysis of lineage-related genes and their expression across pseudotime between neutrophils (black line) and eosinophils (grey line) (See also FIG. 8E). (F) Total transcripts of CMP and GMP subsets, and sorted downstream neutrophil (preNeus) and monocyte (TpMos) precursors, are subjected to a PCA projection. (G) Smart-Seq2 sequenced cells dataset was integrated with the 10x (Tabula Muris) whole mouse BM dataset using Seurat v3. Data integration quality is represented in the overlay plot and (H) respective plots of each dataset indicating cell identities and relative cluster localisations. (See also FIG. 8).

FIG. 2. InfinityFlow resolves GMP heterogeneity on a proteomic level. (A) InfinityFlow workflow. Whole mouse BM cells were stained with a backbone panel of lineage markers (see methods) to allow separation of each lineage for UMAP generation. Cells are then aliquoted into wells containing PE-conjugated antibodies. Each well information was then recorded by flow cytometry and processed using

InfinityFlow pipeline. (B) t-SNE dimensional reduction analysis of 81 GMP-staining markers from InfinityFlow data. Phenograph clustering was performed and clusters are grouped to each myeloid lineage according to known lineage-restricted markers. (C) Analysis of the top discriminating markers for each cluster derived in (B). Black arrows (top) indicate known lineage-specific surface markers indicating GMP subset identity (right). Data is represented as a Z-score based on predicted Log₂ mean fluorescence intensity (MFI) from high (dark) to low (light). Importantly, each section of markers (demarcated by a space in between heatmaps) are the highly expressed markers that represent a certain progenitor subset of GMPs. (D) UMAP projection of total mouse BM cells, representing measured cKit expression levels from high (dark) to low (light) log₂MFI. Neu = neutrophil, preNeu= pre-neutrophil, Eo= Eosinophil, T,B = T and B cells, NK= natural killer cells, Mo = monocytes, Baso = Basophil, DC = dendritic cell, cMoP = common monocyte progenitor, GMP = granulocyte-monocyte progenitor. (E) Zoomed-in expression level (Log₂MFI) plots of GMP area (dotted box in (D)), representing measured intensities of backbone markers. Expression continuity of CD34 and CD11b expression from GMP to preNeu is shown (highlighted boxes). Cell subset regions are denoted (bottom left), indicating bridge of progenitor cells. (F) Putative markers for GMP subset identification by InfinityFlow. Zoomed-in plots are represented as predicted intensities (Log₂MFI) from low (light) to high (dark) levels. (See also FIG. 9)

FIG. 3. Flow cytometric analysis of BM GMPs reveal a CD106⁺CD11b^{lo} and a CD106⁺CD11b^{hi} neutrophil progenitor population. (A) Gating and sorting strategy of mouse BM myeloid cell populations. CMP = common myeloid progenitor, cMoP = common monocyte progenitor. (B) Representative micrographs (n=3) of sorted populations indicated in (A). Scale bars= 10um. Grey arrows indicate hollowing. (C) PCA analysis using bulk RNA-seq data from sorted neutrophil and monocytic precursors according to gating strategy in (A). (D-E) Normalised expression comparing the various key monocyte (D) and neutrophil (E) transcription factors. Results are expressed as mean Log₂RPKM (n=3) ±SD. (F) Heatmap of the top 5% most variable mouse transcription factors among indicated subsets. Known key factors are highlighted in bold. ProNeu-specific genes are highlighted as shown (black box). Data is represented as a Z-Score of expression (Log₂RPKM), calculated per gene from high (dark) to low (light) levels. Importantly, the critical differential genes of each lineage are indicated in bold on the right. (See also FIG. 10)

FIG. 4. Neutrophil development is dependent on two committed proNeu populations. (A) Representative FACS plot of gated proNeu1 and proNeu2 in WT and *Cebpe*^{-/-} mice. (B) Absolute counts of BM myeloid progenitors from WT and *Cebpe*^{-/-} mice. Data is expressed as mean $\pm$ SD (n=5 per group) and is representative of two independent experiments** = p< 0.01, *** = p<0.001, **** = p<0.0001 (Student t-test). (C) BM chimeras were made by reconstituting irradiated mice with equal ratios of WT CD45.1⁺ and *Cebpe*^{-/-} CD45.2⁺ cells. The percentage contributions of various hematopoietic cells by WT CD45.1⁺ or *Cebpe*^{-/-} CD45.2⁺ cells are expressed as mean $\pm$ SD (n = 5) and are representative of two independent experiments. **** = p<0.0001 (Student t-test). (D) UMAP analysis of total live BM cells from WT and *Cebpe*^{-/-} mice. Cells were manually gated and overlaid onto the UMAP plot, representing the localization of each subset on the UMAP space. (E) *In vitro* stimulation of sorted populations with CSF-1, quantified for F4/80⁺ macrophages at day 3 and 5. Results are represented as mean (n=4-5 per subset) $\pm$ SD and are representative of three independent experiments. (F) (top) Experimental setup of (bottom) Intra-BM transfer of sorted uGFP⁺ proNeu1, CD45.2⁺ proNeu2 and ROSA^{mT/mG} RFP⁺ preNeus into wildtype CD45.1⁺ recipients. Black dots represent transferred subset after 3 days. Data are representative of five recipient mice from two independent experiments. Mo = monocytes, Eo = eosinophils. (See also FIG. 11)

FIG. 5. proNeu1 and proNeu2 are functionally distinct progenitors. (A) Heatmap of total ANOVA-corrected variable genes from bulk RNA-Seq transcripts of sorted precursor subsets. Data is represented as a Z-Score, from low (light) to high (dark) expression. Gene clusters (1 to 3) were defined according to hierarchical clustering and exported for (B) gene ontology (GO) enrichment analysis showing the top GO terms (biological process, cellular component and molecular function) using EnrichR. (C) *In vitro* proliferation assay of sorted populations over 4 days in culture. Results are expressed as mean fold change (n=3 per subset) $\pm$ SD and are representative of at least three independent experiments. (D) *In vitro* colony forming potential of sorted populations. Results are expressed as mean (n=3 per subset) $\pm$ SD and are representative of at least three independent experiments. * = p<0.05 (Student t-test). (E) Percentage of cells in the proliferating S-G2-M phase of the cell cycle denoted by Fucci-S/G2/M positive cells. Data are representative of two independent experiments. (F) GO enrichment and biofunction enrichment analysis comparison of proNeu1 and proNeu2. Biofunction enrichment analysis was performed with Ingenuity

Pathway Analysis (IPA) tool. (G) Volcano plot comparing BM proNeu1 and proNeu2. Selected DEGs corresponding to proNeu1 and proNeu2 function are labelled on the plot. (H) Experimental set-up of mid-grade cecal ligation and puncture (CLP) sepsis kinetics and analysis timepoints. (I) Absolute counts of total bone marrow GMPs (left) and GMP subsets based on Ly6C expression (right) at indicated timepoints. (J) FACS analysis of BM Ly6C⁺GMP composition at the indicated timepoints. * = $p < 0.05$ (One-way ANOVA). (K) Absolute counts of total BM GMPs (left) and Ly6C⁺GMP subsets (right) at indicated timepoints. (H-K) Data are expressed as mean ($n=4-9$ per timepoint) $\pm$ SD and are representative of two independent experiments. *= $p < 0.05$ (One-way ANOVA). (See also FIG. 12)

FIG. 6. Skewed specification of GMPs towards neutrophil commitment is directed by G-CSF during emergency granulopoiesis. (A) Transcriptomic comparison between day 3 septic BM Ly6C⁺GMPs against WT sham control BM Ly6C⁺GMPs. Selected genes represent various genes critical for the various myeloid lineages. (B) Cytokine analysis of serum from mid-grade septic mice at various timepoints. Data is expressed as mean ($n=4-9$ per timepoint) $\pm$ SD from two independent experiments. *= $p < 0.05$, ***= $p < 0.001$, ****= $p < 0.0001$ (One-way ANOVA). (C) Analysis of Ly6C⁺GMP subsets after G-CSF complex (G-CSFcx) administration. Data are expressed as mean ($n=5$ per timepoint) $\pm$ SD and are representative of two independent experiments. * = $p < 0.05$ (One-way ANOVA) (D) Myeloablative treatment experimental plan (left) and (right) analysis of Ly6C⁺GMP composition with or without G-CSFcx intervention at day 9 of 5FU treatment. Lines represent absolute counts of total Ly6C⁺GMPs at the indicated timepoints. (E) Absolute numbers of bone marrow mature neutrophils and blood neutrophils at the indicated timepoints. (D-E) Data are expressed as mean ($n=5$ per timepoint) $\pm$ SD and are representative of two independent experiments. ** = $p < 0.01$ (One-way ANOVA). (See also FIG. 13)

FIG. 7. InfinityFlow of human cord blood characterises 2 proNeu subsets. (A) UMAP of total (red blood cell lysed) umbilical cord blood cells. Each cluster is annotated according to their marker profiles. T,B,NK cells were clustered together as their markers were grouped together as lineage exclusion makers. (B) Expression plots of backbone markers denoting the various neutrophil subsets. Results are expressed as scaled fluorescence intensity. (C) InfinityFlow predicted expression levels of putative markers for proNeu identification. Results are expressed as scaled predicted fluorescence intensity from low (light) to high (dark) levels. (D) Gating of

proNeus (left) reveals a rare $SSC^{lo}CD49d^{+}proNeu1$ and $SSC^{hi}CD49d^{int}proNeu2$ subset (right). As proNeu1 cells were rare, the FACS plot of proNeu subsets was generated from a concatenation of all LEGENDScreen™ sample files. (E) Histogram plots of fluorescence intensities of proNeu subsets. (F) FACS analysis of neutrophil subsets from cord blood, peripheral blood and fetal bone marrow samples. (G) Taking the top-expressing markers from each subset ($MFI > 10^3$), a side-by-side comparative heatmap was generated and plotted. Expression is represented as a Z-score of $\log_2$ MFI normalised per marker, from low (light) to high (dark) levels. (See also FIG. 14).

FIG. 8. GMPs are a heterogeneous group of progenitors including known monocyte progenitors. (A) (top) Gating strategy of MDPs (monocyte-dendritic cell progenitors) (in dark grey) and cMoPs (common monocyte progenitor) (in light grey) according to (Hettinger et al., 2013). (bottom) Back-gating and overlap of each population onto traditionally gated GMPs (granulocyte-monocyte progenitors) is shown. (B) (left) Gating strategy of BM progenitor populations according to (Liu et al., 2019) and (right) overlap of gated population with conventional GMP gating strategy. (C) Sorting strategy of preNeus and TpMo used for single-cell sequencing. (D-E) Branch expression analysis modelling (BEAM) of (D) branchpoint #1 and (E) branchpoint #2 using Monocle2. Gene expression is represented from low (light) to high (dark) levels. (F) Gene expression analysis of neutrophil-related genes in the indicated subsets. (G) mRNA and corresponding protein marker expression level plots of the index-sorted cells.

FIG. 9. UMAP, with InfinityFlow, reveals the expression continuity of the various BM lineages. (A) Developmental continuity of cell lineages of the various myeloid lineages expression. (B) Unbiased clustering with PhenoGraph of InfinityFlow dataset. Cluster identity is identified using typical lineage markers and is represented in Table 2. (C) UMAP plots of known lineage-restricted markers from low (light) to high (dark) expression levels, indicating each cell type.

FIG. 10. proNeu2 are $CD34^{lo}CD11b^{hi}$ progenitors found outside the GMP phenotype. (A) Localisation of proNeu1 and preNeus in integrated dataset and (B) differential expression profile of clusters indicated. This expression data is only derived from the unintegrated Tabula Muris dataset. (C) GMP gating strategy without $CD11b$ exclusion showing a $CD11b^{lo}$ and $CD11b^{hi}$ proNeus. (D) FACS plot of $CD115$ (CSF-1R) expression down-regulation with increased temperatures during tissue

processing. Data is representative of at least three independent experiments. (E) Frequency and absolute counts of neutrophil progenitors in the mouse bone marrow. Data is expressed as mean $\pm$ SD (n=5) and is representative of three experiments.

FIG. 11. Neutrophil development is dependent on two committed proNeu populations. (A) UMAP plot of total BM from WT (left) and *Cebpe*^{-/-} (right) mice indicating the various cell lineages. Cells were manually gated and overlaid onto the UMAP plot to view each population's location and relationship with their adjacent cell types. (B) (top) UMAP expression plots of CD81 and CD106, indicating the localisation of proNeu2. (bottom) UMAP expression heatmap plots of CD115 and SiglecF indicating the atypical granulocyte population in *Cebpe*^{-/-} BM cells. (C) *In vitro* stimulation of sorted populations with M-CSF, quantified for Ly6G⁺ neutrophils at day 3 and 5. Results are represented as mean (n=3 per subset) $\pm$ SD and are representative of three independent experiments. (D) Intra-BM transfer of sorted uGFP⁺ Ly6C⁺GMP, proNeu1 or proNeu2 into wildtype recipients. Results represent progeny of transferred proNeu1 subset after 1 day of transfer. (E) Composition of the progeny of each transferred subset was analyzed and represented in a bar graph. Data is expressed as mean $\pm$ SD (n=4 per group) from two independent experiments.

FIG. 12. Characterisation of two functionally distinct proNeu subsets. (A) Genes encoding for granules and (B) ATP metabolic processes among neutrophil precursor subsets are shown as heatmaps, expressed as normalised values (Z-score) from low (light) to high (dark) levels. Importantly, development from proNeu1 to proNeu2 to preNeu is associated with an increase in ATP metabolic processes. (C) Representative colonies of progenitor subsets at the indicated timepoints. Scale bars = 50 μ m. (D) mRNA and protein expression of various markers among cMoP and neutrophil precursor subsets are shown as heatmaps, expressed as normalised values (Z-score of log₂RPKM (for mRNA expression) or MFI (for protein expression)) from low (light) to high (dark) levels. (E) Representative FACS plots of BM Ly6C⁺GMPs during mid-grade sepsis at the indicated time points. (F) Representative FACS plot showing *Csf1r*-GFP expression of BM Ly6C⁺ GMPs during sepsis. (G) Subset percentages within Ly6C⁺GMPs at the indicated timepoints after sepsis onset. Data are expressed as mean (n=3 per timepoint) $\pm$ SD and are representative of two independent experiments. * = p<0.05 (Student t-test). (H) Frequency of *Csf1r*-GFP

cells three days after mid-grade sepsis onset. Data are expressed as mean (n=3) $\pm$ SD and are representative of two independent experiments. * = $p < 0.05$ (Student t-test).

FIG. 13. Skewed specification of GMPs towards neutrophil commitment is directed by G-CSF during emergency granulopoiesis. (A) Pearson correlation of the percentage of cMoPs among Ly6C⁺GMPs against spleen sizes measured in grams. (B) Cytokine profile of septic mice at the indicated timepoints of sepsis onset. Results are expressed as relative concentrations (normalised per analyte) from low (light) to high (dark) levels.

FIG. 14 (related to FIG. 7). CD71 specifically isolates proNeu from cord blood. (A) Expression plot of CD71 visualised on UMAP of total cord blood cells. Expression is represented as relative fluorescence intensity (predicted). (B) Flow cytometric validation of CD71 as putative marker for proNeu isolation. (C) Spearman rank correlation plots of mouse surface marker MFI against human surface marker MFI. Each dot represents a common surface marker expressed both in mouse and human (n=140).

FIG. 15. *In vitro* differentiation capacity of proNeu subsets. Sorted cells of CD34^{hi} (stem and progenitors), proNeu1 and proNeu2 were cultured for three days in serum-free media containing myeloid expansion supplement (Stem Cell Technologies). Cells were then harvested and analysed for mature neutrophil (CD66b⁺CD16⁺CD10⁺) progeny. Data is representative of 4 donor frozen fetal marrow samples.

EXAMPLES

Example embodiments of the disclosure will be better understood and readily apparent to one of ordinary skill in the art from the following discussions and if applicable, in conjunction with the figures. It will be appreciated that the example embodiments are illustrative, and that various modifications may be made without deviating from the scope of the invention. Example embodiments are not necessarily mutually exclusive as some may be combined with one or more embodiments to form new exemplary embodiments.

The granulocyte-monocyte progenitor (GMP) is a Lin^{neg}cKit⁺CD34^{hi}CD16/32^{hi} lineage-primed progeny derived from common myeloid progenitors (CMPs) that forms characteristic granulocyte-macrophage (GM) colonies in culture. GMPs are known for

their potential to generate various myeloid progenies such as neutrophils and monocytes. Since GMPs generate both monocytes and neutrophils, their potential raises important questions during circumstances that demand conflicting needs of these two cell subsets.

In particular, neutrophils are produced in much larger quantities compared to monocytes and their lineage selection requires the repression of monocyte fate with Gfi1. Furthermore, kinetic labelling studies have demonstrated a much longer transit time of neutrophils in the bone marrow compared to monocytes. It thus remains unclear how GMPs adjust their functional output according to different demands, and whether the disparity in transit time between neutrophils and monocytes is due to distinctions between progenitor characteristics downstream or heterogeneity that already exists within GMPs.

To address these questions, recent advances in single cell transcriptomics have attempted to determine the developmental cell states of each cell, which has led to a discovery of heterogeneity in myeloid lineages and cell fate decisions. Specifically, it has been proposed that GMPs undergo a mixed-lineage state prior to granulocyte and monocyte specification. While these results provided insights into the lineage priming program within the GMP hierarchy, there is a lack of functional validation on how expression or suppression of lineage-affiliated genes will translate into cellular heterogeneity. Furthermore, although lineage heterogeneity within GMPs has been proposed, it is unclear if committed progenitors already exist among these progenitors and how they may behave differently during inflammation.

An attempt was made to resolve this heterogeneity with Ly6C and CD115 (CSF-1R) being utilized to subset GMPs into Ly6C⁻GMPs and Ly6C⁺CD115^{hi} monocyte (MPs) and Ly6C⁺CD115^{lo} granulocyte progenitors (GPs). However, these markers alone were not sufficient to fully resolve the strict lineage commitment of each progenitor subset, indicating that better markers are required to evaluate the heterogeneity of these progenitors. Particularly, better markers are required for identifying a neutrophil progenitor.

Neutrophils are important immune cells which provide protection against bacterial and fungal infections. Due to their short lifespan, neutrophils are continuously produced by specialised bone marrow progenitors to meet the daily demand of 100 million cells per day. Neutrophil development begins with long-lived hematopoietic stem cells, which give rise to highly proliferative progenitors that expand in numbers

to generate an adequate supply of mature effector neutrophils for immune surveillance and protection against microbial threats. Despite their importance, the identity and characteristics of a committed neutrophil progenitor are yet to be found.

The inventors have previously reported the identification of neutrophil precursors known as preNeus. While these precursors developed into neutrophils, they were unable to form colonies *in vitro*, indicating that they are not the true progenitor cells for neutrophils.

In this disclosure, through a combination of single-cell transcriptomic and proteomic analyses, the inventors successfully identified an early committed progenitor within the GMPs responsible for the strict production of neutrophils, which they have termed as proNeu1. The comprehensive dissection of GMP hierarchy led to the further identification of a previously unknown intermediate proNeu2 population. Similar populations could be detected in human samples.

The early committed neutrophil progenitor, proNeu1, is already present within the heterogenous population of GMPs. ProNeu1 is shown to give rise to the intermediate progeny proNeu2, which subsequently differentiates into downstream populations. Importantly, it was found that proNeu1 but not proNeu2, expanded extensively and specifically in the early phase of septic inflammation at the expense of monocytic differentiation.

The two subsets of neutrophil progenitors, proNeu1 and proNeu2, which are responsible for the extensive production of neutrophils in humans, are characterised by distinct protein surface marker expression profiles such as CD71 expression. Based on these surface markers, the inventors were able to isolate these progenitors and demonstrate their development into mature CD16⁺CD10⁺ neutrophils *in vitro*.

Collectively, the findings complete the neutrophil maturation trajectory roadmap and call for a revision of the classical GMP nomenclature. The disclosure further exemplifies the importance of understanding progenitor identities to study their function in health and disease.

Results

GMPs contain a heterogeneous group of lineage-committed progenitors

GMPs are known to give rise to granulocytes, such as neutrophils, and monocytes. As such, GMPs were commonly assumed to possess oligo-potent

differentiation potential. However, contrary to this assumption, recent studies have suggested that the GMPs are instead, a heterogeneous mixture containing lineage-committed precursors. To resolve these two viewpoints and to provide a comprehensive understanding of the true hematopoietic potential of cells within this population of cells, the inventors investigated the GMP population by performing an analysis with published repositories of single-cell transcriptomic datasets (Giladi et al., 2018; Olsson et al., 2016). By extracting the annotated GMPs (based on Lin⁻cKit⁺Sca-1⁻CD34^{hi}CD16/32^{hi}), the inventors performed t-Distributed Stochastic Neighbour Embedding (t-SNE) (Maaten and Hinton, 2008) analysis and confirmed that GMPs contained a mixed population of monocytic, neutrophilic, eosinophilic and basophilic progenitor subsets (FIG. 1A). Notably, in addition to current published literature, the inventors further discovered multiple clusters pertaining to different granulocyte and monocyte lineages based on known lineage restricted genes. These multiple clusters possessed key monocytic genes like *Irf8*, *Ly86* and *Csf1r* (FIG. 1A), and their gene expression correlated with the presence of common monocytic progenitors (cMoPs), monocyte progenitors (MPs) and monocyte-dendritic progenitors (MDPs) (FIG. 8A and 8B). The inventors also observed a neutrophil-like cluster containing high expressions of neutrophil elastase (*Ela*) and ficollin (*Fcfn*), which are known genes of the neutrophil lineage. These analyses provide further insight into the heterogeneity of progenitors within GMPs and indications of their cell lineage commitment at the transcriptomic level.

To further look into the various GMP developmental and transitional states leading towards lineage commitment, single-cell RNA-Seq analysis was performed on index-sorted CMPs and total GMPs. The inventors also sorted late precursors, preNeus and TpMos (transitional pre-monocytes), as reference points for neutrophil and monocyte differentiation respectively (FIG. 8C). Back gating of the sorted GMP population showed the presence of Ly6C⁺ and Ly6C⁻ subsets (FIG. 1B). Monocle (Qiu et al., 2017) was then utilised to order the cells in pseudotime, from CMPs to fully specified progenitors, allowing for the understanding of the various developmental and trajectory states. Monocle then revealed two distinct branchpoints (FIG. 1C). Deeper analysis on these branchpoints showed key fate-determining genes that change over the course of development suggestive of a monocyte-granulocyte at point 1 (FIG. 1D) and a neutrophil-eosinophil determination point at point 2 (FIG. 1E). These analyses revealed an initial down-regulation of monocytic fate-determining genes (*Ly86*, *Csf1r*,

Irf8), followed by a combinatorial and selective up-regulation of neutrophil-related genes (*Gfi1*, *Rgcc*, *Vcam1*) that are both important for initiating the neutrophil fate (FIG. 8D and S8E).

Upon further analysis with Monocle, the inventors noticed the presence of a specified progenitor population at the terminal end state of the neutrophil branchpoint within the Ly6C⁺ fraction of GMPs (FIG. 1C). These cells possessed the highest expression of known neutrophil-specific genes, such as *Gfi1* and *S100a8* among others (FIG. 8F). To determine if this population of cells could be putative neutrophil progenitors, these cells were mapped onto a principal component analysis (PCA) of total transcripts and it was discovered that they clustered closer to preNeus along PC2 away from TpMos (FIG. 1F). These putative neutrophil progenitors possessed low levels of CD115 and high levels of Ly6C, both at the mRNA and protein level (FIG. 8G). Furthermore, by performing single-cell data integration (Stuart et al., 2018) with the Tabula Muris (TM) BM dataset (Schaum et al., 2018), the inventors were able to confirm the cell identities from the dataset (FIG. 1G). Visualisation of the integration revealed a precise mapping of preNeus and TpMos to their respective lineages, while CMPs mapped towards the erythrocytic lineage as well as the dendritic cell lineage (FIG. 1H). Importantly, it was observed that the putative neutrophil progenitors associated with the initial neutrophil branch point, suggesting that this population can be the early progenitors for neutrophil development (FIG. 1H). Taken together, the data suggests a population of putative neutrophil progenitors exist within the GMP and are responsible for the generation of neutrophils.

InfinityFlow resolves GMP heterogeneity and identifies population discriminating surface markers

Although transcriptomic signatures provide a useful means in determining cell states, they do not confirm a cell's identity or allow further downstream analysis. Therefore, to validate the GMP heterogeneity on a proteomic level, the expression of 261 surface markers (LEGENDScreen™, Biolegend) (Table 1) on total mouse BM cells was evaluated by flow cytometry. The InfinityFlow (Dutertre et al., 2019) pipeline was then used to predict the co-expression of every surface marker tested and this predicted expression information was concatenated into a single analysis file (FIG. 2A). GMPs stained positively for 81 of these markers. The analysis was first restricted to these 81 markers, which were then used to perform a t-SNE analysis on GMPs only.

With PhenoGraph (Levine et al., 2015), the inventors could subset GMPs into 10 subpopulations, confirming on a proteomic level that GMPs are a mix of progenitors (FIG. 2B). Using the top discriminating markers for each cluster, the inventors were able to ascertain the identities of the known monocyte (cluster 2, 6 and 7), eosinophil (cluster 5) and basophil (cluster 4) committed progenitors by the expression of lineage-specific surface markers (FIG. 2C) (Arinobu et al., 2005; Hettinger et al., 2013; Iwasaki et al., 2005). Neutrophils, however, lacked lineage-specific markers to allow for a proper characterisation of this dedicated progenitor.

Therefore, to circumvent this challenge, UMAP analysis of the InfinityFlow dataset was performed to understand the developmental relationships between cell types in the BM based on their protein expression (Becht et al., 2018; McInnes et al., 2018). One major strength of UMAP is that it preserves the continuity of cell subsets, which allow for the identification of rare and/or transitional populations that would be masked in a t-SNE analysis (Becht et al., 2018). The UMAP analysis was expanded to total BM cells which discriminated the various cell lineages within the BM (FIG. 2D), and allowed for the observation of a clear developmental continuum of cells from early cKit⁺ progenitors to mature cKit⁻ cells of the basophil, eosinophil, monocyte and neutrophil lineages (FIG. 2D and 9A). Utilising the markers from InfinityFlow, each cell type was identified and annotated through the co-localisation of each marker on the UMAP space (FIG. 9B, Table 1 and 9C). From the UMAP analysis, the inventors observed GMPs (cKit⁺Sca-1⁻CD34⁺CD16/32^{hi}) with a gradual upregulation of Ly6C and a subsequent bifurcation into CD115⁻ and CD115⁺ cells, representing preNeus and cMoPs respectively (FIG. 2D and 2E). While it is evident that cMoPs are a subset of the GMP, the branch connecting GMPs with preNeus revealed a progressive upregulation of CD11b and downregulation of CD34 expression along the developmental continuity from GMPs (FIG. 2E). This suggests that an early neutrophil progenitor exist within GMPs and extend along the developmental trajectory towards mature neutrophils. Closer inspection revealed several differentially expressed cell surface markers, including CD81, CD49a, CD106 and CD63, which highlight this branch point (FIG. 2F). These markers potentially serve as positive or exclusion markers to isolate and characterize the putative early neutrophil progenitor.

Identification of an early neutrophil progenitor within the GMP

Using the newly identified markers CD81 and CD106, the inventors characterised two phenotypically distinct neutrophil progenitors (termed as proNeus). This included a CD34^{hi}CD106⁻CD11b^{lo} proNeu1 subset and a CD34^{lo}CD106⁺CD11b^{hi} proNeu2 subset (FIG. 3A). This observation is in line with the scRNA-seq data integration, which also unveiled a gap between the mapped neutrophil progenitor and preNeu cells (FIG. 10A), and cells within this gap expressed a continual progression of neutrophilic genes (FIG. 10B). This suggested that an intermediate subset exists within the developmental trajectory of neutrophil development and is absent from the currently defined GMP population. Indeed, proNeu2 escaped the GMP definition, as CD11b⁺ cells are excluded from the GMPs (FIG. 10C). The inventors also utilized CD81 to exclude cMoPs as this marker allows for the circumvention of the technical concern of CD115 down-regulation during cell preparation (FIG. 10D). Similar to cMoPs, these neutrophil progenitors are rare and they account for 0.05-0.1% of total BM cells (FIG. 10E).

Morphological analysis revealed an initial hollowing of the nucleus in the proNeu1 stage, which increases in diameter in the proNeu2 stage (FIG. 3B) and subsequent maturation stages. Using the same sorting strategy for neutrophil progenitors in FIG. 3A, the inventors isolated these cells and performed bulk RNA-seq of myeloid progenitors, including Ly6C⁻GMP, proNeu1, proNeu2, preNeu, cMoP and TpMo, to examine their differentiation and population inter-relationship. PCA analysis revealed a clear separation of the neutrophil and monocytic lineages (FIG. 3C). Deeper analysis of key neutrophilic and monocytic genes revealed similar expression levels of monocytic genes in Ly6C⁻GMPs and proNeu1 (FIG. 3D). These genes were then up-regulated in cMoPs, signifying their commitment to the monocyte fate. Interestingly, the expression level of key neutrophilic genes *Gfi1* and *Per3* in proNeu1 are also similar in Ly6C⁻GMPs (FIG. 3E). The inventors further extended the knowledge of myeloid transcriptional regulation by selecting the top 20% variable transcription factors between these progenitor subsets and plotted them on a heatmap. The analysis featured groups of transcription factors solely expressed in cMoPs or Ly6C⁻GMPs. Specifically, the data also suggests that *Jag1* and *Sox13* to be exclusive neutrophil lineage-committing factors (FIG. 3F). *Jag1*, in particular, was shown to be associated with G-CSF-mediated neutrophil differentiation.

It has been shown that a deficiency in C/EBP ϵ leads to a disruption in neutrophil development. Here, the RNA-Seq analysis showed an upregulation of *Cebpe* from proNeu1 to proNeu2, suggesting that C/EBP ϵ could be critical for the development of early neutrophil progenitors (FIG. 3E). To test this, the BM of wildtype (WT) and *Cebpe*^{-/-} mice was analysed. The analysis revealed that C/EBP ϵ is critical for proNeu2 development (FIG. 4A). Together with the loss of proNeu2, an accumulation of cMoPs was also observed, suggesting a block in neutrophil development resulted in a skewed differentiation towards the monocyte fate (FIG. 4B). These observations were further confirmed by generating BM chimeras with CD45.1⁺ WT and CD45.2⁺ *Cebpe*^{-/-} BM cells in a 50:50 ratio. Analysis of these mice BM showed that proNeu2, and downstream populations, were mostly generated from WT CD45.1⁺ cells (FIG. 4C). This suggests the importance of proNeu2 as a bridging point between proNeu1 and preNeu. UMAP further illustrates this, showing that proNeu2 acts as a bridge between proNeu1 and preNeu development, and the absence of these cells leads to an aberrant differentiation pathway generating atypical granulocytes that are SiglecF⁺CD115⁺ (FIG. 4D and 11B).

Since proNeu1s possessed both monocytic and neutrophilic transcription factors (FIG. 3D and 3E), it was hypothesized that proNeu1s may not be fully specified to neutrophil lineage and could give rise to monocytes depending on lineage cues. To assess the commitment fate of proNeus, the various sorted progenitor populations were cultured with CSF-1, which is known to skew differentiation towards the monocytic lineage. The results showed that both proNeu1 and proNeu2 gave rise to Ly6G⁺ neutrophils and not macrophages with CSF-1 (FIG. 11C), while both cMoPs and Ly6C⁻GMPs mostly gave rise to macrophages by day 5 of culture with CSF-1 (FIG. 4E). To further affirm the neutrophil commitment capacity of proNeu1 and proNeu2, the inventors adoptively transferred sorted Ly6C⁻GMPs, proNeu1s, proNeu2s and tracked their differentiation potential. While Ly6C⁻GMPs gave rise to both proNeu1s and cMoPs after 1 day, proNeu1 gave rise to proNeu2s and subsequent neutrophil subsets (FIG. 11D). ProNeu2s similarly only specifically gave rise to preNeus and immature Neus (FIG. 11E).

As these transfer experiments were performed in separate WT recipients, the inventors wanted to assess the differentiation potential of proNeus within the same microenvironment. To do this, the inventors co-transferred sorted and labelled

proNeus (1&2) and preNeus into WT recipients and tracked their development after three days. The results showed that both proNeu1s and proNeu2s could only give rise to neutrophils with negligible monocytic differentiation potential (FIG. 4F). Moreover, from proNeu1s to preNeus, a step-wise differentiation progression with a sequential upregulation of Ly6G and CXCR2 was observed. Together, the data highlights a programmed neutrophil lineage commitment that exists within the GMP and this begins with proNeu1s, which exclusively gives rise to downstream neutrophil subsets through a C/EBP ϵ -dependent proNeu2 development.

ProNeu1 and proNeu2 are functionally distinct early neutrophil progenitor populations.

To dissect the functional heterogeneity and progression of these neutrophil progenitors, the transcriptomic signatures from Ly6C⁺GMPs to preNeus was analyzed. By plotting the top variable genes among each subset, the inventors derived three distinct clusters of genes differentially regulated between each stage of neutrophil development (FIG. 5A). From the heatmap, the inventors observed a sequential increase in granule production (Cluster 1) (FIG. 5B and 12A) and a concomitant loss of mitochondrial and ribosomal genes (Cluster 3), in line with their progressive acquisition of neutrophil functionality (FIG. 5B and FIG. 12B).

As CD34 is a known marker for hematopoietic progenitors, it was wondered if progenitor properties are lost in a CD34^{lo} proNeu2 subset. To test this, the proliferative potential of proNeu1 and proNeu2 was evaluated. *In vitro* cultures showed that, unlike the minimal colony-forming activity of preNeus, both proNeu1 and proNeu2 possessed high proliferative potential (FIG. 5C). Notably, a lower proliferative capacity in proNeu2 compared to proNeu1 was observed, which is further supported by the fewer colonies generated *in vitro* (FIG. 5D and 12C). ProNeu1, distinct from proNeu2, possessed enhanced colony forming potential with a cell cycle activity similar to uncommitted Ly6C⁺GMPs, as shown by the Fucci cell-cycle transgenic reporter line (Sakaue-Sawano et al., 2008) (FIG. 5E). This suggests that proNeu1 possess higher self-renewing properties, which is then exchanged for effector functions in proNeu2. Furthermore, transcriptomic pathway analyses support this decrease in progenitor function of proNeu2, showing an exclusive enrichment in neutrophil effector functions, while proNeu1 are enriched in cellular components and cell survival (FIG. 5F).

Collectively, the data shows that proNeu1 and proNeu2 are two distinct progenitor subsets with unique function pertaining to the development of neutrophils. Similar to their surface marker expression, *Cd34* was found to be upregulated in proNeu1 while genes encoding CD106 (*Vcam1*) and CD11b (*Itgam*) were significantly enriched in proNeu2 (FIG. 5G). These results hence confirm the scRNA-seq and UMAP (FIG. 10A) analysis that proNeu2 is downstream in the neutrophil lineage from proNeu1.

To understand how these progenitors might play differential roles in inflammatory states, the inventors utilized a model of sepsis and tracked the BM progenitor composition at various timepoints (FIG. 5H). Through the course of infection, the inventors observed an expansion of Ly6C⁺GMPs but minimal changes in Ly6C⁺GMP numbers (FIG. 5I). Further analysis of Ly6C⁺GMPs revealed a skewed differentiation effect towards the neutrophil lineage on day 3, with a specific increase in proNeu1 frequency at the expense of cMoP numbers (FIG. 5J). Unlike proNeu1, proNeu2 remained largely unchanged during the course of infection, suggesting differential roles for each progenitor during sepsis. The inventors also showed that this skewing effect can be observed from day 1 of sepsis onset (FIG. 12E and 12G), and confirmed that CD115 was not down-regulated during inflammation using a *Csf1r*-GFP MaFIA transgenic mouse (FIG. 12F and 12H). The shift in myeloid progenitor potential then returns to physiological frequencies by day 9, together with a specific expansion of cMoPs (FIG. 5K), suggesting their importance during the resolution phase of sepsis. Importantly, this critical information would have been lost if total GMPs were analysed, exemplifying the need for the analysis of specific progenitor subsets to understand the dynamics of progenitor function in inflammatory conditions.

G-CSF-mediated BM skewing of myeloid progenitors can modulate neutrophil expansion

The ability of BM progenitors to replenish the circulating pool of immune cells is a critical step in infections and diseases when a large demand of mature cells is needed. In particular, neutralization of invading pathogens and microbial insults requires emergency granulopoiesis, which is a mechanism whereby the immune system adapts to produce and mobilize neutrophils in a prompt and efficient manner.

To determine if the increased proNeu1 frequencies during sepsis are pre-determined in early progenitors, transcriptomic analysis of Ly6C⁺GMPs was performed at day 3 of sepsis onset, revealing a down-regulation of multiple lineage associated

genes, such as *Mcpt8*, *Ly86*, *Prss34* and *Fcra1*. An up-regulation of granule protein genes such as *S100a9*, *S100a8*, *Chil3* and *Lcn2* was also observed (FIG. 6A). These results not only verify the importance of the proNeu findings, but further confirms the skewing effect of proNeu1 differentiation. Interestingly, at day 6 where variability of BM skewing is at its peak, the inventors observed a correlation of spleen size with the recovery of the skewed progenitor compartment (FIG. 13A), indicating a possible role of extra-medullary haematopoiesis in the resolution of sepsis.

To determine the factors and signals that enabled the expansion of proNeu1 and proNeu2, the inventors performed a screen of inflammatory analytes on mice serum at the various time points (FIG. 13B). Specifically, high levels of G-CSF and IL-6 were found at day 3, which may account for the skewed neutrophil potential (FIG. 6B). This is supported by a significant increase in proNeu frequencies through *in vivo* administration of G-CSF (FIG. 6C). To demonstrate this instructive skewing effect, myelo-ablated mice BM cells were analysed using a chemotherapeutic drug 5-Fluorouracil, and G-CSF was introduced during the recovery of the myeloid compartment. Introduction of G-CSF at day 9 showed a marked increase in proNeu1 frequency at day 12 after myeloablation (FIG. 6D), and this translated into much higher neutrophil production on day 15 (FIG. 6E). Taken together, the data highlights the relevance of the proNeu findings by providing a framework to understand the biology of sepsis through the comprehension of progenitor skewing. Notably, the results support the G-CSF-mediated skewing effect of proNeu commitment in the early phase of the sepsis inflammatory phase, which is subsequently compensated by high monocyte production and commitment for effective resolution of sepsis.

InfinityFlow of human cord blood cells reveal a proNeu1 and a proNeu2 subset

To determine if such progenitors exist in human, the same workflow (i.e., the InfinityFlow pipeline) was applied to examine whole human cord blood cells to screen for differential surface markers that can help identify these progenitor cells.

Briefly, Red blood cells (RBC)-lysed cord blood cells are stained with a common backbone panel of surface markers that denote the various cell lineages present in the cord blood. Thereafter, cells are aliquoted into wells, each containing a unique PE-conjugated surface marker (LEGENDScreen™). Samples are acquired and the expression profiles of each PE marker was processed with InfinityFlow, a machine learning application that predicts the co-expression profile of each marker with every

tested marker. This allowed for the discovery of new subsets with high dimensional phenotypic characterisation.

UMAP analysis of total cord blood cells showed distinct populations representing the various lineages (FIG. 7A). Deeper analysis into the neutrophil cluster revealed the previously identified three bone marrow neutrophil subsets, with similar protein expression profiles as described in mouse. Importantly, through UMAP, a putative proNeu population possessing low expression levels of CD11b and high levels of CD49d was identified (FIG. 7B). Mining on the InfinityFlow dataset, the inventors further identified several potential markers for human proNeu identification. These included CD71, LOX-1, CD164, CD112, CD181 and TACSTD2 (FIG. 7C). Validation of one of these markers, CD71, showed that it was exclusively expressed by proNeus among total RBC-lysed cord blood cells (FIG. 14A). This suggests a possible isolation strategy of proNeus (FIG. 14B). This strategy is an easy 2-step process, which utilizes large volumes of donor cord blood commonly available for clinical use. These cells are typically discarded after enrichment for CD34⁺ stem and progenitor cells which are used for cell therapeutic purposes.

The inventors could further subset proNeus into proNeu1 and proNeu2 by their CD49d expression level and side-scatter properties (FIG. 7D). CD49d^{hi}SSC^{lo} proNeu1 were rare cells as compared to CD49d^{int}SSC^{hi} proNeu2, and they expressed higher levels of CD34, CD38 and CD81 (FIG. 7E). The expression of CD34 and CD38 suggests that proNeu1 cells exist within the human GMP nomenclature of Lin⁻CD34⁺CD38⁺CD45RA⁺ cells. To validate the findings, cord and adult peripheral blood, as well as fetal bone marrow samples were analysed. From the analysis, the inventors were able to detect proNeu1 and proNeu2 in cord blood donor samples but not in the peripheral blood. In the fetal bone marrow, the inventors could detect a higher frequency of proNeu1, along with higher frequencies of proNeu2, preNeus and immature Neus (FIG. 7F).

The data suggest a similarity in neutrophil development between mouse and human with the identification of human equivalents of proNeu1 and proNeu2. To further show this correlation, the inventors utilized and compared the protein expression profiles of each neutrophil subset from both the mouse and human InfinityFlow datasets. The MFIs were extracted from each subset and it was observed that each subset had moderate correlation between the mouse and human equivalent neutrophil subset (FIG. 14C). Moreover, by comparing the top-expressing markers of

each subset in both mouse and human, 16 markers that share similar expression progression from proNeu1 to mature neutrophils were identified (FIG. 7G). This further suggests a similar acquisition of function and maturation in both mice and human. Taken together, the data provides a complete analysis of the human neutrophil pathway, identifying the human equivalents of proNeu1 and proNeu2 with their associated surface marker expression profiles.

Finally, to show that the identified progenitors proNeu1 and proNeu2 have the potential to generate mature neutrophils, *in vitro* cultures of sorted proNeu1 and proNeu2 cells were performed. The data showed that both proNeu1 and proNeu2 were capable of generating CD16⁺CD10⁺ mature neutrophils (FIG.15). This generation takes 3 days, which provides a quick supply of neutrophils if transferred into patients who undergo myeloablative treatments. This is observed in the CD34^{hi} cultured cells, which generate very low numbers of mature neutrophils after 3 days of culture. This finding also allows for the further development of culture conditions that will allow for the extended proliferation and expansion of donor neutrophils for therapeutic purposes. Taken together, the data strongly supports the existence of two distinct neutrophil progenitors and the method to isolate these cells for therapeutic uses.

Discussion

The classical model of haematopoiesis is a hierarchical and step-wise differentiation program, led by instructive transcription factors that govern each cell's lineage fate. The generation of myeloid cells are through GMPs (Lin⁻cKit⁺Sca-1⁻CD34⁺CD16/32^{hi}) as shown by the formation of both granulocytic and monocytic colonies in methylcellulose colony-forming assays (Akashi et al., 2000). Yet, whether GMPs are a bona fide homogenously multipotent subset or a heterogenous blend of lineage-restricted populations remains a long-standing question. To gain better insights into this, the inventors employed a combinatorial approach of transcriptomic, proteomic and bioinformatic tools to develop a phenotyping scheme for identifying putative progenitors within the GMP populations. By subjecting the currently defined GMPs through optimized subset-defining surface markers derived from InfinityFlow (Dutertre et al., 2019) and bioinformatic approaches, the inventors identified a population of neutrophil-committed progenitors (i.e. CD81⁺CD106⁻ proNeu1) that exist within GMP population. Furthermore, with deep surface marker phenotyping and RNA-Seq profiling, the inventors further characterized a downstream CD81⁺CD106⁺

proNeu2 progenitor subset. Together, these data serve as a missing link in the early stages of the neutrophil developmental pathway, which allows for the mapping of their development from proNeu1→proNeu2 →preNeu →immature neutrophil →mature neutrophil.

The ability to delineate early neutrophil progenitors from GMPs allows for the assessment of transcription factors that are involved in instructing myeloid progenitor lineage specification and commitment. Inspection of a list of TFs (*Irf8*, *Klf4*, *Irf5*, *Gfi1*, *Cebpe* and *Per3*) that are known to govern monocytic and granulocytic lineage commitment consistently showed that Ly6C⁺GMPs and proNeu1 express similar levels of these TFs, while differential expression of these TFs only became evident at the proNeu2 and cMoP stage. This indicates that a neutrophil-monocyte lineage bifurcation may occur at this hierarchical level. This raises the question about the extent of lineage commitment by proNeu1 population, i.e., whether this subset of progenitor is specified or committed towards the neutrophil lineage. The *in vitro* and *in vivo* functional studies revealed that proNeu1 is committed to the neutrophil lineage, as proNeu1 can only give rise to neutrophils even when they were cultured with a strong monocytic lineage promoting cytokine such as M-CSF. Additionally, the RNA-Seq data showed that cMoP, Ly6C⁺GMP, proNeu1 and proNeu2 possess distinct “TF signatures”, providing an overview of putative TFs that could be important for lineage specification and commitment of these progenitors. For instance, the inventors observed increased levels of *Jag1* and *Sox13* expression specifically only in proNeu1, suggesting that these two TFs can be developed as markers for tagging proNeu1 and to be examined as key regulators for proNeu1 differentiation. Collectively, the data not only confirmed previous lineage-associated TFs, but also extended the list of putative TFs for myeloid lineage specification and commitment.

A previous *in vivo* tracking study demonstrated that granulocytes and monocytes are closely related in terms of their clonal origin. Here, the disclosure describes a transcriptionally and molecularly defined neutrophil progenitor in the GMP hierarchy, allowing for a better understanding of the lineage relationships and the dynamics of neutrophil/monocyte production. Given that myeloid progenitors can be sustained without the input from HSCs, it is conceivable that these progenitors are highly adaptable to varying inflammatory perturbations, according to the demands of the immune response. The study revealed that there is a selective expansion of proNeu1 and disappearance of cMoP during the acute phase of sepsis onset. This phenomenon

could be a consequence of skewed differentiation potential of progenitor cells, conceivably at the Ly6C⁺GMP level, and a preferential proliferation of early neutrophil progenitors to meet the immediate requirements for neutrophils.

In summary, the disclosure provides new insight into the divergent pathways of myeloid progenitor development towards neutrophils and monocytes from GMPs, and how the balance between neutrophil/monocyte production is important for host homeostasis. Moreover, the identification of early neutrophil progenitors opens up new avenues for therapeutic strategy for the management of neutropenia in hematopoietic stem cell transplantation or high dose chemotherapy, by the infusion of an expanded proNeu subset. This can serve a source for rapid neutrophil repopulation to help confer protection against infection during this critical period of need.

Materials and methods

Mice

Eight to twelve-week-old C57BL/6 mice were bred and maintained under specific pathogen-free (SPF) conditions in the Biological Resource Centre (BRC) of A*STAR, Singapore. Both males and females were used for experiments, but animals were sex- and age-matched in each experiment as much as possible. uGFP (C57BL/6-Tg(UBC-GFP)30Scha/J), CD45.1 (B6.SJL-Ptprc^a Pepc^b/BoyJ), MaFIA (C57BL/6-Tg(Csf1r-EGFP-NGFR/FKBP1A/TNFRSF6)2Bck/J) and *Rosa26^{mT/mG}* (STOCK Gt(ROSA)^{26Sortm4}(ACTB-tdTomato,-EGFP)Luo/J mice were obtained from The Jackson Laboratory. Fucci-S/G2/M (#474) were obtained from the RIKEN BioResource Center (Ibaraki, Japan; (Tomura et al., 2013)). *Cebpe*^{-/-} provided by P. Koeffler (Cancer Science Institute of Singapore, NUS, Singapore) (Yamanaka et al., 1997). All transgenic mice were maintained on a C57BL/6 background and experiments were performed under the approval of the Institutional Animal Care and Use Committee (IACUC), in accordance with the guidelines of the Agri-Food and Veterinary Authority (AVA) and the National Advisory Committee for Laboratory Animal Research (NACLAR) of Singapore.

Human Blood, Cord Blood and Fetal Bone Marrow

All samples were obtained in accordance with a favorable ethical opinion from SingHealth CIRB or A*STAR, the Singapore Immunology Network. Fresh (<24 hours

from collection) human umbilical cord blood (UCB) units were collected through a collaboration with National Cancer Centre Singapore (NCCS). NCCS obtained the UCB units through Singapore Cord Blood Bank (SCBB), from research consented units failing to meet the criteria for public clinical banking. Usage of the samples were approved by the Centralized Institutional Review Board (CIRB) of Singapore Health Services (that covers NCCS) as well as the dedicated Research Advisory Ethics Committee of SCBB.

Treatments

For 5-Fluorouracil (5-FU) myeloablative treatment, mice were injected once intraperitoneally with 150mg/kg 5-FU (Sigma-Aldrich) or PBS control. For G-CSF treatment, mice were injected once intraperitoneally with 1.5µg of G-CSF/anti-G-CSF antibody complex (G-CSFcx) as previously described (Rubinstein et al., 2013). Briefly, G-CSFcx were generated by incubating G-CSF (Neupogen) and anti-G-CSF (BVD11-37G10; Southern-Biotech) at 1:5 cytokine to antibody ratio for 20 min at 37°C, and were next diluted at least 10-fold in PBS before injection.

Tissue preparation and data analysis for flow cytometry and cell sorting

Blood was obtained via an incision in the submandibular region and was then lysed in red blood cell lysis buffer (eBioscience). For BM cells, mice femurs were flushed using a 23-gauge needle in PBS containing 2mM EDTA and 2% fetal bovine serum (FBS) and passed through a 70-µm nylon mesh sieve. Spleens were harvested and homogenized into single-cell suspensions using 70-µm nylon mesh sieves and syringe plungers. Antibodies were purchased from BD, Biolegend, eBioscience or R&D. For the identification of BM myeloid progenitor cell subsets, cells were stained with fluorophore-conjugated anti-mouse antibodies against CD34 (RAM34), CD11b (M1/70), CD16/32 (2.4G2), CD115 (AFS598), cKit (2B8), CXCR2 (SA044G4), CXCR4 (2B11), Gr1 (RB6-8C5), Ly6C (HK1.4) CD106 (429), CD81 (Eat-2), and Flt3 (A2F10), together with exclusion lineage markers that include Ly6G (1A8), CD90.2 (53-2.1), B220 (RA3-6B2), NK.1.1 (PK136), and Sca-1 (D7). After exclusion of cell doublets and dead cells with DAPI, proNeu1 were identified as (Lin/CD115/Flt3)⁻cKit^{hi}CD16/32^{hi}Ly6C⁺CD34^{hi}CD11b^{lo}CD106⁻, proNeu2 were identified as (Lin/CD115/Flt3)⁻cKit^{hi}CD16/32^{hi}Ly6C⁺CD34^{hi}CD11b^{hi}CD106⁺, preNeus were

identified as (Lin/CD115/Siglec-F)⁻Gr1⁺CD11b⁺CXCR4^{hi} cKit^{int}CXCR2⁻, CMPs were identified as Lin⁻cKit⁺Sca-1⁻CD16/32^{int}CD34^{int}, Ly6C⁻GMPs were identified as Lin⁻cKit⁺Sca-1⁻CD16/32^{hi}CD34^{hi}, MDPs were identified as Lin⁻cKit⁺Sca-1⁻CD115⁺Flt3⁺Ly6C⁻ or and cMoPs were identified as Lin⁻cKit⁺Sca-1⁻CD115⁺CD81⁻Flt3⁻Ly6C⁺. Flow cytometry acquisition was performed on a 5-laser BD LSR II (BD) using FACSDiva software, and data was subsequently analyzed with FlowJo software (Tree Star). Cell numbers were quantified with count beads (CountBright; Life Technologies) according to the manufacturer's instructions. Sorting of BM neutrophil subsets were performed using a BD ARIAll (BD) to achieve >98% purity.

LEGENDScreen™ and InfinityFlow pipeline

Mice femurs, tibias, pelvic bones, humeri and spinal bone marrow were harvested and crushed in PBS containing 2mM EDTA and 2% foetal bovine serum (FBS) and passed through a 70-µm nylon mesh sieve. For human cord blood, RBC lysis was performed on one unit of cord blood donor sample for 10min at room temperature. Cells were spun down at 400g for 10min. This process was repeated once to remove most RBC cells. Mouse cells were first stained with fixable live/dead for 30min before staining with a backbone panel cocktail of mouse antibodies to define the various lineages in the BM. These markers include: CD34 (RAM34), CD11b (M1/70), CD16/32 (2.4G2), CD115 (AFS598), cKit (2B8), CXCR2 (SA044G4), Ly6C (HK1.4), Flt3 (A2F10), Sca-1 (D7). CD48 (HM48-1), CD43 (S11), CD62L (MEL-14) and FITC-conjugated lineage markers (Ly6G (1A8), CD90.2 (53-2.1), B220 (RA3-6B2), NK.1.1 (PK136)). After 90min of staining at 4°C, cells were lysed in 1X RBC lysis (eBioscience) for 5 min, then spun down at 400g for 5 min. Cells were then stained with secondary streptavidin for 30min and washed as before. To enrich the BM for progenitor identification, mature neutrophils, B, T and NK cells were partially depleted using FITC selection kit (Stem Cell Technologies) according to the manufacturer protocol. For cord blood cells, cells were stained with a backbone panel of antibodies for 30min at 4°C, including: CD3 (UCHT1), CD56 (HCD56), CD19 (HIB19), CD10 (HI10a), CD49d (9F10), CD34 (581), CD66b (G10F5), cKit (104D2), CD38 (HIT2), CD15 (HI98), CD14 (M5E2), CD101 (BB27), CD45 (HI30), CD11b (ICRF44), CD16 (3G8). Cells were then counted and aliquoted into individual wells containing specific PE-conjugated marker. (Tables 1 and 3). After staining for 30 min, plates were washed

and fixed before FACS acquisition was performed on a 5-laser BD LSR II (BD) using FACSDiva software, and data was subsequently processed through the InfinityFlow pipeline as described elsewhere (Dutertre et al., 2019).

Cytospin and Wright-Giemsa staining

Sorted neutrophil subsets (5×10^4 cells each) were spun onto glass slides using Cytospin 4 Cytocentrifuge (Thermo scientific), dried for 10 minutes, fixed in methanol and stained with the Hema 3 manual staining system (Fisher Diagnostics) according to the manufacturer's protocol. Images were acquired with an Olympus BX43 equipped with a 100x oil immersion objective, and image brightness was adjusted with Photoshop (Adobe).

Transcriptomics

For single-cell transcriptomic analysis, BM CMPs, GMPs, preNeus and TpMos cells were sorted based on the gating strategy depicted in FIG. 1B and 8C. Single cell cDNA libraries were using the SMARTSeq v2 protocol (Picelli et al., 2014) with the following modifications: 1. 1mg/ml BSA Lysis buffer (Ambion® Thermo Fisher Scientific, Waltham, MA, USA); 2. Use of 200 pg cDNA with 1/5 reaction of Illumina Nextera XT kit (Illumina, San Diego, CA, USA). The length distribution of the cDNA libraries was monitored using a DNA High Sensitivity Reagent Kit on the Perkin Elmer Labchip (Perkin Elmer, Waltham, MA, USA). All samples were subjected to an indexed paired-end sequencing run of 2x151 cycles on an Illumina HiSeq 4000 system (Illumina, San Diego, CA, USA) (250 samples/lane).

For total-mRNA bulk RNA-seq analysis, BM Ly6C⁺GMP, proNeu1, proNeu2 and cMoP were sorted based on the gating strategy of FIG. 4A. preNeus were sorted as described previously. Total RNA was extracted using Arcturus PicoPure RNA Isolation kit (Applied Biosystems™ Thermo Fisher Scientific) according to manufacturer's protocol. All human RNAs were analyzed on Agilent Bioanalyser for quality assessment with a median RNA Integrity Number (RIN) of 9.4. cDNA libraries were prepared using 2 ng of total RNA with 1ul of 1:50,000 dilution of ERCC RNA Spike in Controls respectively (Ambion® Thermo Fisher Scientific) using the SMARTSeq v2 protocol (Picelli et al., 2014) with the following modifications: 1. Addition of 20 μ M TSO; 2. Use of 200pg cDNA with 1/5 reaction of Illumina Nextera XT kit (Illumina, San Diego, CA, USA). The length distribution of the cDNA libraries was monitored using a DNA

High Sensitivity Reagent Kit on the Perkin Elmer Labchip (Perkin Elmer, Waltham, MA, USA). All samples were subjected to an indexed paired-end sequencing run of 2x151 cycles on an Illumina HiSeq 4000 system (Illumina) (26 samples/lane). Raw reads were aligned to mouse genome build GRCm38 using STAR aligner. Read counts per gene were then calculated using the featureCounts (part of the Subread package) based on GENCODE gene annotation version M20. Log 2 transformed reads per kilobase per million mapped reads (log2 RPKM) normalization was done to account for transcript length and the total number of reads. Differentially expressed genes (DEGs) analysis was done using edgeR (Robinson et al., 2009) on protein coding genes only. DEGs with FDR (False Discovery Rate) less than 0.05 were selected as statistically significant.

***In vitro* cell culture**

Sorted cells (1×10^4 for each subset) were plated onto 96-well plates in triplicates and cultured at 37°C, 5% CO₂ in StemSpan™ SFEM II (Stem Cell Technologies) containing penicillin (100 U/ml), streptomycin (100 ug/ml), a combination of cytokines (50ng/ml SCF, 10ng/ml LIF, 20 ng/ml IL-3, 20 ng/ml IL-6) and with or without 50 ng/ml CSF-1. For human fetal marrow cultures, frozen samples were thawed and sorted before plating in 96-well plates in StemSpan™ SFEM II containing penicillin (100 U/ml), streptomycin (100 ug/ml) and StemSpan™ myeloid expansion supplement (Stem Cell Technologies). For colony assays, sorted cells (3×10^4) were cultured for in Iscove's modified Dulbecco's medium (Sigma) with 25mM HEPES and L-Glutamine (Chemtron) containing 10% (vol/vol) FBS, 1 mM sodium pyruvate, penicillin (100 U/ml) and streptomycin (100 ug/ml), 1% (wt/vol) methylcellulose (MethoCult M3234, Stem Cell Technologies) and the same cytokine combination as above. Representative colony images were collected with an Olympus IX-81 microscope (Olympus). Image brightness was adjusted with Photoshop.

Adoptive cell transfer

Sorted *uGFP*⁺ proNeu1, RFP⁺ (*Rosa26*^{mT/mG}) proNeu2 and CD45.1 preNeus (5×10^4 cells each) were transferred intra-BM into wildtype recipients as described previously (Chong et al., 2016). Briefly, recipient mice were anesthetized with ketamine (150mg/kg) /xylazine (10mg/kg), and had their right leg shaved to expose the kneecap. Sorted proNeu1, proNeu2 and preNeus were mixed and resuspended in

1X PBS in equal proportions and a volume of 10 μ L was administered into the tibia through the kneecap using a 29–gauge insulin needle. For single population transfers, sorted uGFP⁺ Ly6C⁺GMP, proNeu1 and proNeu2 (5 x 10⁴ cells) were used. After 24hr or 72 hours after cell transfer, tibias were collected, stained and analyzed by flow cytometry.

CLP-induced sepsis

Cecal ligation and puncture was performed as described previously (Rittirsch et al., 2009). Briefly, the peritoneal cavity was exposed under ketamine/xylazine anesthesia and the cecum was exteriorized. 50% (mid-grade) or 80% (high-grade) of the cecum was ligated distal of the ileo-cecal valve using a non-absorbable 7-0 suture. A 26–gauge needle was used to perforate the distal end of the cecum, and a small drop of feces was extruded through the puncture before being relocated into the peritoneal cavity. The peritoneum was closed and mice were subsequently treated with saline and Buprenorphine (5-20 mg/kg) by subcutaneous injection. For sham-operated controls, the peritoneum was exposed and the cecum was exteriorized before closing the peritoneum as mentioned above. Mice were euthanized and harvested 2 weeks after the surgery.

Quantification and statistical analysis

Statistical analyses were done using Prism software (Graphpad). Student's t-test or one-way analysis of variance (ANOVA) with Bonferroni correction were performed.

Tables

Table 1. (Related to FIG.2) List of surface markers tested in mouse BM LEGENDScreen™

Location	Marker	Clone (Biolegend)	Isotype
Plate_1_A1_013	Blank		
Plate_1_A10_022	CD103	2E7	Armenian Hamster IgG
Plate_1_A11_023	Delta_like 4	HMD4-1	Armenian Hamster IgG
Plate_1_A12_024	CD195	HM-CCR5	Armenian Hamster IgG

Plate_1_A2_014	Isotype_AHlgG	HTK888	
Plate_1_A3_015	CD3e	145-2C11	Armenian Hamster IgG
Plate_1_A4_016	CD80	16-10A1	Armenian Hamster IgG
Plate_1_A5_017	CD81	Eat-2	Armenian Hamster IgG
Plate_1_A6_018	CD154	MR1	Armenian Hamster IgG
Plate_1_A7_019	Notch 1	HMN1-12	Armenian Hamster IgG
Plate_1_A8_020	CD30	mCD30.1	Armenian Hamster IgG
Plate_1_A9_021	CD178	MFL3	Armenian Hamster IgG
Plate_1_B1_025	Notch 4	HMN4-14	Armenian Hamster IgG
Plate_1_B10_034	CD11c	N418	Armenian Hamster IgG
Plate_1_B11_035	Delta_like 1	HMD1-3	Armenian Hamster IgG
Plate_1_B12_036	CD196	29-2L17	Armenian Hamster IgG
Plate_1_B2_026	CD229	Ly9ab3	Armenian Hamster IgG
Plate_1_B3_027	CD69	H1.2F3	Armenian Hamster IgG
Plate_1_B4_028	Notch 3	HMN3-133	Armenian Hamster IgG
Plate_1_B5_029	JAML	4E10	Armenian Hamster IgG
Plate_1_B6_030	Notch 2	HMN2-35	Armenian Hamster IgG
Plate_1_B7_031	CD194	2G12	Armenian Hamster IgG
Plate_1_B8_032	CD152	UC10-4B9	Armenian Hamster IgG
Plate_1_B9_033	CD120a	55R-286	Armenian Hamster IgG
Plate_1_C1_037	CD29	HMB1-1	Armenian Hamster IgG
Plate_1_C10_046	CD16.2	mDcR2-1	Armenian Hamster IgG
Plate_1_C11_047	CD36	HM36	Armenian Hamster IgG
Plate_1_C12_048	DcTRAIL_R1	mDcR1-3	Armenian Hamster IgG
Plate_1_C2_038	CD55	RIKO-3	Armenian Hamster IgG
Plate_1_C3_039	Jagged 2	HMJ2-1	Armenian Hamster IgG
Plate_1_C4_040	CD79b	HM79-12	Armenian Hamster IgG

Plate_1_C5_041	IFNgR b chain	MAR1-5A3	Mouse IgG1, k
Plate_1_C6_042	CD61	2C9.G2 (HMβ3-1)	Armenian Hamster IgG
Plate_1_C7_043	CD121a	JAMA-147	Armenian Hamster IgG
Plate_1_C8_044	TCRb chain	GL3	Hamster IgG
Plate_1_C9_045	FceRIa	MAR-1	Armenian Hamster IgG
Plate_1_D1_049	CD84	mCD84.7	Armenian Hamster IgG
Plate_1_D10_058	CD339	HMJ1-29	Armenian Hamster IgG
Plate_1_D11_059	CD49a	HMa1	Armenian Hamster IgG
Plate_1_D12_060	PD1H	MH5A	Armenian Hamster IgG
Plate_1_D2_050	CD48	HM48-1	Armenian Hamster IgG
Plate_1_D3_051	CD49b	HMa2	Armenian Hamster IgG
Plate_1_D4_052	CD120b	TR75-89	Armenian Hamster IgG
Plate_1_D5_053	CD183	CXCR3-173	Armenian Hamster IgG
Plate_1_D6_054	CD262	MD5-1	Armenian Hamster IgG
Plate_1_D7_055	HVEM	HMHV-1B18	Armenian Hamster IgG
Plate_1_D8_056	TCR Vd1.1_1.2	H57-597	Armenian Hamster IgG
Plate_1_D9_057	B7H4	HMH4-5G1	Armenian Hamster IgG
Plate_1_E1_061	CD85k	H1.1	Armenian Hamster IgG
Plate_1_E10_070	Ly108	330-AJ	Mouse IgG2a, k
Plate_1_E11_071	CD207	4C7	Mouse IgG2a, k
Plate_1_E12_072	CX3CR1	SA011F11	Mouse IgG2a, k
Plate_1_E2_062	Plexin B2	3E7	Armenian Hamster IgG
Plate_1_E3_063	CD27	LG.3A10	Armenian Hamster IgG1,
Plate_1_E4_064	DR3	4C12	Armenian Hamster IgG1
Plate_1_E5_065	TCR gd	4B2.9	Armenian Hamster IgG
Plate_1_E6_066	Isotype_mIgG2a	MOPC-173	Mouse IgG2a, k
Plate_1_E7_067	CD45.1	A20	Mouse (A.SW) IgG2a, k

Plate_1_E8_068	CD45.2	104	Mouse (SJL) IgG2a, k
Plate_1_E9_069	NK1.1	PK136	Mouse IgG2a, k
Plate_1_F1_073	Isotype_mIgG1	MOPC-21	Mouse IgG1, k
Plate_1_F10_082	Trem_like 4	16E5	Mouse IgG1, k
Plate_1_F11_083	CD59a	mCD59.3	Mouse IgG1, k
Plate_1_F12_084	Ly49H	3D10	Mouse IgG1, k
Plate_1_F2_074	CD66a	MAb-CC1	Mouse IgG1, k
Plate_1_F3_075	IFNAR1	MOB-47	Armenian Hamster IgG
Plate_1_F4_076	Tim2	F37-2C4	Mouse IgG1, k
Plate_1_F5_077	CD272	8F4	Mouse IgG1, k
Plate_1_F6_078	CD64	X54-5/7.1	Mouse IgG1, k
Plate_1_F7_079	CD351	TX61	Mouse IgG1, k
Plate_1_F8_080	LAP	TW7-20B9	Mouse IgG1, k
Plate_1_F9_081	TIGIT	1G9	Mouse IgG1, k
Plate_1_G1_085	CD90.1	OX-7	Mouse IgG1, k
Plate_1_G10_094	Siglec H	551	Rat IgG1, K
Plate_1_G11_095	CD255	MTW-1	Rat IgG1, k
Plate_1_G12_096	CD202b	TEK4	Rat IgG1, k
Plate_1_G2_086	Isotype_mIgG2b	MPC-11	Mouse IgG2b, k
Plate_1_G3_087	CD157	BP-3	Mouse IgG2b, k
Plate_1_G4_088	CD159a	16A11	Mouse IgG2b, k
Plate_1_G5_089	XCR1	ZET	Mouse IgG2b, k
Plate_1_G6_090	Isotype_mIgM	MM-30	Mouse IgM, k
Plate_1_G7_091	SSEA_1	MC-480	Mouse IgM, k
Plate_1_G8_092	Isotype_rIgG1	RTK 2071	Rat IgG1, k
Plate_1_G9_093	Ig light chain κ	RMK-45	Rat IgG
Plate_1_H1_097	GITR Ligand	YGL 386	Rat IgG1, k

Plate_1_H10_106	Mac3	M3/84	Rat IgG1, k
Plate_1_H11_107	CD223	C9B7W	Rat IgG1, k
Plate_1_H12_109	CD134	OX-86	Rat IgG1, k
Plate_1_H2_098	CD147	OX-114	Rat IgG1, k
Plate_1_H3_099	CD73	TY/11.8	Rat IgG1, k
Plate_1_H4_100	CD51	RMV-7	Rat IgG1, k
Plate_1_H5_101	NKG2D	CX5	Rat IgG1, k
Plate_1_H6_102	CD96	3.3	Rat IgG1, k
Plate_1_H7_103	Integrin b7	FIB27	Rat IgG1, k
Plate_1_H8_104	CD210	1B1.3a	Rat IgG1, k
Plate_1_H9_105	CD83	Michel-19	Rat IgG1, k
Plate_2_A12_119	Isotype_rIgG2a	RTK2758	Rat IgG2a, k
Plate_2_A2_111	CD41	MWReg30	Rat IgG1, k
Plate_2_A3_112	CD268	7H22-E16	Rat IgG1, k
Plate_2_A4_113	CD144	BV13	Rat IgG1, k
Plate_2_A5_114	CD370	7H11	Rat IgG1, k
Plate_2_A6_115	CD369	RH1	Rat IgG1, k
Plate_2_A7_116	PIR A/B	6C1	Rat IgG1, k
Plate_2_A8_117	CD22	OX-97	Rat IgG1, k
Plate_2_A9_118	E_Cadherin	DECMA-1	Rat IgG1, k
Plate_2_B1_120	MAIRV	TX70	Rat IgG2a, k
Plate_2_B10_129	CD197	4B12	Rat IgG2a, k
Plate_2_B11_130	CD47	miap301	Rat IgG2a, k
Plate_2_B12_131	CD98	RL388	Rat IgG2a, k
Plate_2_B2_121	CD146	ME-9F1	Rat IgG2a, k
Plate_2_B3_122	VISTA	MIH63	Rat IgG2a, k
Plate_2_B4_123	CD8a	53-6.7	Rat IgG2a, k

Plate_2_B5_124	CD275	HK5.3	Rat IgG2a, k
Plate_2_B6_125	CD34_MEC14.7	MEC14.7	Rat IgG2a, k
Plate_2_B7_126	Sca_1	D7	Rat IgG2a, k
Plate_2_B8_127	CD40	3/23	Rat IgG2a, k
Plate_2_B9_128	B220	RA3-6B2	Rat IgG2a, k
Plate_2_C1_132	CD14	Sa14-2	Rat IgG2a, k
Plate_2_C10_141	Tim4	RMT4-54	Rat IgG2a, k
Plate_2_C11_142	CD71	RI7217	Rat IgG2a, k
Plate_2_C12_143	H2	M1/42	Rat IgG2a, k
Plate_2_C2_133	CD107a	1D4B	Rat IgG2a, k
Plate_2_C3_134	CD18	M18/2	Rat IgG2a, k
Plate_2_C4_135	Ly6G	1A8	Rat IgG2a, k
Plate_2_C5_136	CD21,CD35	7E9	Rat IgG2a, k
Plate_2_C6_137	Mac2	M3/38	Rat IgG2a, k
Plate_2_C7_138	CD199	9B1	Rat IgG2a, k
Plate_2_C8_139	Ly51	6C3	Rat IgG2a, k
Plate_2_C9_140	IgD	11-26c.2a	Rat IgG2a, k
Plate_2_D1_144	CD45RB	C363-16A	Rat IgG2a, k
Plate_2_D10_153	CD105	MJ7/18	Rat IgG2a, k
Plate_2_D12_154	4_1BB Ligand	TKS-1	Rat IgG2a, k
Plate_2_D2_145	CD326	G8.8	Rat IgG2a, k
Plate_2_D3_146	IgM	RMM-1	Rat IgG2a, k
Plate_2_D4_147	CD155	TX56	Rat IgG2a, k
Plate_2_D5_148	CD200R	OX-110	Rat IgG2a, k
Plate_2_D6_149	CD254	IK22/5	Rat IgG2a, k
Plate_2_D7_150	IL21R	4A9	Rat IgG2a, k
Plate_2_D8_151	CD276	RTAA15	Rat IgG2a, k

Plate_2_D9_152	CD9	MZ3	Rat IgG2a, k
Plate_2_E1_155	CD265	R12-31	Rat IgG2a, k
Plate_2_E10_164	F4/80	BM8	Rat IgG2a, k
Plate_2_E11_165	CD94	18d3	Rat IgG2a, k
Plate_2_E12_166	CD267	8F10	Rat IgG2a, k
Plate_2_E2_156	TLR4	MTS510	Rat IgG2a, k
Plate_2_E3_157	CD19	6D5	Rat IgG2a, k
Plate_2_E4_158	LPAM_1	DATK32	Rat IgG2a, k
Plate_2_E5_159	CD62L	MEL-14	Rat IgG2a, k
Plate_2_E6_160	CD23	B3B4	Rat IgG2a, k
Plate_2_E7_161	CD5	53-7.3	Rat IgG2a, k
Plate_2_E8_162	CD273	TY25	Rat IgG2a, k
Plate_2_E9_163	CD31	390	Rat IgG2a, k
Plate_2_F1_167	Ly_49A	YE1/48.10.6	Rat IgG2a, k
Plate_2_F10_176	PDC_TREM	4A6	Rat IgG2a, k
Plate_2_F11_177	CD135	A2F10	Rat IgG2a, k
Plate_2_F12_178	CD127	A7R34	Rat IgG2a, k
Plate_2_F3_169	CD11a	M17/4	Rat IgG2a, k
Plate_2_F4_170	LT beta R	5G11	Rat IgG2a, k
Plate_2_F6_172	CD106	429 (MVCAM.A)	Rat IgG2a, k
Plate_2_F7_173	CD365	RMT1-4	Rat IgG2a, k
Plate_2_F8_174	CD115	AFS98	Rat IgG2a, k
Plate_2_F9_175	CD140a	APA5	Rat IgG2a, k
Plate_2_G1_179	CD140b	APB5	Rat IgG2a, k
Plate_2_G10_188	CD200R3	Ba13	Rat IgG2a, k
Plate_2_G11_189	MAIR_IV	TX69	Rat IgG2a, k
Plate_2_G12_190	Ly49D	4E5	Rat IgG2a, k

Plate_2_G2_180	ESAM	1G8/ESAM	Rat IgG2a, k
Plate_2_G3_181	CD200	OX-90	Rat IgG2a, k
Plate_2_G4_182	CD309	Avas12	Rat IgG2a, k
Plate_2_G5_183	TLT_2	MIH47	Rat IgG2a, k
Plate_2_G6_184	CD253	N2B2	Rat IgG2a, k
Plate_2_G7_185	CD335	29A1.4	Rat IgG2a, k
Plate_2_G8_186	CD205	NLDC-145	Rat IgG2a, k
Plate_2_G9_187	Galectin9	108A2	Rat IgG2a, k
Plate_2_H1_191	CD123	5B11	Rat IgG2a, k
Plate_2_H10_200	CD63	NVG-2	Rat IgG2a, k
Plate_2_H11_201	CD49e	5H10-27(MFR5)	Rat IgG2a, k
Plate_2_H12_202	CD193	J073E5	Rat IgG2a, k
Plate_2_H2_192	CD355	11-5/CRTAM	Rat IgG2a, k
Plate_2_H3_193	CD169	3D6.112	Rat IgG2a, k
Plate_2_H4_194	CD138	281-2	Rat IgG2a, k
Plate_2_H5_195	CD160	7H1	Rat IgG2a, k
Plate_2_H6_196	CD39	Duha59	Rat IgG2a, k
Plate_2_H7_197	GARP	F011-5	Rat IgG2a, k
Plate_2_H8_198	CD179a	R3	Rat IgG2a, k
Plate_2_H9_199	CD371	5D3/CLEC12A	Rat IgG2a, k
Plate_3_A10_212	MAdCAM1	MECA-367	Rat IgG2a, k
Plate_3_A11_213	MERTK	2B10C42	Rat IgG2a, k
Plate_3_A12_214	CD226	TX42.1	Rat IgG2a, k
Plate_3_A2_204	CD300LG	ZAQ5	Rat IgG2a, k
Plate_3_A3_205	CD301	LOM-8.7	Rat IgG2a, k
Plate_3_A4_206	IL33Ra	DIH9	Rat IgG2a, k
Plate_3_A5_207	CD304	3E12	Rat IgG2a, k

Plate_3_A6_208	CD6	OX-129	Rat IgG2a, k
Plate_3_A7_209	CD100	BMA-12	Rat IgG2a, k
Plate_3_A8_210	CD104	346-11A	Rat IgG2a, k
Plate_3_A9_211	CD182	SA044G4	Rat IgG2a, k
Plate_3_B1_215	Ly6K	MK34	Rat IgG2a, k
Plate_3_B10_224	CD43	S11	Rat IgG2b, k
Plate_3_B11_225	FR4	12A5	Rat IgG2b, k
Plate_3_B12_226	CD1d	1B1	Rat IgG2b, k
Plate_3_B2_216	CD16/32	93	Rat IgG2a, λ
Plate_3_B3_217	CD150	TC15-12F12.2	Rat IgG2a, λ
Plate_3_B4_218	CD25	PC61	Rat IgG2a, λ
Plate_3_B5_219	CD38	90	Rat IgG2a, λ
Plate_3_B6_220	CD133	315-2C11	Rat IgG2a, λ
Plate_3_B7_221	CD301b	URA-1	Rat IgG2a, λ
Plate_3_B8_222	CD34_SA376A4	SA376A4	Rat IgG2a, λ
Plate_3_B9_223	Isotype_rlgG2b	RTK4530	Rat IgG2b, k
Plate_3_C1_227	CD70	FR70	Rat IgG2b, k
Plate_3_C10_236	CD24	M1/69	Rat IgG2b, k
Plate_3_C11_237	Gr1	RB6-8C5	Rat IgG2b, k
Plate_3_C12_238	CD86	PO3	Rat IgG2b, k
Plate_3_C2_228	CD4	GK1.5	Rat IgG2b, k
Plate_3_C3_229	IA/IE	M5/114.15.2	Rat IgG2b, k
Plate_3_C4_230	CD153	RM153	Rat IgG2b, k
Plate_3_C5_231	CD54	YN1/1.7.4	Rat IgG2b, k
Plate_3_C6_232	33D1	33D1	Rat IgG2b, k
Plate_3_C7_233	CD90.2	30-H12	Rat IgG2b, k
Plate_3_C8_234	TER119	TER-119	Rat IgG2b, k

Plate_3_C9_235	CD49d	R1-2	Rat IgG2b, k
Plate_3_D1_239	CD11b	M1/70	Rat IgG2b, k
Plate_3_D10_248	CD3	17A2	Rat IgG2b, k
Plate_3_D11_249	CD274	10F.9G2	Rat IgG2b, k
Plate_3_D12_250	CD117	2B8	Rat IgG2b, k
Plate_3_D2_240	CD45	30-F11	Rat IgG2b, k
Plate_3_D3_241	CD279	RMP1-30	Rat IgG2b, k
Plate_3_D4_242	RAE1y	CX1	Rat IgG2b, k
Plate_3_D5_243	CD8b	YTS156.7.7	Rat IgG2b, k
Plate_3_D7_245	CD126	D7715A7	Rat IgG2b, k
Plate_3_D8_246	CD317	927	Rat IgG2b, k
Plate_3_D9_247	CD132	TUGm2	Rat IgG2b, k
Plate_3_E1_251	CD88	20/70	Rat IgG2b, k
Plate_3_E10_260	CD130	4H1B35	Rat IgG2b, k
Plate_3_E11_261	CD198	SA214G2	Rat IgG2b, k
Plate_3_E12_262	CD20	SA275A11	Rat IgG2b, k
Plate_3_E2_252	CD93	AA4.1	Rat IgG2b, k
Plate_3_E3_253	CD252	RM134L	Rat IgG2b, k
Plate_3_E4_254	MD1	MD-113	Rat IgG2b, k
Plate_3_E5_255	CD357	YGITR 765	Rat IgG2b, k
Plate_3_E6_256	CD185	L138D7	Rat IgG2b, k
Plate_3_E7_257	CD37	Duno85	Rat IgG2b, k
Plate_3_E8_258	CD300c/d	TX52	Rat IgG2b, k
Plate_3_E9_259	CD186 (CXCR6)	SA051D1	Rat IgG2b, k
Plate_3_F1_263	CD124	I015F8	Rat IgG2b, k
Plate_3_F10_272	GL7	GL7	Rat IgM, k
Plate_3_F11_273	Isotype_SHIgG	SHG-1	Syrian Hamster IgG

Plate_3_F12_274	CD28	37.51	Syrian Hamster IgG
Plate_3_F2_264	IL23R	12B2B64	Rat IgG2b, k
Plate_3_F3_265	CD184	L276F12	Rat IgG2b, k
Plate_3_F4_266	CD2	RM2-5	Rat IgG2b, λ
Plate_3_F5_267	Isotype_rIgG2c	RTK4174	Rat IgG2c, k
Plate_3_F6_268	Ly6C	HK1.4	Rat IgG2c, k
Plate_3_F7_269	Ly6D	49-H4	Rat IgG2c, k
Plate_3_F8_270	Isotype_rIgM	RTK2118	Rat IgM, k
Plate_3_F9_271	CD49b_IgM	DX5	Rat IgM, k
Plate_3_G1_275	Podoplanin	8.1.1	Syrian Hamster IgG
Plate_3_G2_276	CD137	17B5	Syrian Hamster IgG
Plate_3_G3_277	CD278	15F9	Syrian Hamster IgG
Plate_3_G4_278	KLRG1	2F1/KLRG1	Syrian Hamster IgG
Plate_3_G5_279	Ly49CFIH	14B11	Syrian Hamster IgG

Table 2 (related to FIG. 2). Cell type identification of PhenoGraph clusters

Pheno-graph	Label	Markers
1	Transitional Pre-Monocytes (tpMo)	CD184 ^{hi} , Ly6C ^{hi} , CD115, CX3CR1, CD62L ^{hi}
2	Pro B cells	B220, CD19, CD16/32 ^{lo} , CD48 ^{lo}
3	cMoP, tpMo	CD34, Ly6C, CD115, CD62L
4	IgM+ B Cells	IgM, B220
5	Dendritic Cells/ preDCs	FLT3, CD11c, SiglecH, IFNAR1, CD317
6	Eosinophils	SiglecF, F4/80, CD41
7	NK, T cells, NK T cells	NK1.1, CD3, NKG2D
8	Megakaryocytes	CD41, CD61, SSC ^{hi}
9	Immature Neutrophils	CD371, CD63, Ly6G, CD81

10	Mature Neutrophils	CD182 ^{hi} , Ly6G, Gr1, CD11b
11	Classical monocytes (Ly6C ^{hi} Monocytes)	CD115, Ly6C, CD11b
12	Mature Neutrophils	CD182 ^{hi} , Ly6G, Gr1, CD11b
13	Mature Neutrophils	CD182 ^{hi} , Ly6G, Gr1, CD11b
14	Recirculating/Aged Neutrophils	CD43 ^{lo} , Ly6G, CD11b ^{hi} , CD62L ^{lo} , CD182 ^{lo}
15	Basophils, Mast cells	CD220R, CD49b, IL33R, CD16/32 ^{hi} , cKit, CD123
16	Ly6C ^{hi} Monocytes downre-gulated in CD115 during harvest	CD115, Ly6C, CD11b
17	Mature Neutrophils	CD182 ^{hi} , Ly6G, Gr1, CD11b
18	HSPCs	cKit, Sca-1, Flt3, CD48, CD11b-
19	Pre-pro B Cells	B220 ^{lo} , CD2-, CD14, CD19-, CD103
20	Stromal Cells	CD140a, CD140b, CD45-, CD144
21	Transitional Pre-Monocytes (tpMo)	CD184 ^{hi} , Ly6C ^{hi} , CD115, CX3CR1, CD62L ^{hi}
22	Red Blood Cells	CD147, Ter119
23	pre-Neutrophils	cKit, Gr1, CD11b, CD43, Ly6C
24	Ly6C- Monocytes (non-classical monocytes)	CD43, CD115, CX3CR1, CD11c
25	Recirculating Ly6C ^{hi} Classical Monocytes	Ly6C, CX3CR1, CD62L, CD16/32, CD115
26	pre B cells	CD16/32 ^{hi} , B220, CD19
27	IgD+ B cells	IgD, B220

Table 3 (Related to FIG. 7). List of surface markers tested in human cord blood LEGENDScreen™.

Location	Marker	Clone	Isotype
Plate_1_A1_001	Blank		
Plate_1_A10_009	CD2	RPA-2.10	mouse IgG1, k
Plate_1_A12_011	B7-H4	MIH43	mouse IgG1, k
Plate_1_A3_003	CCR10	6588-5	Armenian Hamster IgG

Plate_1_A4_004	CD278	C398.4A	Armenian Hamster IgG
Plate_1_A5_005	IFN- γ R b chain	2HUB-159	Hamster IgG
Plate_1_A7_006	CD46	TRA-2-10	Mouse IgG1
Plate_1_A8_007	CD70	113-16	Mouse IgG1
Plate_1_A9_008	CD1a	HI149	mouse IgG1, k
Plate_1_B1_012	Cadherin 11	16G5	mouse IgG1, k
Plate_1_B10_021	CD111	R1.302	mouse IgG1, k
Plate_1_B11_022	CD112	TX31	mouse IgG1, k
Plate_1_B12_023	CD114	LMM741	mouse IgG1, k
Plate_1_B2_013	CD10	HI10a	mouse IgG1, k
Plate_1_B3_014	CD100	A8	mouse IgG1, k
Plate_1_B4_015	CD103	Ber-ACT8	mouse IgG1, k
Plate_1_B5_016	CD105 (Endoglin)	SN6h	mouse IgG1, k
Plate_1_B6_017	CD106	STA	mouse IgG1, k
Plate_1_B7_018	CD107a	H4A3	mouse IgG1, k
Plate_1_B8_019	CD107b	H4B4	mouse IgG1, k
Plate_1_B9_020	CD109	W7C5	mouse IgG1, k
Plate_1_C1_024	CD116	4H1	mouse IgG1, k
Plate_1_C10_033	CD13	WM15	mouse IgG1, k
Plate_1_C11_034	CD131	1C1	mouse IgG1, k
Plate_1_C12_035	CD134	Ber-ACT35 (ACT35)	mouse IgG1, k
Plate_1_C2_025	CD117	104D2	mouse IgG1, k
Plate_1_C3_026	CD119	GIR-208	mouse IgG1, k
Plate_1_C4_027	CD11a	HI111	mouse IgG1, k
Plate_1_C5_028	CD11b	ICRF44	mouse IgG1, k
Plate_1_C6_029	CD122	TU27	mouse IgG1, k
Plate_1_C7_030	CD123	6H6	mouse IgG1, k
Plate_1_C8_031	CD126	UV4	mouse IgG1, k
Plate_1_C9_032	CD127	A019D5	mouse IgG1, k
Plate_1_D1_036	CD135	BV10A4H2	mouse IgG1, k
Plate_1_D10_045	CD143	5-369	mouse IgG1, k
Plate_1_D11_046	CD146	P1H12	mouse IgG1, k
Plate_1_D12_047	CD148	A3	mouse IgG1, k
Plate_1_D2_037	CD137	4B4-1	mouse IgG1, k
Plate_1_D3_038	4-1BB Ligand	5F4	mouse IgG1, k
Plate_1_D4_039	CD138	MI15	mouse IgG1, k
Plate_1_D5_041	CD14	63D3	mouse IgG1, k
Plate_1_D6_040	CD140a	16A1	mouse IgG1, k
Plate_1_D7_042	CD140b	18A2	mouse IgG1, k
Plate_1_D8_043	CD141	M80	mouse IgG1, k
Plate_1_D9_044	CD142	NY2	mouse IgG1, k

Plate_1_E1_048	CD15	W6D3	mouse IgG1, k
Plate_1_E10_057	CD163	GHI/61	mouse IgG1, k
Plate_1_E11_058	CD164	67D2	mouse IgG1, k
Plate_1_E12_059	CD165	SN2 (N6- D11)	mouse IgG1, k
Plate_1_E2_049	CD150	A12 (7D4)	mouse IgG1, k
Plate_1_E3_050	CD151	50-6	mouse IgG1, k
Plate_1_E4_051	CD154	24-31	mouse IgG1, k
Plate_1_E5_052	CD156c	SHM14	mouse IgG1, k
Plate_1_E6_053	CD158e1	DX9	mouse IgG1, k
Plate_1_E7_054	CD16	3G8	mouse IgG1, k
Plate_1_E8_055	CD161	HP-3G10	mouse IgG1, k
Plate_1_E9_056	CD162	KPL-1	mouse IgG1, k
Plate_1_F1_060	CD166	3A6	mouse IgG1, k
Plate_1_F10_069	CD180	MHR73-11	mouse IgG1, k
Plate_1_F11_070	CD182	5E8/CXCR2	mouse IgG1, k
Plate_1_F12_071	CD183	G025H7	mouse IgG1, k
Plate_1_F2_061	CD169	7-239	mouse IgG1, k
Plate_1_F3_062	CD170	1A5	mouse IgG1, k
Plate_1_F4_063	CD172a/b (SIRPα/β)	SE5A5	mouse IgG1, k
Plate_1_F5_064	CD172g (SIRPγ)	LSB2.20	mouse IgG1, k
Plate_1_F6_065	CD178	NOK-1	mouse IgG1, k
Plate_1_F7_066	CD179a	HSL96	mouse IgG1, k
Plate_1_F8_067	CD179b	HSL11	mouse IgG1, k
Plate_1_F9_068	CD18	TS1/18	mouse IgG1, k
Plate_1_G1_072	CD185	J252D4	mouse IgG1, k
Plate_1_G10_081	CD203c	NP4D6	mouse IgG1, k
Plate_1_G11_082	CD205	HD83	mouse IgG1, k
Plate_1_G12_083	CD206	15-2	mouse IgG1, k
Plate_1_G2_073	CD19	HIB19	mouse IgG1, k
Plate_1_G3_074	CD191	5F10B29	mouse IgG1, k
Plate_1_G4_075	CD194	L291H4	mouse IgG1, k
Plate_1_G5_076	CD1b	SN13 (K5- 1B8)	mouse IgG1, k
Plate_1_G6_077	CD1c	L161	mouse IgG1, k
Plate_1_G7_078	CD200	OX-104	mouse IgG1, k
Plate_1_G8_079	CD200R	OX-108	mouse IgG1, k
Plate_1_G9_080	CD202b	33.1 (Ab33)	mouse IgG1, k
Plate_1_H10_093	CD229	HLy-9.1.25	mouse IgG1, k
Plate_1_H11_094	CD23	EBVCS-5	mouse IgG1, k
Plate_1_H2_085	CD21	Bu32	mouse IgG1, k
Plate_1_H3_086	CD213α1	SS12B	mouse IgG1, k
Plate_1_H4_087	CD213α2	SHM38	mouse IgG1, k
Plate_1_H5_088	CD218a	H44	mouse IgG1, k

Plate_1_H6_089	CD221	1H7/CD221	mouse IgG1, k
Plate_1_H7_090	CD223 (LAG-3)	11C3C65	mouse IgG1, k
Plate_1_H8_091	CD226	11A8	mouse IgG1, k
Plate_1_H9_092	CD227	16A	mouse IgG1, k
Plate_2_A10_009	CD268	11C1	mouse IgG1, k
Plate_2_A11_010	CD27	M-T271	mouse IgG1, k
Plate_2_A2_001	CD244 (2B4)	C1.7	mouse IgG1, k
Plate_2_A3_002	CD245	DY12	mouse IgG1, k
Plate_2_A4_003	CD25	M-A251	mouse IgG1, k
Plate_2_A5_004	CD252	11C3.1	mouse IgG1, k
Plate_2_A6_005	CD261	DJR1	mouse IgG1, k
Plate_2_A7_006	CD262	DJR2-4 (7-8)	mouse IgG1, k
Plate_2_A8_007	CD263	DJR3	mouse IgG1, k
Plate_2_A9_008	CD266	ITEM-1	mouse IgG1, k
Plate_2_B1_012	CD275	9F.8A4	mouse IgG1, k
Plate_2_B10_021	CD30	BY88	mouse IgG1, k
Plate_2_B11_022	CD300c	TX45	mouse IgG1, k
Plate_2_B12_023	CD309	7D4-6	mouse IgG1, k
Plate_2_B2_013	CD276	MIH42	mouse IgG1, k
Plate_2_B3_014	CD277	BT3.1	mouse IgG1, k
Plate_2_B4_015	CD279	EH12.2H7	mouse IgG1, k
Plate_2_B5_016	CD28	CD28.2	mouse IgG1, k
Plate_2_B6_017	CD29	TS2/16	mouse IgG1, k
Plate_2_B7_018	CD290	3C10C5	mouse IgG1, k
Plate_2_B8_019	CD298	LNH-94	mouse IgG1, k
Plate_2_B9_020	CD3	UCHT1	mouse IgG1, k
Plate_2_C1_024	CD31	WM59	mouse IgG1, k
Plate_2_C10_033	CD336	P44-8	mouse IgG1, k
Plate_2_C11_034	CD337	P30-15	mouse IgG1, k
Plate_2_C12_035	CD34	581	mouse IgG1, k
Plate_2_C2_025	CD314	1D11	mouse IgG1, k
Plate_2_C3_026	CD317	RS38E	mouse IgG1, k
Plate_2_C4_027	CD324	67A4	mouse IgG1, k
Plate_2_C5_028	CD325	8C11	mouse IgG1, k
Plate_2_C6_029	CD328	6-434	mouse IgG1, k
Plate_2_C7_030	CD33	WM53	mouse IgG1, k
Plate_2_C8_031	CD334	4FR6D3	mouse IgG1, k
Plate_2_C9_032	CD335	9E2	mouse IgG1, k
Plate_2_D1_036	CD340	24D2	mouse IgG1, k
Plate_2_D10_045	CD38	HIT2	mouse IgG1, k
Plate_2_D11_046	CD39	A1	mouse IgG1, k
Plate_2_D12_047	CD4	RPA-T4	mouse IgG1, k

Plate 2 D2 037	CD344	CH3A4A7	mouse IgG1, k
Plate 2 D3 038	CD35	E11	mouse IgG1, k
Plate 2 D4 039	CD354	TREM-26	mouse IgG1, k
Plate 2 D5 040	CD360	17A12	mouse IgG1, k
Plate 2 D6 041	CD365	1D12	mouse IgG1, k
Plate 2 D7 042	CD366	F38-2E2	mouse IgG1, k
Plate 2 D8 043	CLEC4A	9E8	mouse IgG1, k
Plate 2 D9 044	CD36L1	m1b9	mouse IgG1, k
Plate 2 E1 048	CD40	5C3	mouse IgG1, k
Plate 2 E10 057	CD49b	P1E6-C5	mouse IgG1, k
Plate 2 E11 058	CD49c	ASC-1	mouse IgG1, k
Plate 2 E12 059	CD49d	9F10	mouse IgG1, k
Plate 2 E2 049	CD41	HIP8	mouse IgG1, k
Plate 2 E3 050	CD42b	HIP1	mouse IgG1, k
Plate 2 E4 051	CD43	CD43-10G7	mouse IgG1, k
Plate 2 E5 052	CD44	BJ18	mouse IgG1, k
Plate 2 E6 053	CD45	HI30	mouse IgG1, k
Plate 2 E7 054	CD47	CC2C6	mouse IgG1, k
Plate 2 E8 055	CD48	BJ40	mouse IgG1, k
Plate 2 E9 056	CD49a	TS2/7	mouse IgG1, k
Plate 2 F1 060	CD5	UCHT2	mouse IgG1, k
Plate 2 F10 069	CD62L	DREG-56	mouse IgG1, k
Plate 2 F11 070	CD62P	AK4	mouse IgG1, k
Plate 2 F2 061	CD50	CBR-IC3/1	mouse IgG1, k
Plate 2 F3 062	CD54	HA58	mouse IgG1, k
Plate 2 F4 063	CD55	JS11	mouse IgG1, k
Plate 2 F5 064	CD56 (NCAM)	5.1H11	mouse IgG1, k
Plate 2 F6 065	CD58	TS2/9	mouse IgG1, k
Plate 2 F7 066	CD6	BL-CD6	mouse IgG1, k
Plate 2 F8 067	CD61	VI-PL2	mouse IgG1, k
Plate 2 G10 081	CD83	HB15e	mouse IgG1, k
Plate 2 G11 082	CD85	17G10.2	mouse IgG1, k
Plate 2 G12 083	CD85k	ZM4.1	mouse IgG1, k
Plate 2 G2 073	CD69	FN50	mouse IgG1, k
Plate 2 G4 075	CD74	LN2	mouse IgG1, k
Plate 2 G5 076	CD79b	CB3-1	mouse IgG1, k
Plate 2 G6 077	CD8a	SK1	mouse IgG1, k
Plate 2 G7 078	CD80	2D10	mouse IgG1, k
Plate 2 G8 079	CD81	5A6	mouse IgG1, k
Plate 2 G9 080	CD82	ASL-24	mouse IgG1, k
Plate 2 H1 084	CD87	VIM5	mouse IgG1, k
Plate 2 H10 093	CD97	VIM3b	mouse IgG1, k

Plate 2 H11_094	CD99	3B2/TA8	mouse IgG1, k
Plate 2 H12_095	CXCL16	22-19-12	mouse IgG1, k
Plate 2 H2_085	CD89	A59	mouse IgG1, k
Plate 2 H3_086	CD8a	RPA-T8	mouse IgG1, k
Plate 2 H4_087	CD9	HI9a	mouse IgG1, k
Plate 2 H5_088	CD90	5E10	mouse IgG1, k
Plate 2 H6_089	CD93	VIMD2	mouse IgG1, k
Plate 2 H7_090	CD94	DX22	mouse IgG1, k
Plate 2 H8_091	CD95	DX2	mouse IgG1, k
Plate 2 H9_092	CD96	NK92.39	mouse IgG1, k
Plate 3 A10_009	HVEM	122	mouse IgG1, k
Plate 3 A11_010	Ig light chain κ	MHK-49	mouse IgG1, k
Plate 3 A12_011	IgM	MHM-88	mouse IgG1, k
Plate 3 A2_001	DLL1	MHD1-314	mouse IgG1, k
Plate 3 A3_002	DLL4	MHD4-46	mouse IgG1, k
Plate 3 A4_003	DR3	JD3	mouse IgG1, k
Plate 3 A5_004	EGFR	AY13	mouse IgG1, k
Plate 3 A6_005	CD357	108-17	mouse IgG1, k
Plate 3 A7_006	GPR19	K152D10	mouse IgG1, k
Plate 3 A8_007	GPR56	CG4	mouse IgG1, k
Plate 3 A9_008	HLA-E	3D12	mouse IgG1, k
Plate 3 B1_012	CD360	2G1-K12	mouse IgG1, k
Plate 3 B10_020	TNAP	W8B2	mouse IgG1, k
Plate 3 B11_021	MUC-13	TCC16	mouse IgG1, k
Plate 3 B12_022	NKp80	5D12	mouse IgG1, k
Plate 3 B2_013	Integrin $\alpha\beta 1$	Y9A2	mouse IgG1, k
Plate 3 B3_014	Jagged 2	MHJ2-523	mouse IgG1, k
Plate 3 B4_015	Ksp37	TDA3	mouse IgG1, k
Plate 3 B5_016	LAP	TW4-2F8	mouse IgG1, k
Plate 3 B6_017	LY6G6D	13.8	mouse IgG1, k
Plate 3 B7_018	MERTK	590H11G1E3	mouse IgG1, k
Plate 3 B8_019	MSC	W7C6	mouse IgG1, k
Plate 3 C1_023	Notch 1	MHN1-519	mouse IgG1, k
Plate 3 C10_032	Siglec-8	7C9	mouse IgG1, k
Plate 3 C11_033	Siglec-9	K8	mouse IgG1, k
Plate 3 C12_034	SSEA-5	8E11	mouse IgG1, k
Plate 3 C2_024	Notch3	MHN3-21	mouse IgG1, k
Plate 3 C3_025	Notch 4	MHN4-2	mouse IgG1, k
Plate 3 C4_026	NPC	57D2	mouse IgG1, k
Plate 3 C5_027	CD352	NT-7	mouse IgG1, k
Plate 3 C6_028	PSMA	LNI-17	mouse IgG1, k
Plate 3 C8_030	Siglec-10	5G6	mouse IgG1, k

Plate_3_C9_031	CD328	S7.7	mouse IgG1, k
Plate_3_D1_035	SUSD2	W5C5	mouse IgG1, k
Plate_3_D10_044	VEGFR-3	9D9F9	mouse IgG1, k
Plate_3_D12_045	APCDD1	7.13	mouse IgG2a, k
Plate_3_D2_036	TCR α/β	IP26	mouse IgG1, k
Plate_3_D4_038	Tim-4	9F4	mouse IgG1, k
Plate_3_D5_039	TLT-2	MIH61	mouse IgG1, k
Plate_3_D6_040	TM4SF20	C9	mouse IgG1, k
Plate_3_D7_041	TRA-2-49	TRA-2- 49/6E	mouse IgG1, k
Plate_3_D8_042	TRA-2-54	TRA-2-54/2J	mouse IgG1, k
Plate_3_D9_043	TSLPR	1B4	mouse IgG1, k
Plate_3_E1_046	CD272	MIH26	mouse IgG2a, k
Plate_3_E10_055	CD155	TX24	mouse IgG2a, k
Plate_3_E11_056	CD158b	DX27	mouse IgG2a, k
Plate_3_E12_057	CD184	12G5	mouse IgG2a, k
Plate_3_E2_047	CD198	L263G8	mouse IgG2a, k
Plate_3_E3_048	CCRL2	K097F7	mouse IgG2a, k
Plate_3_E4_049	CD102	CBR-IC2/2	mouse IgG2a, k
Plate_3_E5_050	CD104	58XB4	mouse IgG2a, k
Plate_3_E6_051	CD124	G077F6	mouse IgG2a, k
Plate_3_E7_052	CD130	2E1B02	mouse IgG2a, k
Plate_3_E8_053	CD144	BV9	mouse IgG2a, k
Plate_3_E9_054	CD152 (CTLA-4)	BNI3	mouse IgG2a, k
Plate_3_F1_058	CD186	K041E5	mouse IgG2a, k
Plate_3_F10_067	CD26	BA5b	mouse IgG2a, k
Plate_3_F11_068	CD269	19F2	mouse IgG2a, k
Plate_3_F12_069	CD282	TL2.1	mouse IgG2a, k
Plate_3_F2_059	CD192	K036C2	mouse IgG2a, k
Plate_3_F3_060	CD197	G043H7	mouse IgG2a, k
Plate_3_F4_061	CD199	L053E8	mouse IgG2a, k
Plate_3_F5_062	CD209	9E9A8	mouse IgG2a, k
Plate_3_F6_063	CD217	W15177A	mouse IgG2a, k
Plate_3_F7_064	CD230 (Prion)	3F4	mouse IgG2a, k
Plate_3_F8_065	CD24	ML5	mouse IgG2a, k
Plate_3_F9_066	CD243	UIC2	mouse IgG2a, k
Plate_3_G1_070	CD284	HTA125	mouse IgG2a, k
Plate_3_G10_078	CD370	8F9	mouse IgG2a, k
Plate_3_G11_079	CD371	50C1	mouse IgG2a, k
Plate_3_G12_080	CD45RO	UCHL1	mouse IgG2a, k
Plate_3_G2_071	CD301	H037G3	mouse IgG2a, k
Plate_3_G3_072	CD303	201A	mouse IgG2a, k
Plate_3_G4_073	CD304	12C2	mouse IgG2a, k

Plate 3 G5 074	CD307e	509f6	mouse IgG2a, k
Plate 3 G6 093	CD323	SHM33	mouse IgG2a, k
Plate 3 G7 075	CD357	108-17	mouse IgG2a, k
Plate 3 G8 076	CD36	5-271	mouse IgG2a, k
Plate 3 G9 077	CD369	15E2	mouse IgG2a, k
Plate 3 H1 081	CD51	NKI-M9	mouse IgG2a, k
Plate 3 H10 090	Ganglioside GD2	14G2a	mouse IgG2a, k
Plate 3 H11 091	GPR83	K07JP05	mouse IgG2a, k
Plate 3 H12 092	HLA-A,B,C	W6/32	mouse IgG2a, k
Plate 3 H2 082	CD59	p282 (H19)	mouse IgG2a, k
Plate 3 H3 083	CD7	CD7-6B7	mouse IgG2a, k
Plate 3 H4 084	CD71	CY1G4	mouse IgG2a, k
Plate 3 H5 085	CD84	CD84.1.21	mouse IgG2a, k
Plate 3 H6 086	CD88	S5/1	mouse IgG2a, k
Plate 3 H7 087	CD355	Cr24.1	mouse IgG2a, k
Plate 3 H8 088	erbB3	1B4C3	mouse IgG2a, k
Plate 3 H9 089	FPR3	K102B9	mouse IgG2a, k
Plate 4 A10 010	SUSD2	W3D5	mouse IgG2a, k
Plate 4 A11 011	Notch 2	MHN2-25	mouse IgG2a, k
Plate 4 A12 012	TACSTD2	NY18	mouse IgG2a, k
Plate 4 A2 002	HLA-DR	L243	mouse IgG2a, k
Plate 4 A3 003	Ig light chain λ	MHL-38	mouse IgG2a, k
Plate 4 A4 004	IgD	IA6-2	mouse IgG2a, k
Plate 4 A5 005	IL-28RA	MHLICR2a	mouse IgG2a, k
Plate 4 A6 006	integrin $\beta 5$	AST-3T	mouse IgG2a, k
Plate 4 A7 007	KLRG1	SA231A2	mouse IgG2a, k
Plate 4 A8 008	LOX-1	15C4	mouse IgG2a, k
Plate 4 A9 009	MICA/MICB	6D4	mouse IgG2a, k
Plate 4 B1 013	TIGIT (VSTM3)	A15153G	mouse IgG2a, k
Plate 4 B10 021	CD196	G034E3	mouse IgG2b, k
Plate 4 B11 022	CD1d	51.1	mouse IgG2b, k
Plate 4 B12 023	CD20	2H7	mouse IgG2b, k
Plate 4 B3 014	C3aR	hC3aRZ8	mouse IgG2b, k
Plate 4 B4 015	CCX-CKR (CCRL1)	13E11	mouse IgG2b, k
Plate 4 B5 016	CD11c	S-HCL-3	mouse IgG2b, k
Plate 4 B6 017	CD129	AH9R7	mouse IgG2b, k
Plate 4 B7 018	CD158	HP-MA4	mouse IgG2b, k
Plate 4 B8 019	CD181	8F1/CXCR1	mouse IgG2b, k
Plate 4 B9 020	CD193	5E8	mouse IgG2b, k
Plate 4 C1 024	CD22	S-HCL-1	mouse IgG2b, k
Plate 4 C10 033	CD368	9B9	mouse IgG2b, k
Plate 4 C11 034	CD45RA	HI100	mouse IgG2b, k

Plate 4 C12_035	CD45RB	MEM-55	mouse IgG2b, k
Plate 4 C2_025	CD220	B6.220	mouse IgG2b, k
Plate 4 C3_026	CD235ab	HIR2	mouse IgG2b, k
Plate 4 C4_027	CD258	T5-39	mouse IgG2b, k
Plate 4 C5_028	CD274	29E.2A3	mouse IgG2b, k
Plate 4 C6_029	CD319	162.1	mouse IgG2b, k
Plate 4 C7_030	CD32	FUN-2	mouse IgG2b, k
Plate 4 C8_031	CD326	9C4	mouse IgG2b, k
Plate 4 C9_032	CD338	5D3	mouse IgG2b, k
Plate 4 D1_036	CD49e	NKI-SAM-1	mouse IgG2b, k
Plate 4 D10_045	Dopamine Receptor D1 (DRD1)	L205G1	mouse IgG2b, k
Plate 4 D11_046	EphA2	SHM16	mouse IgG2b, k
Plate 4 D12_047	FcεR1α	AER-37 (CRA-1)	mouse IgG2b, k
Plate 4 D2_037	CD52	HI186	mouse IgG2b, k
Plate 4 D3_038	CD66a/c/e	ASL-32	mouse IgG2b, k
Plate 4 D4_039	CD85h	24	mouse IgG2b, k
Plate 4 D5_040	CD85	GHI/75	mouse IgG2b, k
Plate 4 D6_041	CD86	IT2.2	mouse IgG2b, k
Plate 4 D7_042	CD92	VIM15b	mouse IgG2b, k
Plate 4 D8_043	CXCR7	8F11-M16	mouse IgG2b, k
Plate 4 D9_044	Delta Opioid Receptor	DOR7D2A4	mouse IgG2b, k
Plate 4 E1_048	GARP	7B11	mouse IgG2b, k
Plate 4 E10_056	SSEA-4	MC-813-70	Mouse IgG3, k
Plate 4 E12_057	Sialyl Lewis X (dimeric)	FH6	Mouse IgM, k
Plate 4 E2_049	CD215	JM7A4	mouse IgG2b, k
Plate 4 E3_050	Lymphotoxin β Receptor	31G4D8	mouse IgG2b, k
Plate 4 E4_051	MRGX2	K125H4	mouse IgG2b, k
Plate 4 E5_052	TMEM8A	SA065C3	mouse IgG2b, k
Plate 4 E6_053	CD254	MIH24	mouse IgG2b, k
Plate 4 E7_054	CD318	CUB1	mouse IgG2b, k
Plate 4 E9_055	CD255	CARL-1	Mouse IgG3, k
Plate 4 F1_058	TRA-1-81	TRA-1-81	Mouse IgM, k
Plate 4 F10_066	CD120b	3G7A02	Rat IgG2a, k
Plate 4 F11_067	CD210	3F9	Rat IgG2a, k
Plate 4 F12_068	CD267	1A1	Rat IgG2a, k
Plate 4 F2_059	CD160	BY55	Mouse IgM, k
Plate 4 F3_060	CD57	HNK-1	Mouse IgM, k
Plate 4 F4_061	CD66b	G10F5	Mouse IgM, k
Plate 4 F5_062	TRA-1-60-R	TRA-1-60-R	Mouse IgM, k
Plate 4 F7_063	CD115	9-4D2-1E4	Rat IgG1, k
Plate 4 F8_064	CD201	RCR-401	Rat IgG1, k
Plate 4 G1_069	CD294	BM16	Rat IgG2a, k

Plate 4 G10 077	CD132	TUGh4	Rat IgG2b, k
Plate 4 G11 078	CD195	J418F1	Rat IgG2b, k
Plate 4 G12 079	CX3CR1	2A9-1	Rat IgG2b, k
Plate 4 G2 070	CD49f	GoH3	Rat IgG2a, k
Plate 4 G3 071	CD85	MKT5.1	Rat IgG2a, k
Plate 4 G4 072	CD85d	42D1	Rat IgG2a, k
Plate 4 G5 073	IgG Fc	M1310G05	Rat IgG2a, k
Plate 4 G6 074	Integrin β 7	FIB504	Rat IgG2a, k
Plate 4 G7 075	XCR1	S15046E	Rat IgG2a, k
Plate 4 G8 076	Podoplanin	NC-08	Rat IgG2a, l
Plate 4 H2 080	SSEA-3	MC-631	Rat IgM, k

Table 4. Key resources table

REAGENT	SOURCE	IDENTIFIER
Antibodies		
B220 (Clone RA3-6B2)	Thermo Fisher	Cat# 25-0452; RRID: AB_2341160
CD81 (Clone Eat-2)	Biolegend	Cat# 104906, RRID:AB_2076266
CD106 (Clone 429 (MVCAM.A))	Biolegend	Cat# 105716, RRID:AB_1595489
cKit (Clone 2B8)	Thermo Fisher	Cat# 62-1171-82, RRID:AB_2637141
CD11b (Clone M1/70)	Thermo Fisher	Cat# 63-0112-80, RRID:AB_2637407
CD115 (Clone AFS98)	Biolegend	Cat# 135510, RRID:AB_2085221
Flt3 (Clone A2F10)	Biolegend	Cat# 135308, RRID:AB_1953267
NK1.1 (Clone PK136)	Thermo Fisher	Cat# 13-5941-81, RRID:AB_466803
B220 (Clone RA3-6B2)	Thermo Fisher	Cat# 13-0452-82, RRID:AB_466449
CD90.2 (Clone 53-2.1)	Biolegend	Cat# 140314, RRID:AB_10643274
F4/80 (Clone BM8)	Biolegend	Cat# 123118, RRID:AB_893477
CD16/32 (Clone 2.4G2)	BD Horizon	Cat# 565502, RRID:AB_2739269
CD34 (Clone RAM34)	Thermo Fisher	Cat# 13-0341-81, RRID:AB_466424
Gr1 (Clone RB6-8C5)	Thermo Fisher	Cat# 45-5931-80; RRID: AB_906247
Ly6C (Clone HK1.4)	Biolegend	Cat# 128026; RRID: AB_10640120
Ly6G (Clone 1A8)	Biolegend	Cat# 127618; RRID: AB_1877261
Sca-1 (Clone D7)	Biolegend	Cat# 108114; RRID: AB_493596
Siglec-F (Clone E50-2440)	BD Biosciences	Cat# 562757; RRID: AB_394341
CXCR2 (Clone SA044G4)	Biolegend	Cat# 149306; RRID: AB_2565694
CXCR4 (Clone 2B11)	Thermo Fisher	Cat# 13-9991-82; RRID: AB_10609202

CD45.1 (Clone A20)	Biolegend	Cat#110707; RRID: AB_313496
CD45.2 (Clone 104)	Biolegend	Cat#109807; RRID: AB_313444
CD3 (Clone UCHT1)	Biolegend	Cat#300440; RRID: AB_2562046
CD10 (Clone HI10a)	Biolegend	Cat#312208; RRID: AB_314919
CD19 (Clone HIB19)	Biolegend	Cat#302205; RRID: AB_314235
CD56 (Clone HCD56)	Biolegend	Cat#318304; RRID: AB_604100
CD14 (Clone M5E2)	Biolegend	Cat#301838; RRID: AB_2562909
CD15 (Clone HI98)	BD Biosciences	Cat#564232; RRID: AB_2738686
CD49d (Clone 9F10)	BD Biosciences	Cat#563645; RRID: AB_2738344
CD66b (Clone G105F)	BD Biosciences	Cat#305111; RRID: AB_2563293
CD101 (Clone BB27)	Biolegend	Cat#331007; RRID: AB_2121761
CD16 (Clone 3G8)	BD Biosciences	Cat#560248; RRID: AB_1645467
CD34 (Clone 581)	Biolegend	Cat#343515; RRID: AB_1877252
CD117 (Clone 104D2)	Thermo Fisher	Cat#64-1178-42; RRID: AB_2734860
CD45 (Clone 2D1)	Biolegend	Cat#368515; RRID: AB_2566375
CD11b (ICRF44)	BD Biosciences	Cat#563839; RRID: AB_2716869
CD38 (S17015F)	Biolegend	Cat#303531; RRID: AB_2561527
CD71 (CY1G4)	Biolegend	Cat#334106; RRID: AB_2201481
Chemicals, Peptides, and Recombinant Proteins		
1X RBC Lysis Buffer	eBioscience	Cat# 00-4333-57
5-Fluorouracil	Sigma-Aldrich	Cat# F6627
CountBright Absolute Counting Beads	Life Technologies	Cat# C36950
DAPI	Life Technologies	Cat# D1306
Hema 3 Manual Staining System	Thermo Fisher	Cat# 22-122911
EQ Four Element Calibration Beads	Fluidigm	Cat# 201078
Human G-CSF (Neupogen)	Amgen	Cat# 100696800
MethoCult™ M3234	Stem Cell Technologies	Cat# 03234
StemSpan™SFEM II	Stem Cell Technologies	Cat# 09655
Critical Commercial Assays		
ERCC RNA Spike-In Mix	Thermo Fisher	Cat# 4456740

MILLIPLEX MAP Mouse Cytokine/Chemokine Magnetic Bead Panel - Premixed 32 Plex - Immunology Multiplex Assay	Merck	Cat# MCYTMAG-70K-PX32
LEGENDScreen™ Human Kit	Biolegend	Cat# 700007
LEGENDScreen™ Mouse Kit	Biolegend	Cat# 70000
Arcturus PicoPure RNA isolation kit	Thermo Fisher	Cat# KIT0214
Deposited Data		
Bulk RNA-seq of myeloid progenitor populations	This disclosure	GEO:
Smart-Seq2 Single- cell Dataset of myeloid precursors	This disclosure	GEO:
Tabula Muris BM 10x single-cell RNA-seq dataset	(Schaum et al., 2018)	GEO: GSE109774
Single-cell RNA-seq of BM GMPs	(Olsson et al., 2016)	GEO: GSE70240
Experimental Models: Organisms/Strains		
Mouse: C57BL/6J	The Jackson Laboratory	Stock No: 000664
Mouse: <i>Rosa26^{mT/mG}</i> ; STOCK Gt(ROSA) ^{26Sortm4} (ACTB- tdTomato,-EGFP)Luo/J	The Jackson Laboratory	Stock No: 007676
Mouse: Fucci-S/G2/M (#474)	RIKEN BRC, Ibaraki, Japan	Stock No: RBRC02704
Mouse: C57BL/6- Tg(UBC- GFP)30Scha/J; UBI- GFP	The Jackson Laboratory	Stock No: 04353
Mouse: B6.SJL-Ptprc ^a Pepc ^b /BoyJ; B6 Cd45.1	The Jackson Laboratory	Stock No: 002014
Mouse: <i>Cebpe</i> ^{-/-}	H.P. Koeffler	(Kyme et al., 2012)

Software and Algorithms		
GraphPad Prism 7	GraphPad Software	http://www.graphpad.com
FlowJo 10	TreeStar	http://flowjo.com/
t-SNE	(Van Der Maaten and Hinton, 2008)	https://cran.r-project.org/web/packages/Rtsne/index.html
edgeR	(Robinson et al., 2009)	https://bioconductor.org/packages/release/bioc/html/edgeR.html
pheatmap	R package	https://cran.r-project.org/web/packages/pheatmap/index.html
Enrichr	(Chen et al., 2013)	http://amp.pharm.mssm.edu/Enrichr/
Monocle 2	(Qiu et al., 2017)	http://cole-trapnell-lab.github.io/monocle-release/docs/
Seurat v3	(Stuart et al., 2018)	https://github.com/satijalab/seurat
UMAP	(McInnes et al., 2018)	https://umap-learn.readthedocs.io/en/latest/
SVM regression used in InfinityFlow	The R foundation	https://cran.r-project.org/web/packages/e1071/
R 3.6	The R foundation	https://www.r-project.org/

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It will be appreciated by a person skilled in the art that other variations and/or modifications may be made to the embodiments disclosed herein without departing

from the spirit or scope of the disclosure as broadly described. For example, in the description herein, features of different exemplary embodiments may be mixed, combined, interchanged, incorporated, adopted, modified, included etc. or the like across different exemplary embodiments. The present embodiments are, therefore, to be considered in all respects to be illustrative and not restrictive.