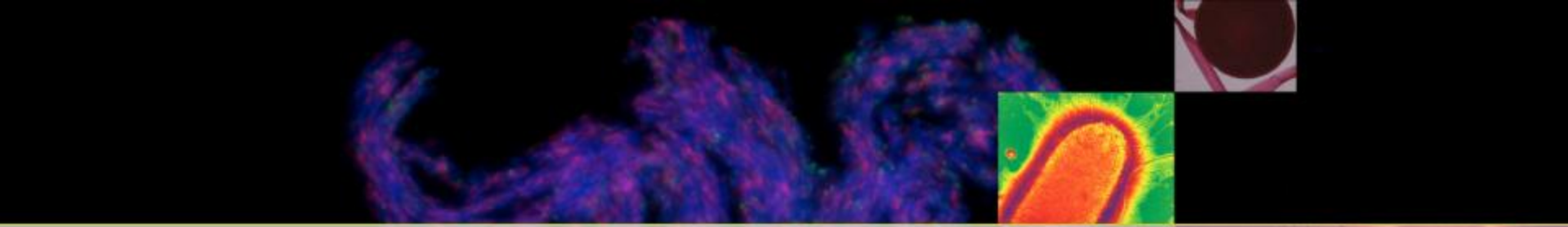
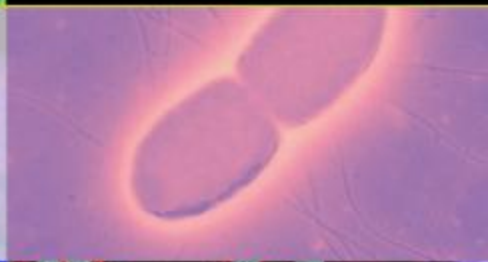
DISCOVERING ATCC IMMUNOLOGICAL CELLS - MODEL SYSTEMS TO STUDY THE IMMUNE AND CARDIOVASCULAR SYSTEMS

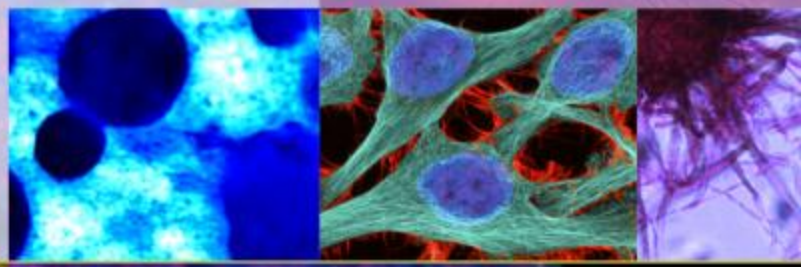
James Clinton, Ph.D.

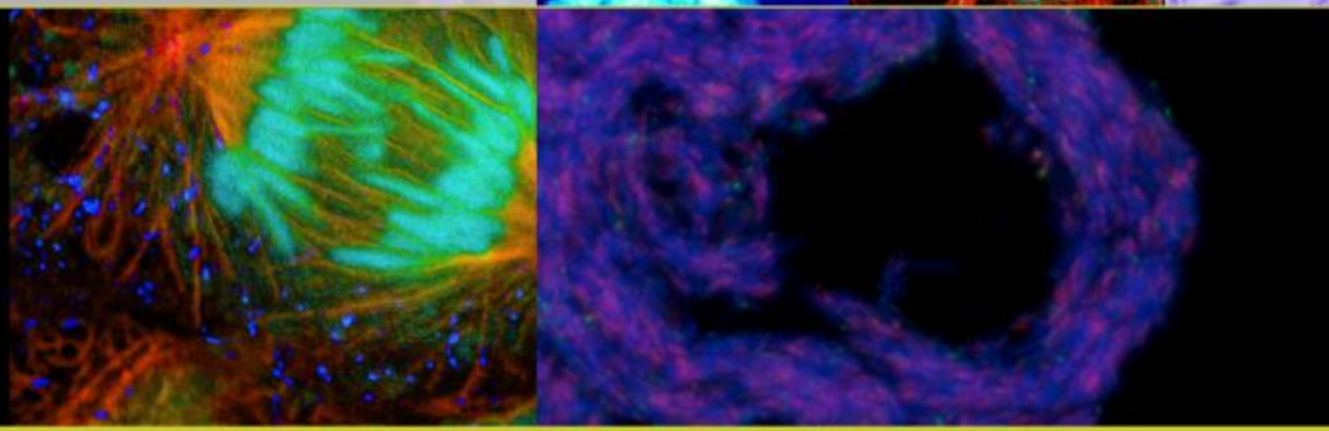
Scientist, ATCC

February 19, 2015









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About ATCC

- Founded in 1925, ATCC is a non-profit organization with headquarters in Manassas, VA
- World's premiere biological materials resource and standards development organization
- ATCC collaborates with and supports the scientific community with industry-standard products and innovative solutions
- Broad range of biomaterials
  - Continuous cell lines, iPSCs, primary cells, and hTERT immortalized cells
  - Bacteria, fungi, yeasts, protists, and viruses
  - Microbial and tumor cell panels
  - Genomic and synthetic nucleic acids
  - Media, sera, and reagents

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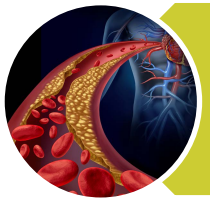
Outline



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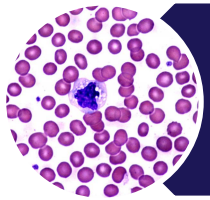
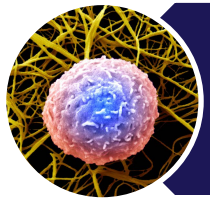
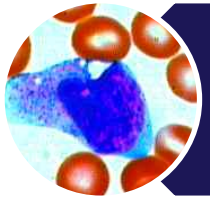


CD34+ hematopoietic stem & progenitor cells



Mononuclear cells



CD14+ monocytes

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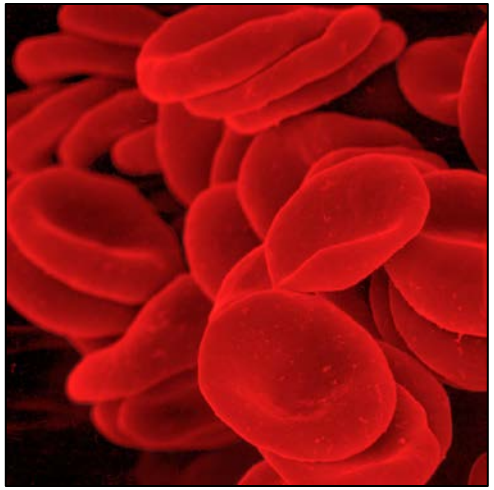
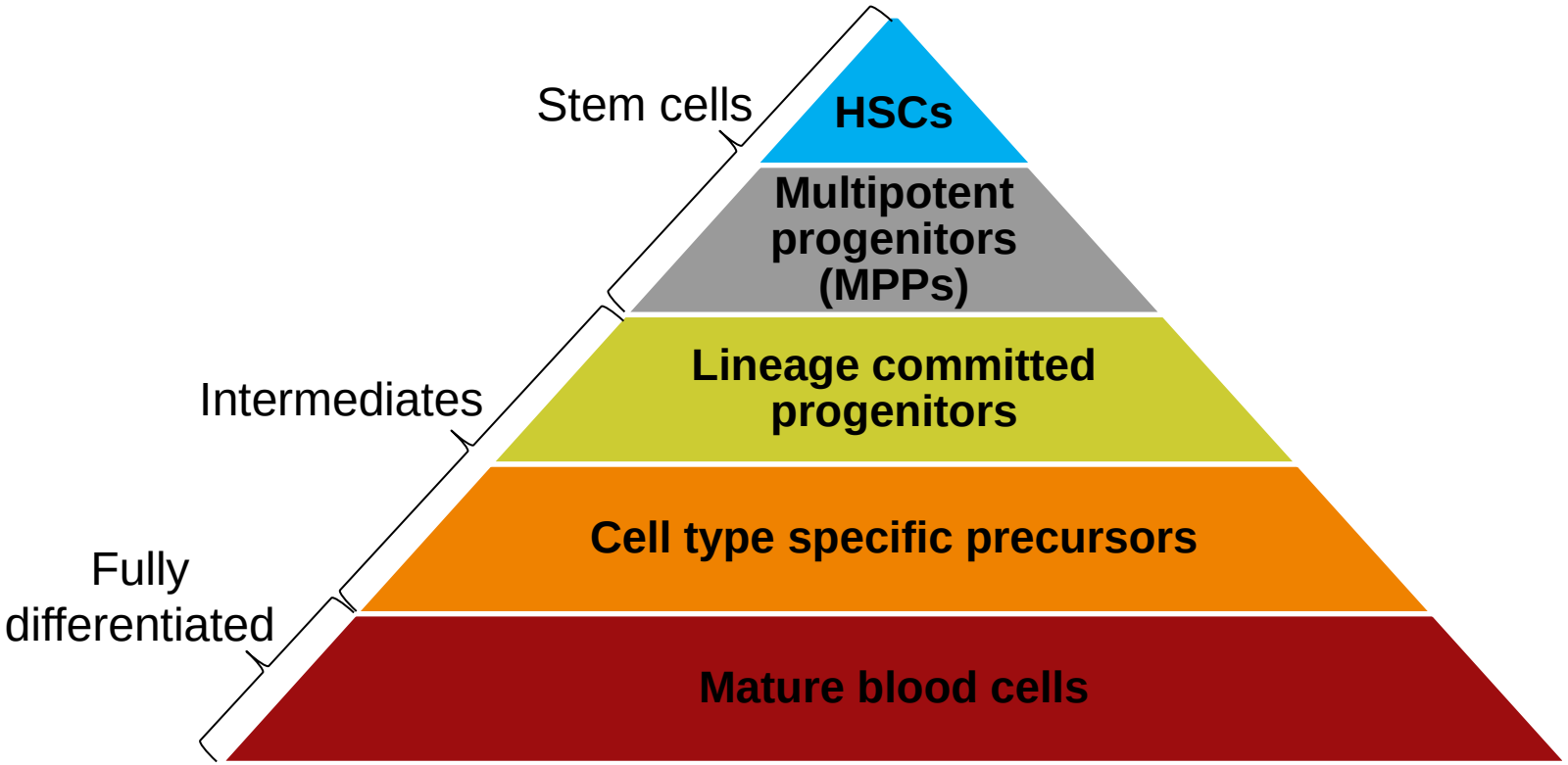
Blood cells

- Blood is comprised of a heterogeneous population of specialized cells
- Three major types
  - **Leukocytes**: Acquired and innate immunity
  - **Erythrocytes**: Gas transport
  - **Thrombocytes**: Wound healing
- Millions of blood cells are generated every second, approximately 1 trillion every day
- Hematopoiesis:
  - Dynamic process
  - Varies in response to injury or infection
  - Individual cells may live for hours to years
- Blood cells arise from hematopoietic stem cells (HSCs)

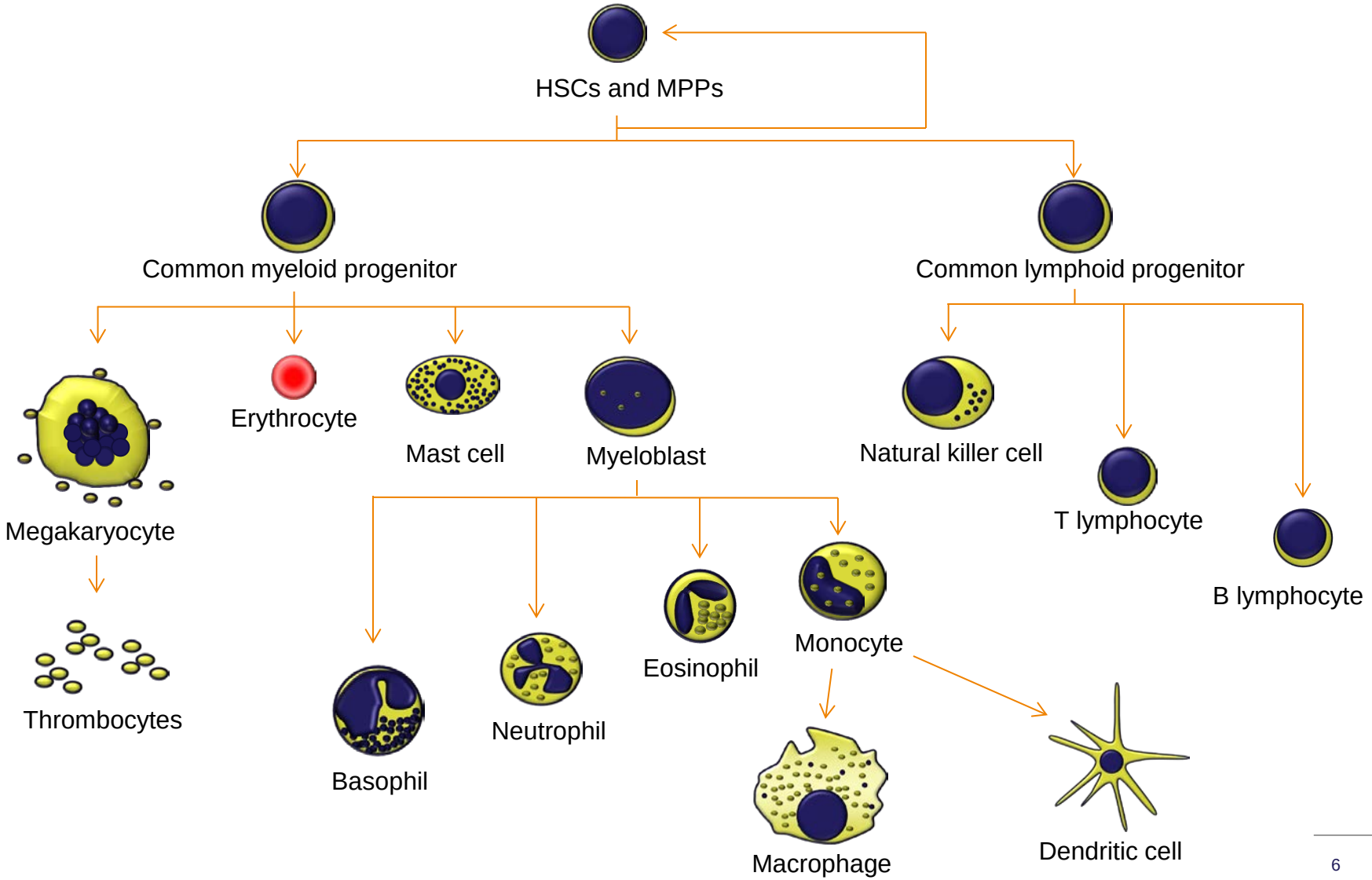


Erythrocytes

Hematopoiesis: A hierarchal system

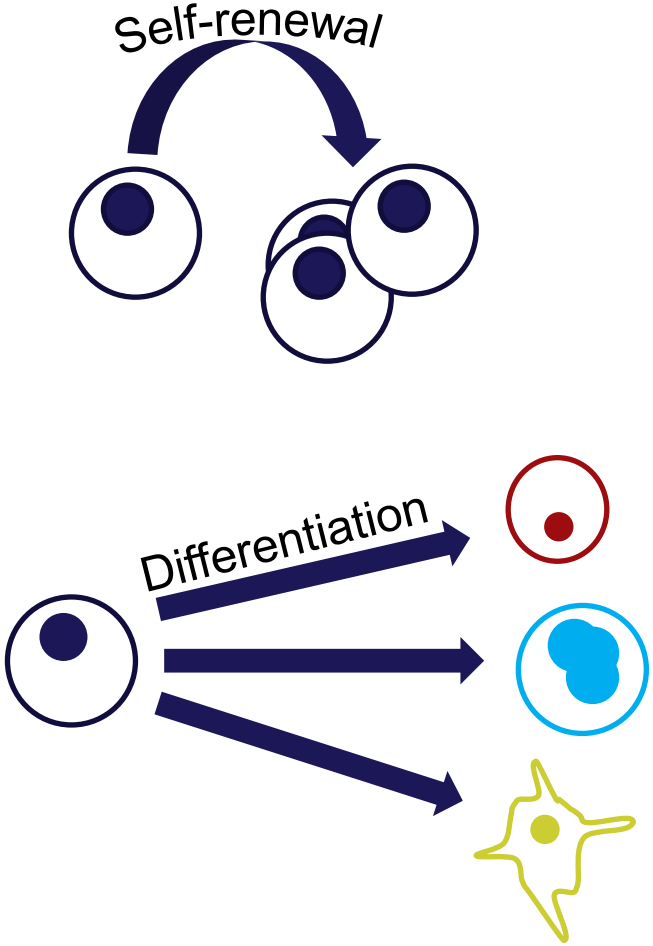


Hematopoietic cell fate and lineage

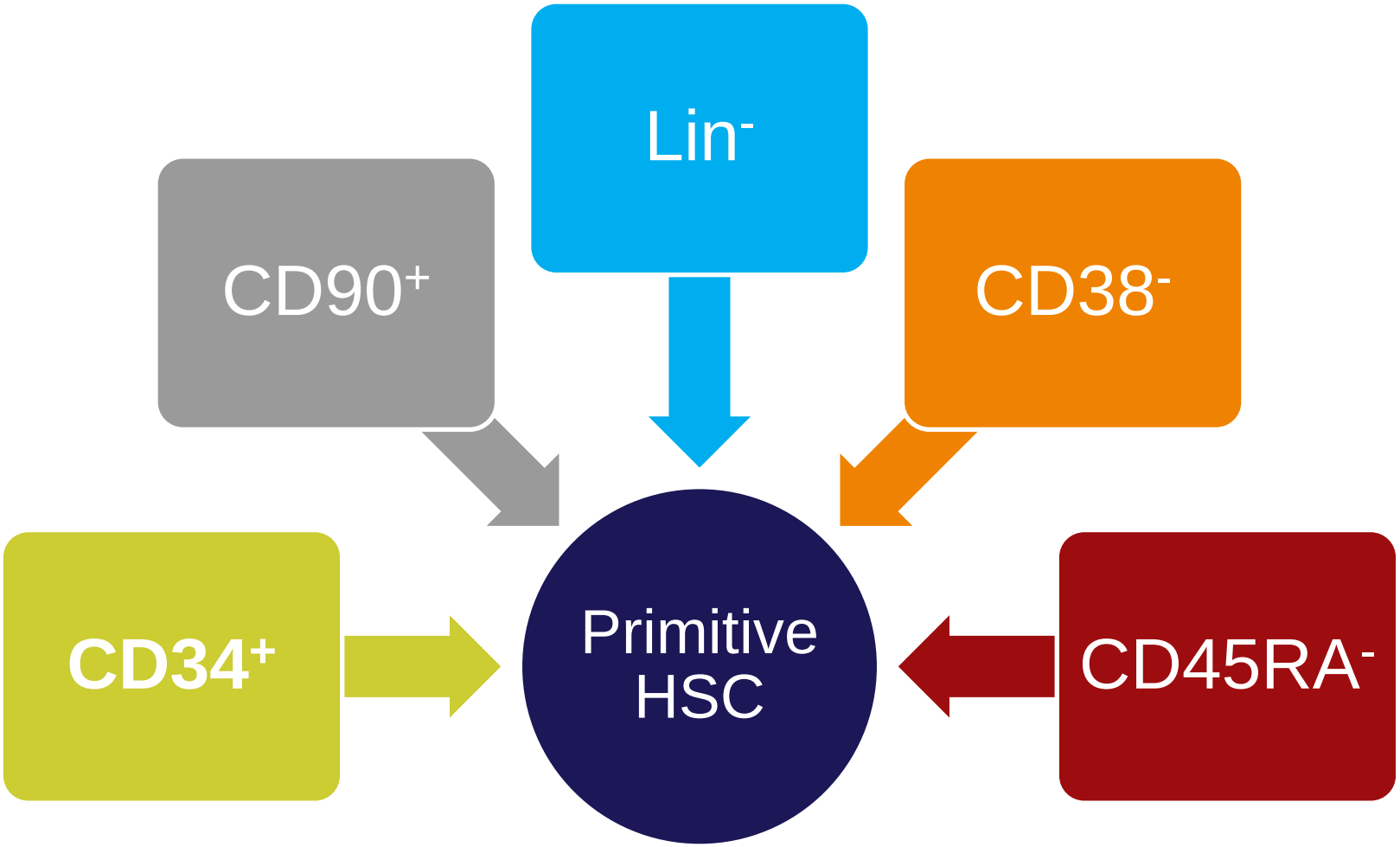


Hematopoietic stem cells: Characteristics

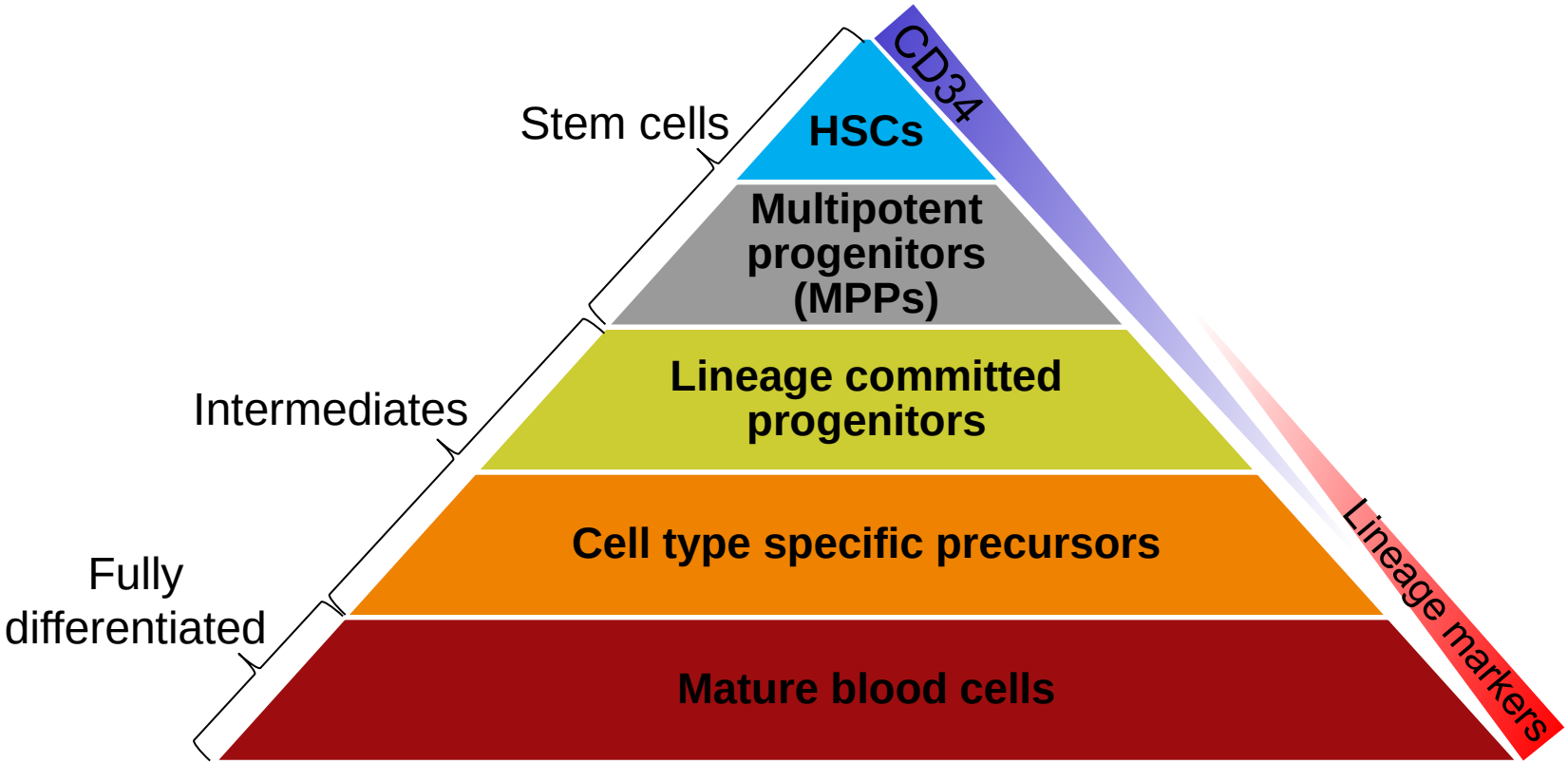
- Hematopoietic stem cells (HSCs) are multipotent cells that give rise to all other blood cells
- HSCs reside primary in bone marrow (major site of hematopoiesis in adults)
- True hematopoietic stem cells are rare
- True hematopoietic stem cells can only be confirmed via *in vivo* functional assays



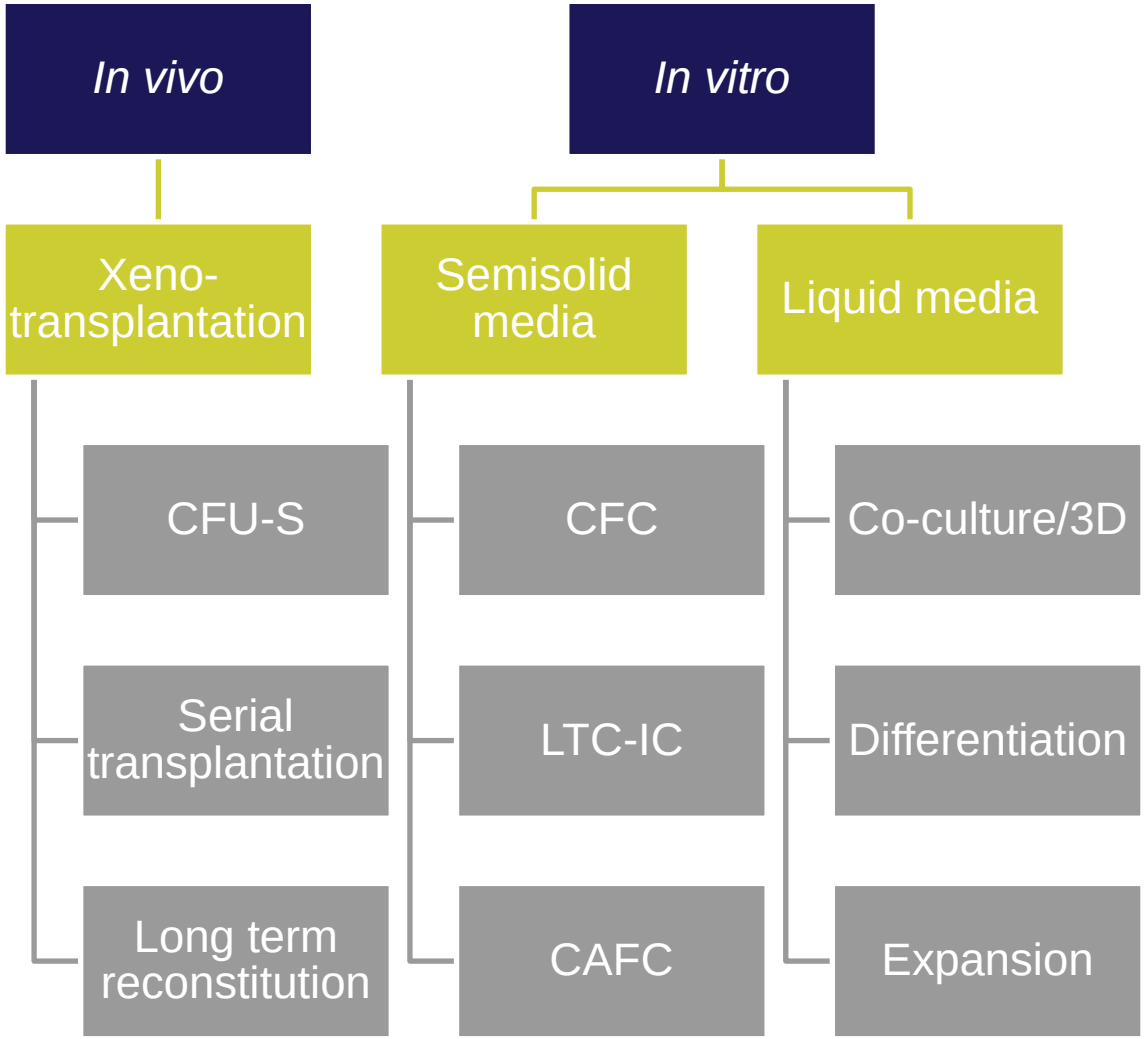
Hematopoietic stem cells: Markers



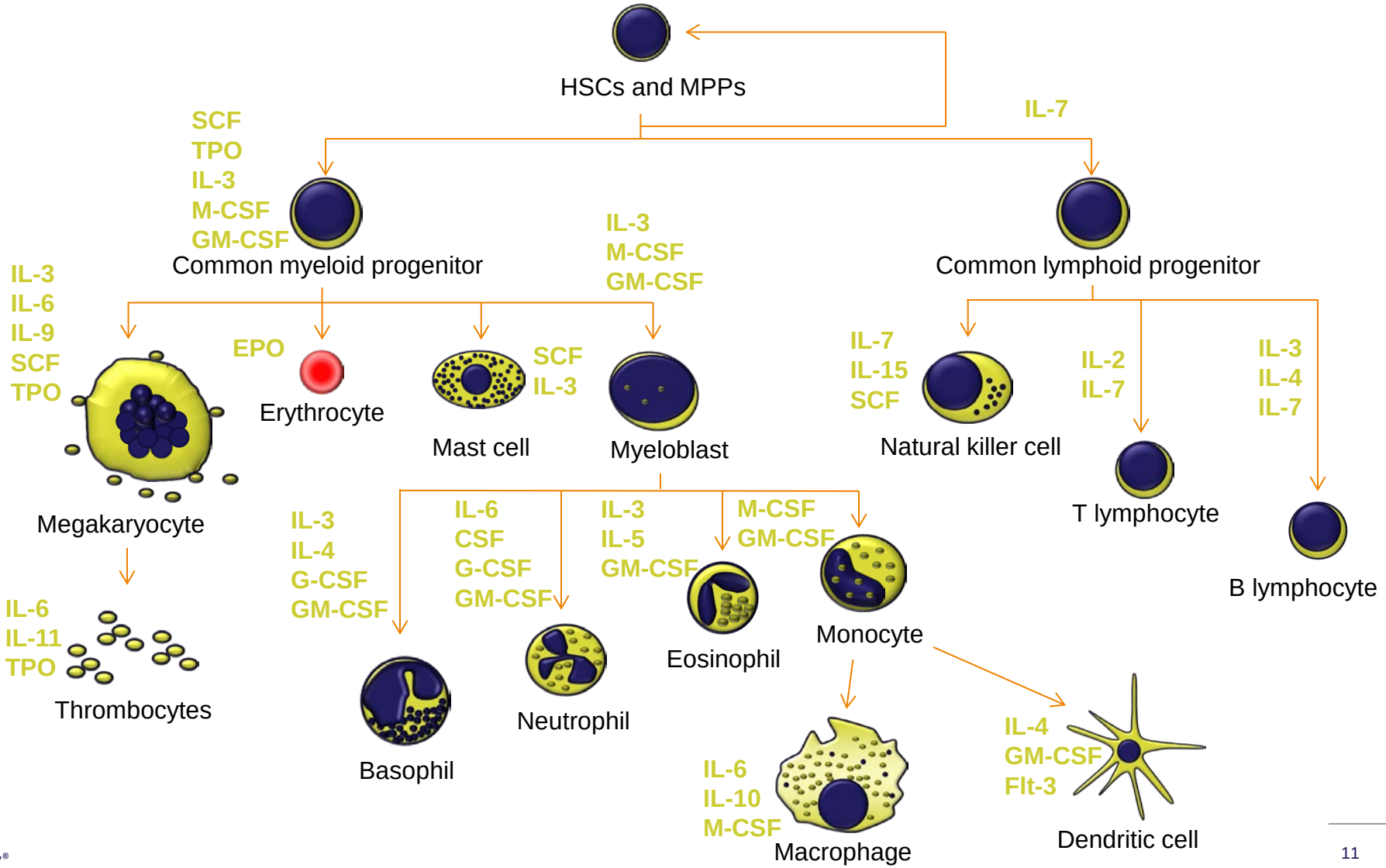
CD34+ cells are a mixed population of stem and progenitor cells



Assays to study hematopoietic stem and progenitor cells



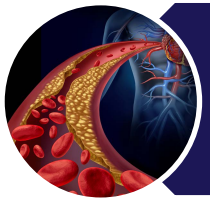
Cytokines influence cell fate and lineage *in-vitro*



Blood and hematopoiesis summary

- Blood is a heterogeneous tissue - its replacement *in vivo* is a complex process
- HSCs are responsible for the generation of all other blood cell types
- Recent advances allow for the identification and isolation of human HSCs as well as other blood cell types
- This process can be studied *in vitro* though the use of lineage directed differentiation of HSPCs

Outline



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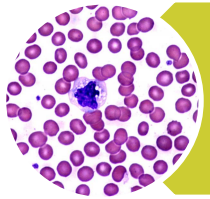
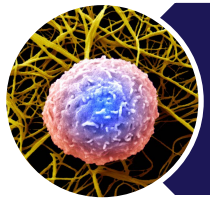
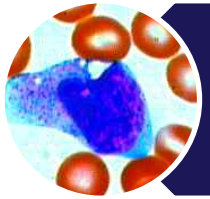


CD34+ hematopoietic stem & progenitor cells



Mononuclear cells



CD14+ monocytes

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Primary CD34+ HSPCs

Applications

- *Ex vivo* expansion and differentiation
- Stem cell markers
- Gene transfer
- Cytokine and chemokine expression and regulation
- Receptor expression

Key research areas

- Safely and efficiently expand HSCs *in vitro* or *in vivo* for transplantation
- Immune response
  - Graft-versus-host disease/transplant rejection
- Cancer
- Cell-fate determination

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Mature lymphocyte generation from CD34+ HSPCs on a 3D matrix

A Simple Model System Enabling Human CD34⁺ Cells to Undertake Differentiation Towards T Cells

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1 Regenerative Medicine Group, Cranfield Health, Cranfield University, Cranfield, United Kingdom, 2 Department of Immunology and Cancer Research, Faculty of Medicine, the Hebrew University of Jerusalem, Jerusalem, Israel

Abstract

Background: Channelling the development of haematopoietic progenitor cells into T lymphocytes is dependent upon a series of extrinsic prompts whose temporal and spatial sequence is critical for a productive outcome. Simple models of human progenitor cells development depend in the main on the use of xenogeneic systems which may provide some limitations to development.

Methods and Findings: Here we provide evidence that a simple model system which utilises both human keratinocyte and fibroblast cell lines arrayed on a synthetic tantalum coated matrix provides a permissive environment for the development of human CD34⁺ haematopoietic cells into mature CD4⁺ or CD8⁺ T lymphocytes in the presence of Interleukin 7 (IL-7), Interleukin 15 (IL-15) and the Fms-like tyrosine kinase 3 ligand (Flt-3L). This system was used to compare the ability of CD34⁺ cells to produce mature thymocytes and showed that whilst these cells derived from cord blood were able to productively differentiate into thymocytes the system was not permissive for the development of CD34⁺ cells from adult peripheral blood.

Conclusions/Significance: Our study provides direct evidence for the capacity of human cord blood CD34⁺ cells to differentiate along the T lineage in a simple human model system. Productive commitment of the CD34⁺ cells to generate T cells was found to be dependent on a three-dimensional matrix which induced the up-regulation of the Notch delta-like ligand 4 (Dll-4) by epithelial cells.

Citation: Lapenna A, B-Lynch C, Kapeni C, Aspinall R (2013) A Simple Model System Enabling Human CD34⁺ Cells to Undertake Differentiation Towards T Cells. PLoS ONE 5(7): e69572. doi:10.1371/journal.pone.0069572

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Competing Interests: The authors have declared that no competing interests exist.

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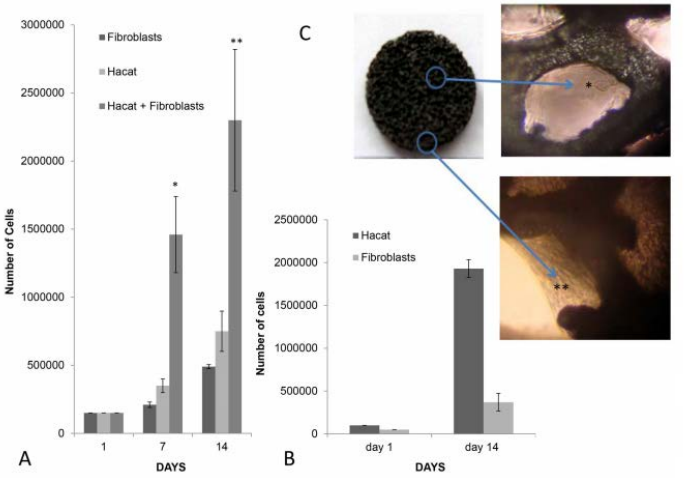
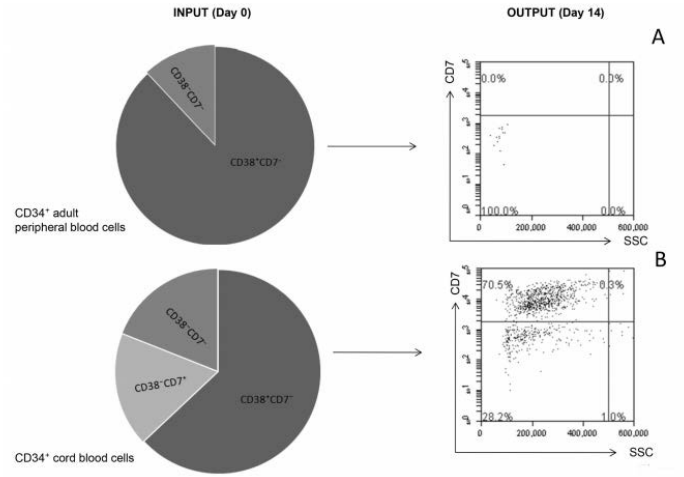
Introduction

The generation of T cells from haematopoietic progenitor cells requires the positioning of progenitors within the thymus where a unique environment induces supports and directs their differentiation [1]. Production of new thymocytes continues throughout life and because the progenitors cannot be stored and maintained indefinitely within the thymus, continuation of production requires seeding of the thymus with these cells. Analysis of thymic output reveal that the rate of production of new T cells declines with age [2] and that as thymocyte production decreases so there is atrophy of the thymus.

In broad terms thymic atrophy has been linked to deficits in (FTOC) systems or allogeneic cell lines such as mouse bone marrow-derived OP9 cells expressing the Notch delta-like ligand 1 (OP9-Dll1) [3–5]. But the experiments in human systems have proved more intractable. Analysis of the capacity of haematopoietic progenitor cell populations to produce T cells have proceeded but has been hampered, mainly through the use of xenogeneic model systems which by their very nature are limited and associated with incomplete or inefficient differentiation of the progenitors [5]. Some studies of thymic stromal cells have indicated changes with age in the thymic environment cell type composition and expression profile but these data were limited by the lack of culture methods which could effectively model the thymic architecture in vitro [6].



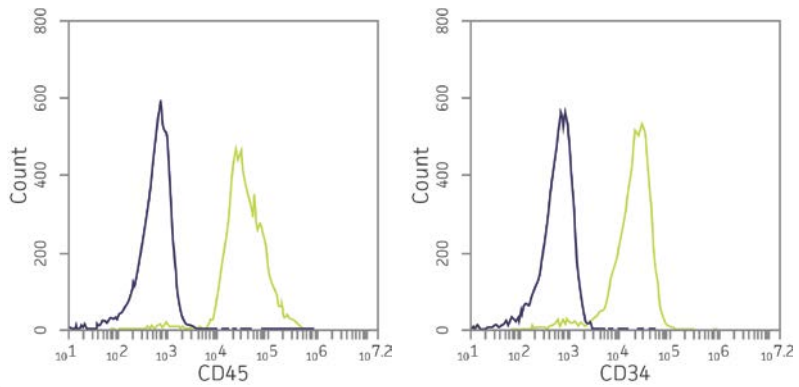


Lapenna A, *et al*. *PloS one* 8.7, 2013: e69572.

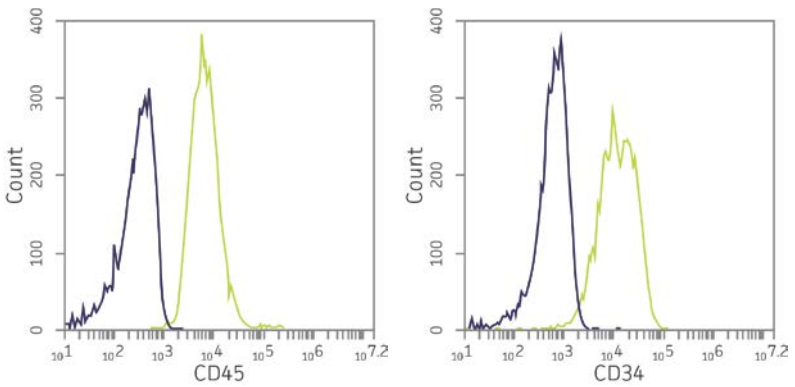
ATCC primary CD34+ HSPCs

- Healthy human volunteer donors; IRB-approved informed consent
- Adult, non-pregnant (excluding cord blood)
- Cryopreserved at P0; Purity: ≥ 90% CD34+
- Age, gender, ethnicity, and blood type on CoA

ATCC® No.	Tissue	Type	Size
PCS-800-012	Bone marrow	Hematopoietic stem/progenitor cells (CD34+); Normal	≥ 0.5 x 10^6
PCS-800-014	Cord blood	Hematopoietic stem/progenitor cells (CD34+); Normal	≥ 0.5 x 10^6



Bone marrow



Cord blood

CD34+ HSPC lineage directed expansion and differentiation

Goals

- Demonstrate primary CD34+ HSPC capacity for lineage-directed expansion and differentiation *in vitro*
- Confirm multilineage differentiation (erythrocyte, megakaryocyte, and pan-myeloid)
- Compare CD34+ differentiation efficiency from multiple tissues
- Utilize a method amenable to high throughput assays

Methods

- Cryopreserved CD34+ primary cells from bone marrow and cord blood
- Serum-free liquid culture
- Analysis of phenotype by surface marker expression
- Commercially available cytokine cocktails

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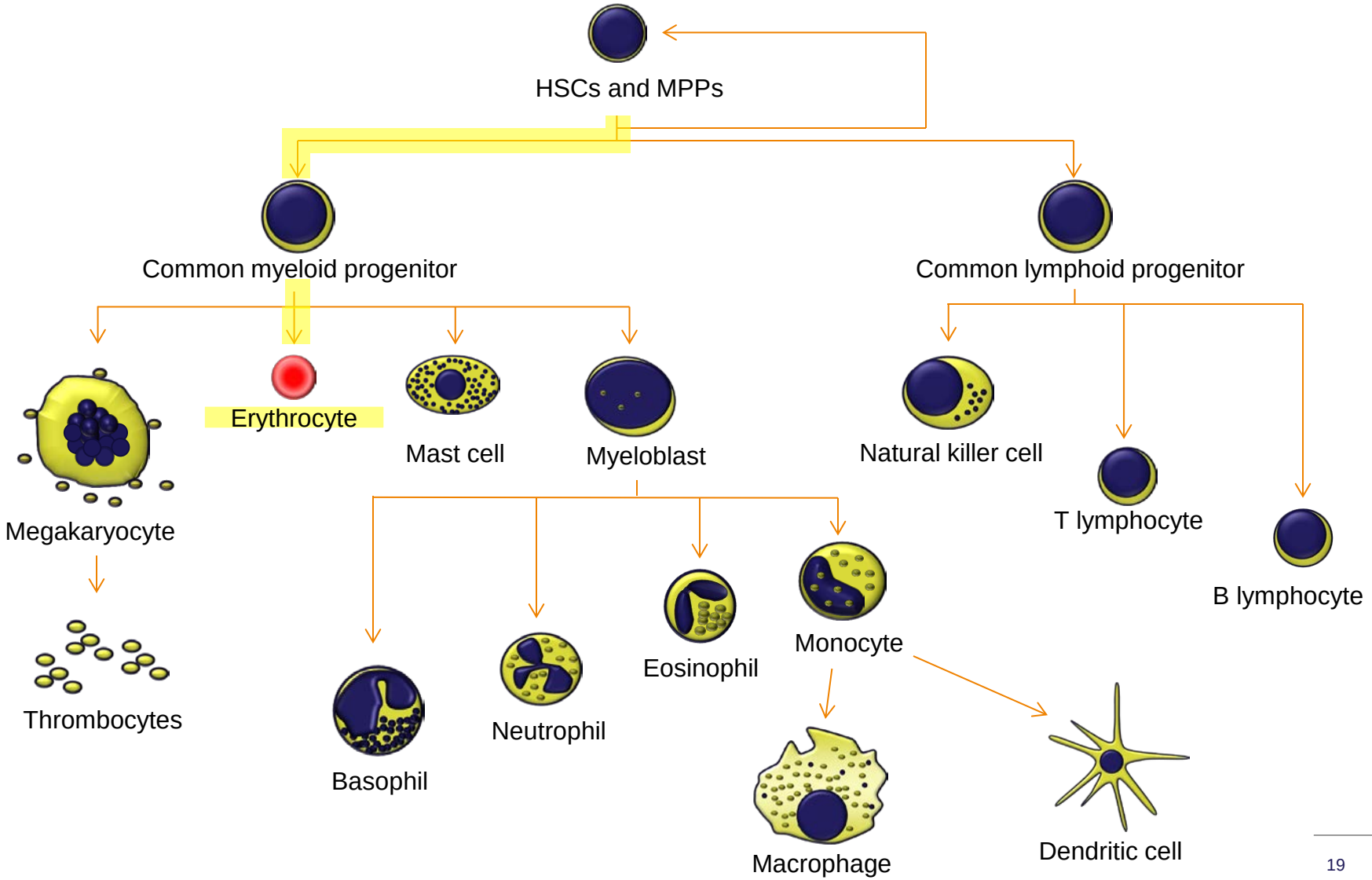
General differentiation protocol workflow

Thaw & seed	Maintenance	Analyze
Thaw vial of CD34+ cells	Add fresh media every ~2 days	Collect cells for analysis
Seed directly into differentiation media	Re-suspend in fresh media every 4-7 days	
Basal media: SFEM II	Maintain concentration below $1x10^6$/mL	
Supplement with cytokines		

For detailed differentiation protocols see the ATCC website

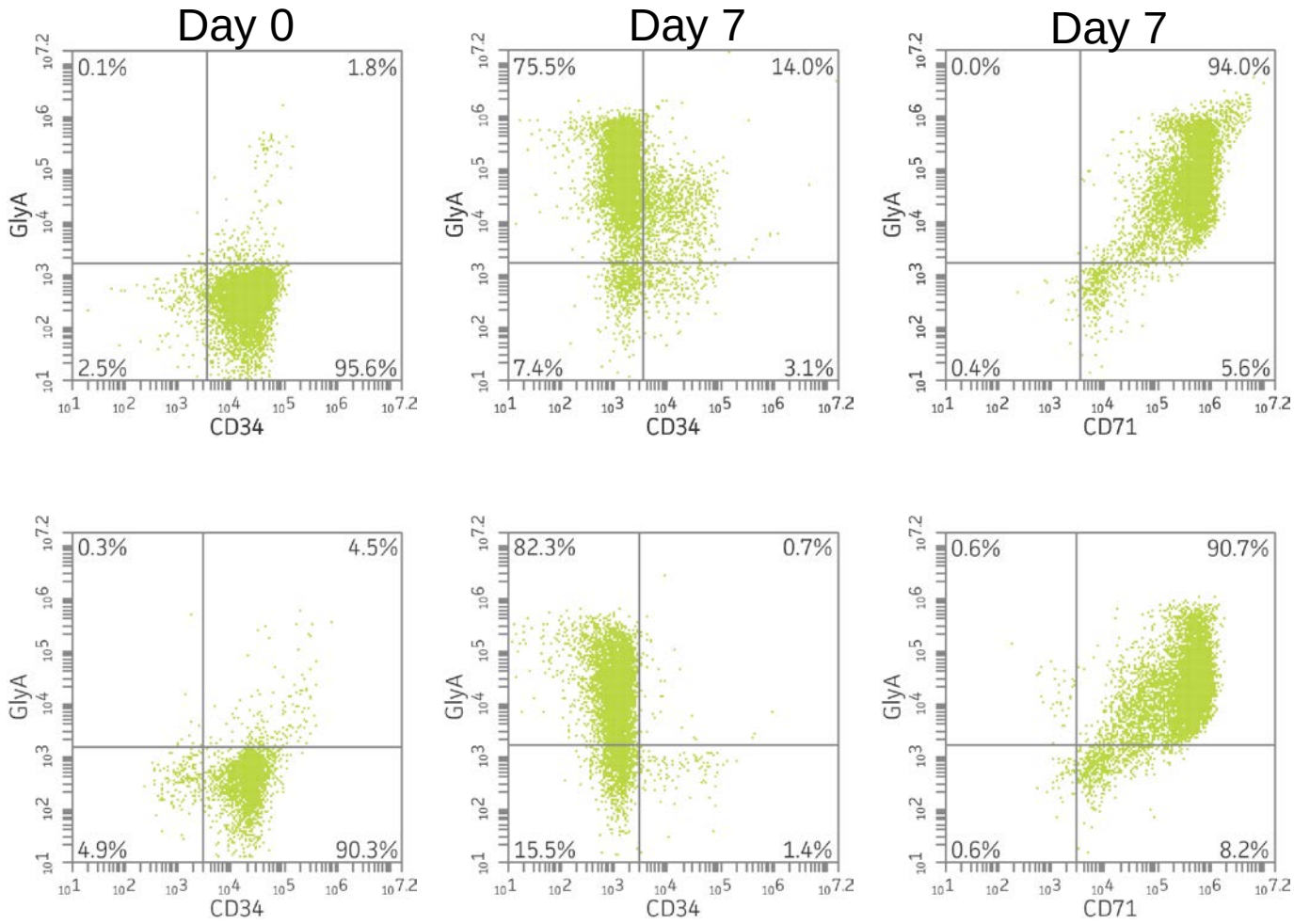
Erythroid differentiation and expansion



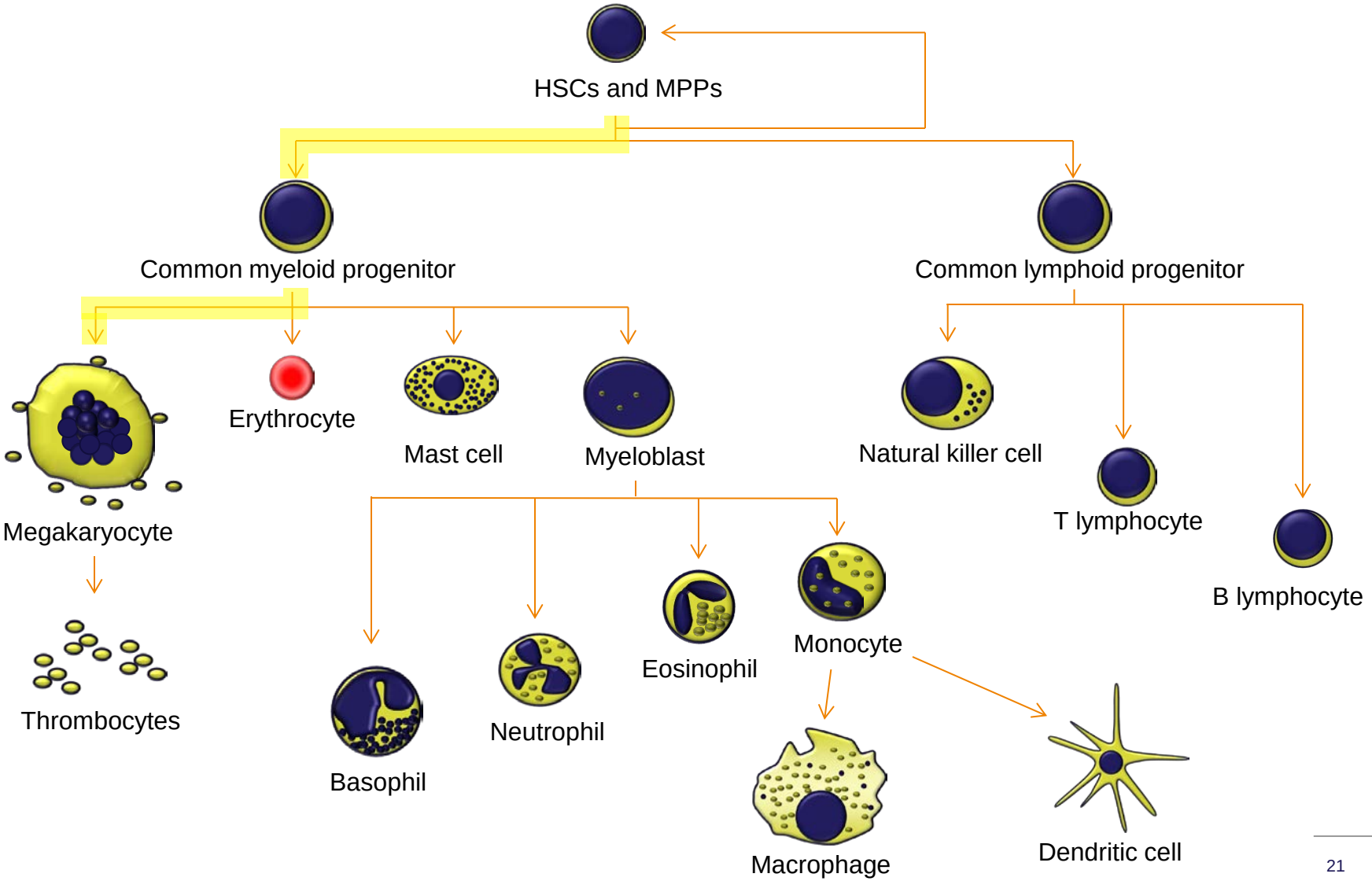
Expression of erythroid lineage markers on differentiated BM and CB CD34+ cells

Bone Marrow CD34+ HSPCs

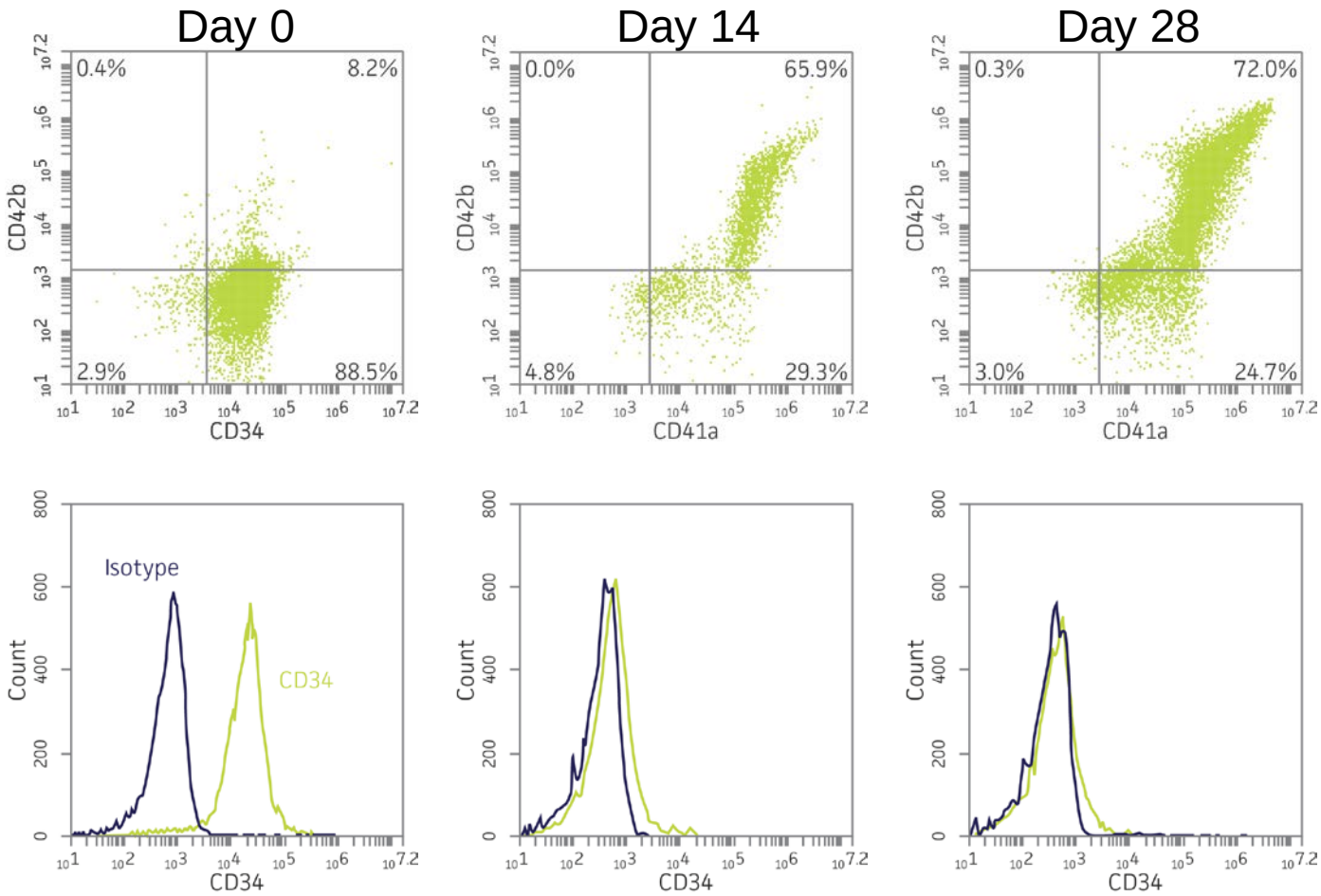
Cord Blood CD34+ HSPCs



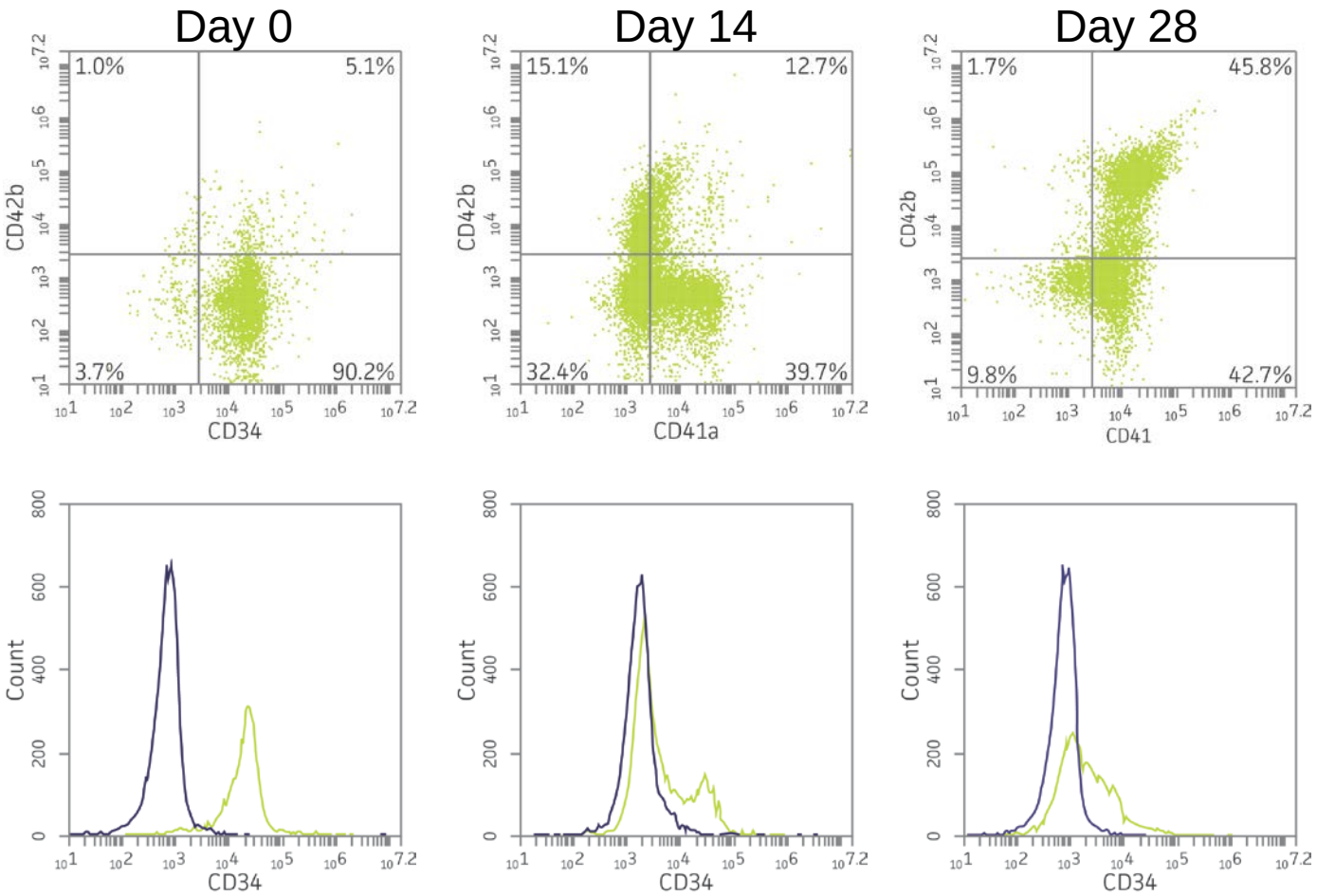
Megakaryocyte differentiation and expansion



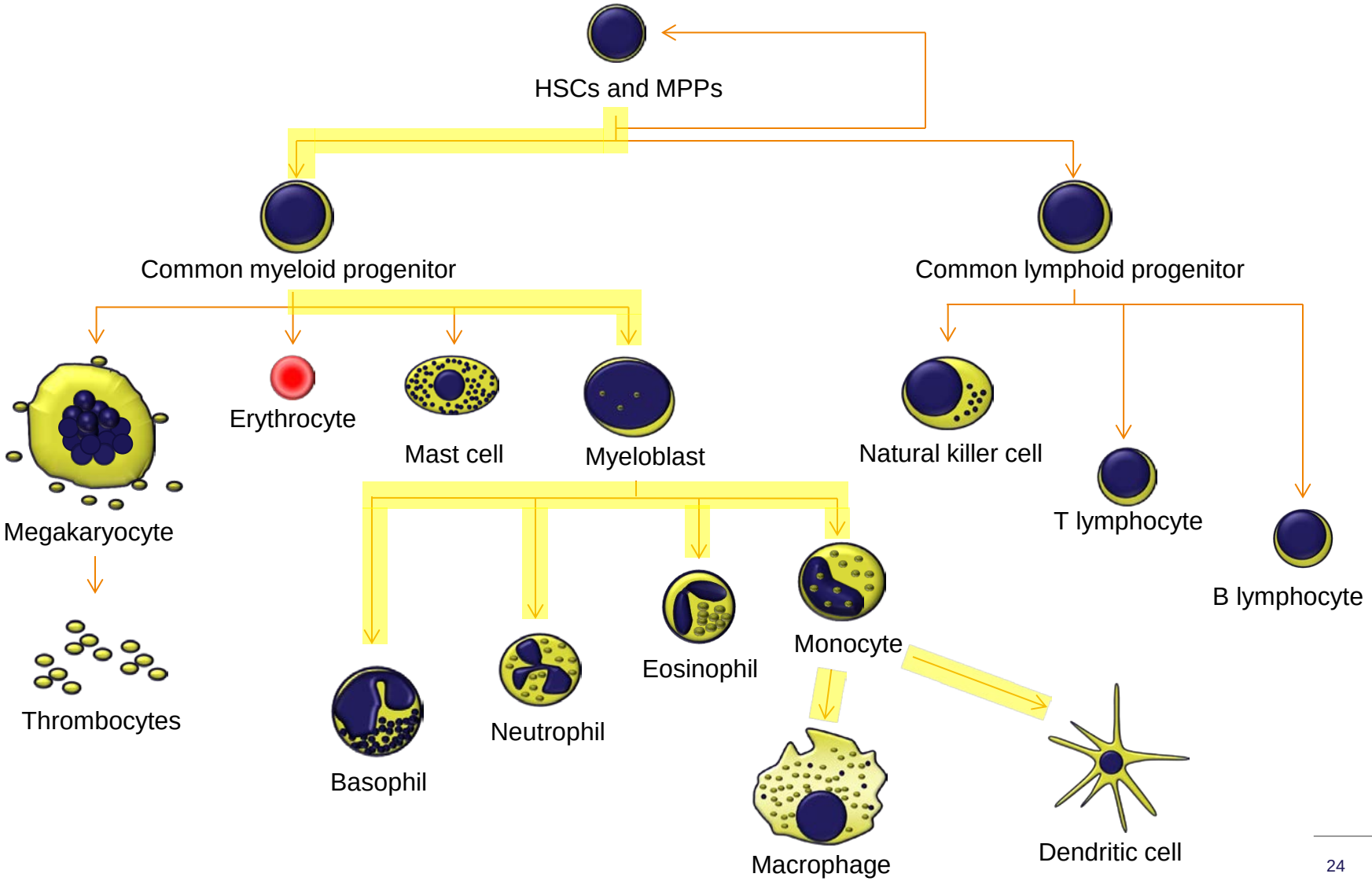
Expression of megakaryocyte lineage markers on differentiated bone marrow CD34+ HPSCs



Expression of megakaryocyte lineage markers on differentiated cord blood CD34+ HPSCs



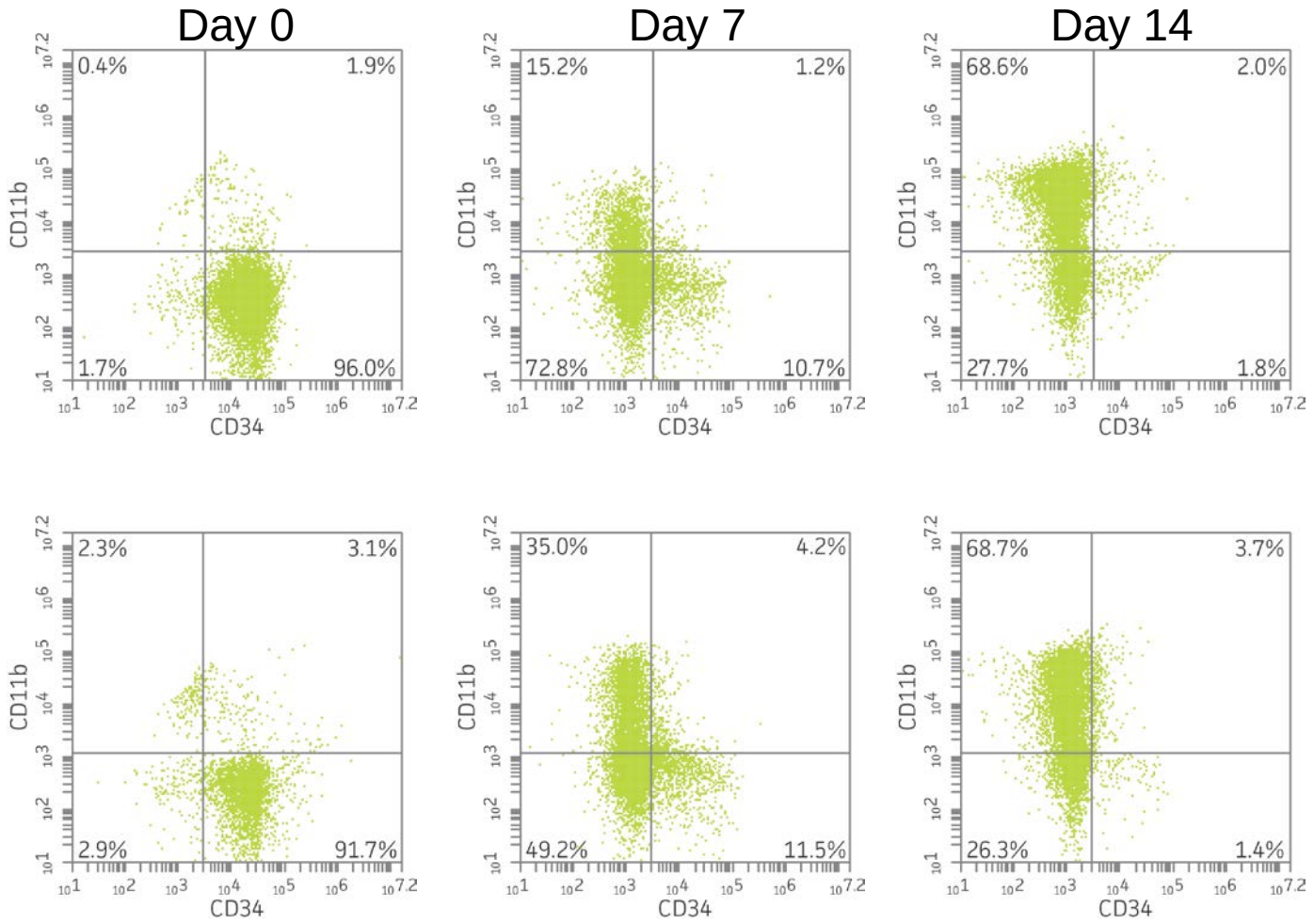
Pan-myeloid differentiation and expansion



Expression of pan-myeloid lineage markers on differentiated BM and CB CD34+ HPSCs

Bone Marrow CD34+ HSPCs

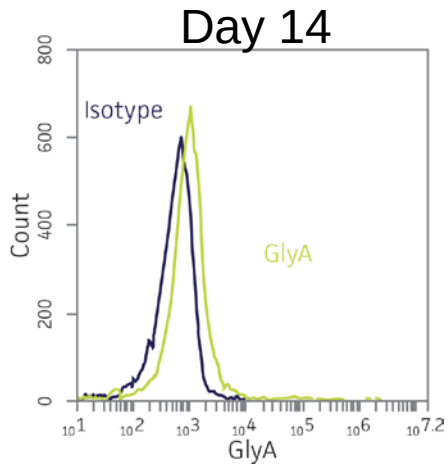
Cord Blood CD34+ HSPCs

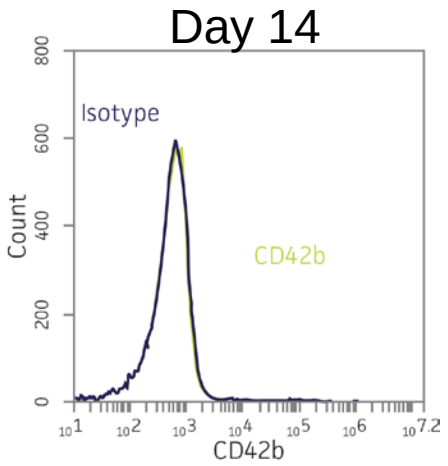


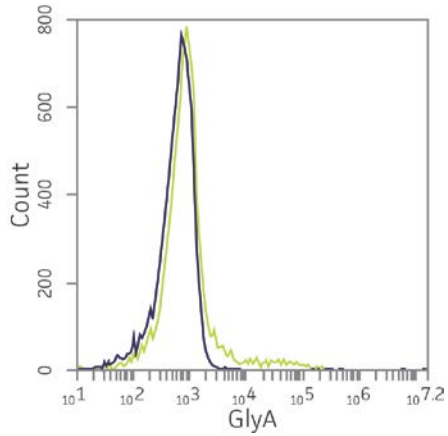
Specificity of pan-myeloid-directed differentiation of CD34+ HPSCs

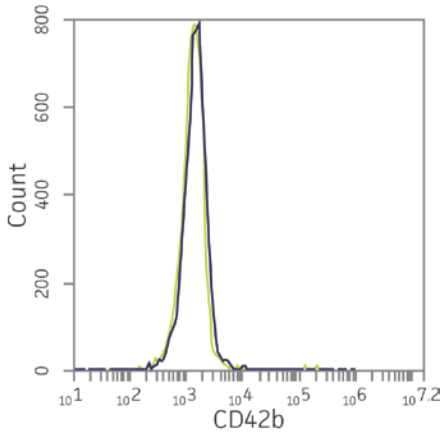
Bone Marrow CD34+ HSPCs

Cord Blood CD34+ HSPCs









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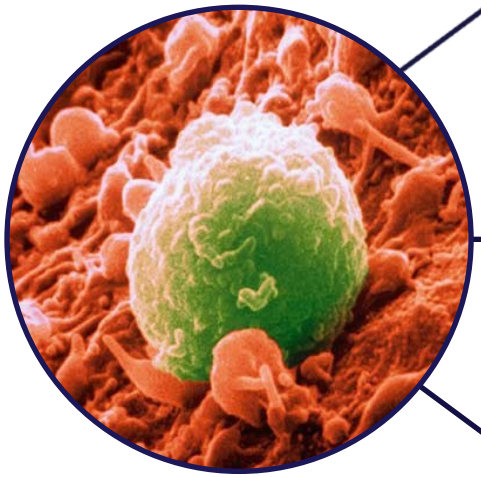
ATCC primary CD34+ HSPCs: Summary



High purity

- Single donor
- ≥ 90% CD34+

Multiple tissues

- Bone marrow
- Cord blood

Multipotent

- Erythroid
- Megakaryocyte
- Pan-myeloid

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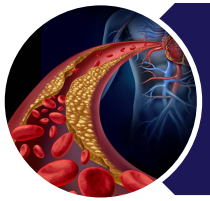
Outline



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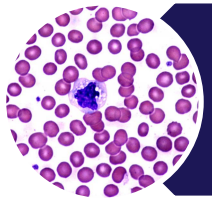
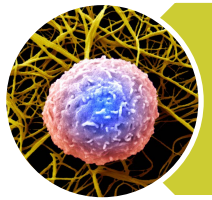
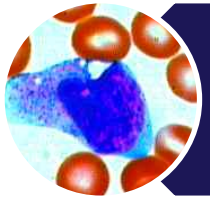


CD34+ hematopoietic stem & progenitor cells



Mononuclear cells



CD14+ monocytes

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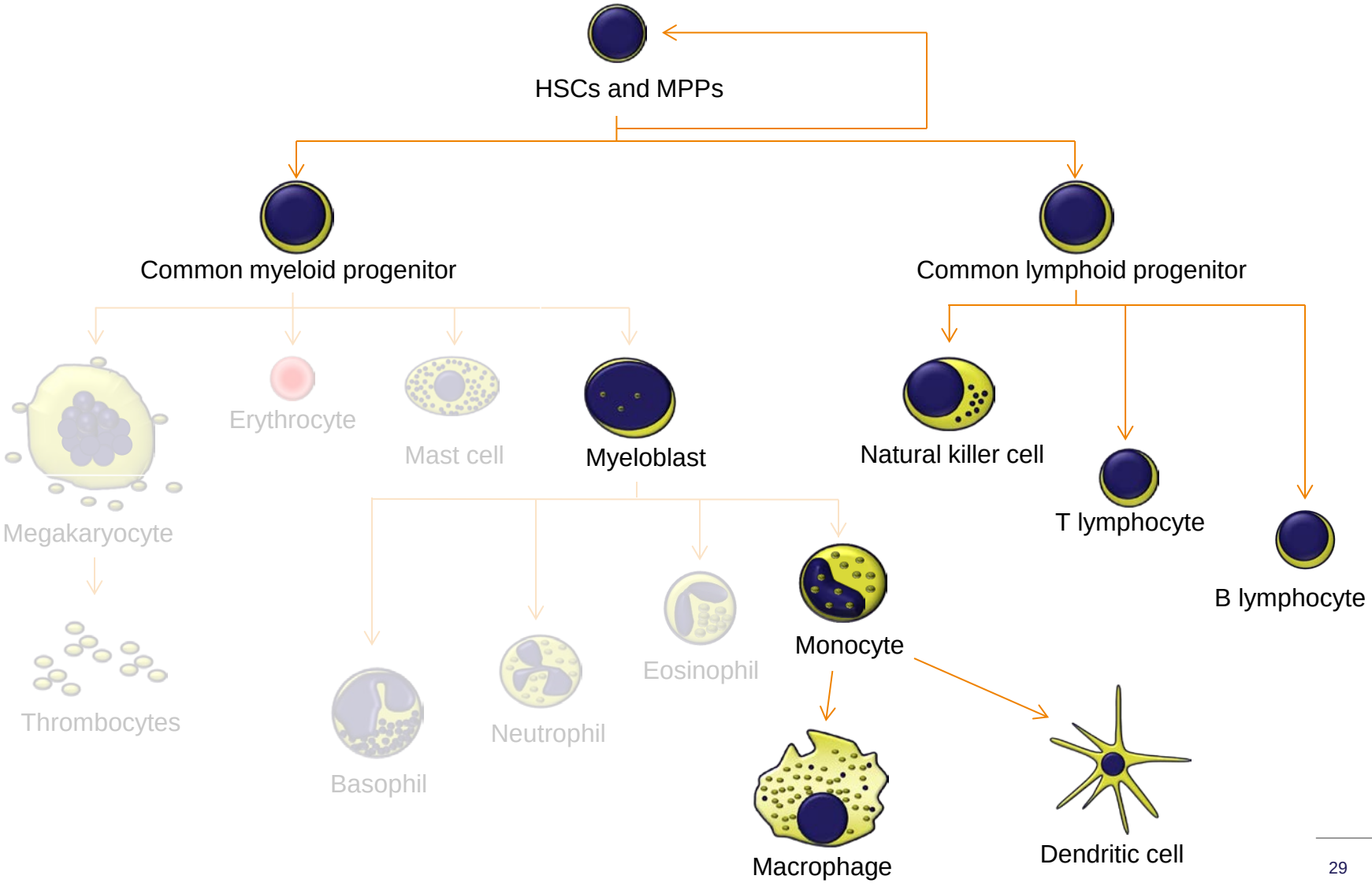
Mononuclear Cells



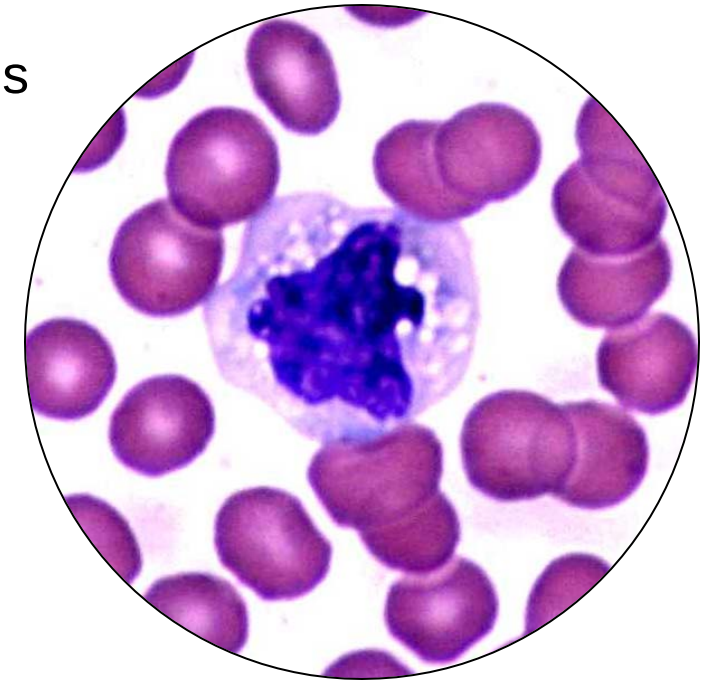
Primary mononuclear cells

Applications

- Isolation and study of cell subpopulations
- Molecular expression profiling

Key research areas

- Infectious disease
- Blood pathologies
- Immunology
- Vaccine development
- Toxicology
- Regenerative medicine



Monocyte and erythrocytes

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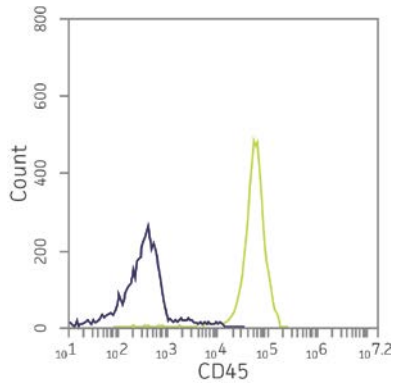
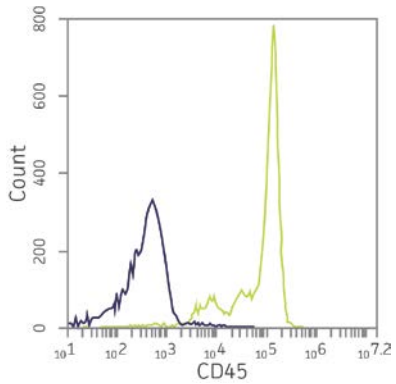
Primary mononuclear cells

- Healthy human volunteer donors; IRB-approved informed consent
- Adult, non-pregnant
- Cryopreserved at P0; Purity: ≥ 90% CD45+
- Age, gender, ethnicity, and blood type on CoA

ATCC® No.	Tissue	Type	Size
PCS-800-011	Peripheral blood	Mononuclear cells (PBMCs); Normal, Human	≥ 25 x 10^6
PCS-800-013	Bone marrow	Mononuclear cells (BMMCs); Normal, Human	≥ 25 x 10^6



Peripheral blood mononuclear cells



Bone marrow mononuclear cells

Primary mononuclear cells

Reported on CoA (lot specific)

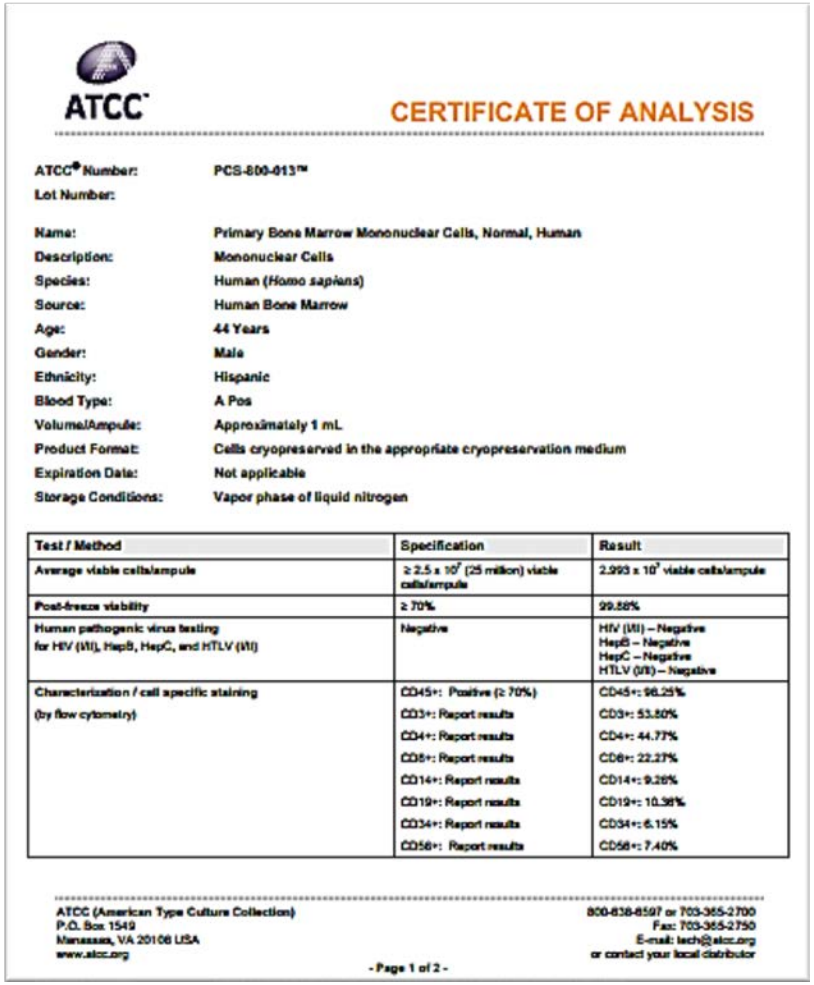
- Specific marker expression

Peripheral Blood

- CD45+ (%)
- CD3+ (%)
- CD8+ (%)
- CD14+ (%)
- CD19+ (%)
- CD56+ (%)

Bone Marrow

- CD45+ (%)
- CD3+ (%)
- CD8+ (%)
- CD14+ (%)
- CD19+ (%)
- CD34+ (%)
- CD56+ (%)



ATCC

CERTIFICATE OF ANALYSIS

ATCC® Number:	PCS-800-013™
Lot Number:	
Name:	Primary Bone Marrow Mononuclear Cells, Normal, Human
Description:	Mononuclear Cells
Species:	Human (*Homo sapiens*)
Source:	Human Bone Marrow
Age:	44 Years
Gender:	Male
Ethnicity:	Hispanic
Blood Type:	A Pos
Volume/Ampule:	Approximately 1 mL
Product Format:	Cells cryopreserved in the appropriate cryopreservation medium
Expiration Date:	Not applicable
Storage Conditions:	Vapor phase of liquid nitrogen

Test / Method	Specification	Result
Average viable cells/ampule	≥ 2.5 x 10^7 (25 million) viable cells/ampule	2.993 x 10^7 viable cells/ampule
Post-freeze viability	≥ 70%	99.58%
Human pathogenic virus testing for HIV (I/II), HepB, HepC, and HTLV (I/II)	Negative	HIV (I/II) – Negative HepB – Negative HepC – Negative HTLV (I/II) – Negative
Characterization / cell specific staining (by flow cytometry)	CD45+: Positive (≥ 70%) CD3+: Report results CD4+: Report results CD8+: Report results CD14+: Report results CD19+: Report results CD34+: Report results CD56+: Report results	CD45+: 98.25% CD3+: 53.80% CD4+: 44.77% CD8+: 22.27% CD14+: 9.26% CD19+: 10.38% CD34+: 6.15% CD56+: 7.40%

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- Page 1 of 2 -

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Non-viral method increases natural killer cells' anti-cancer cell cytotoxicity

Enhanced Cytotoxicity of Natural Killer Cells following the Acquisition of Chimeric Antigen Receptors through Trogocytosis

Fu-Nan Cho[1¶], Tsung-Hsien Chang[2¶], Chih-Wen Shu[2], Ming-Chin Ko[3], Shuen-Kuei Liao[4], Kang-Hsi Wu[5], Ming-Sun Yu[6], Shyh-Jer Lin[6], Ying-Chung Hong[6], Chien-Hsun Chen[7], Chien-Hui Hung[3], Yu-Hsiang Chang[3,8,9]*

1 Department of Obstetrics and Gynecology, Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan, 2 Department of Medical Education and Research, Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan, 3 Department of Pediatrics, Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan, 4 Graduate Institute of Cancer Biology and Drug Discovery and Center of Excellence for Cancer Research, Taipei Medical University, Taipei, Taiwan, 5 Department of Pediatrics, Children's Hospital and School of Chinese Medicine, China Medical University Hospitals, Taichung, Taiwan, 6 Haematology-Oncology Section, Department of Medicine, Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan, 7 Department of Radiation Oncology, Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan, 8 Faculty of Medicine, National Yang-Ming University, Taipei, Taiwan, 9 Department of Nursing, Tajen University, Yanpu Township, Pingtung County, Taiwan

Abstract

Natural killer (NK) cells have the capacity to target tumors and are ideal candidates for immunotherapy. Viral vectors have been used to genetically modify *in vitro* expanded NK cells to express chimeric antigen receptors (CARs), which confer cytotoxicity against tumors. However, use of viral transduction methods raises the safety concern of viral integration into the NK cell genome. In this study, we used trogocytosis as a non-viral method to modify NK cells for immunotherapy. A K562 cell line expressing high levels of anti-CD19 CARs was generated as a donor cell to transfer the anti-CD19 CARs onto NK cells via trogocytosis. Anti-CD19 CAR expression was observed in expanded NK cells after these cells were co-cultured for one hour with freeze/thaw-treated donor cells expressing anti-CD19 CARs. Immunofluorescence analysis confirmed the localization of the anti-CD19 CARs on the NK cell surface. Acquisition of anti-CD19 CARs via trogocytosis enhanced NK cell-mediated cytotoxicity against the B-cell acute lymphoblastic leukemia (B-ALL) cell lines and primary B-ALL cells derived from patients. To our knowledge, this is the first report that describes the increased cytotoxicity of NK cells following the acquisition of CARs via trogocytosis. This novel strategy could be a potential valuable therapeutic approach for the treatment of B-cell tumors.

Citation: Cho F-N, Chang T-H, Shu C-W, Ko M-C, Liao S-K, et al. (2014) Enhanced Cytotoxicity of Natural Killer Cells following the Acquisition of Chimeric Antigen Receptors through Trogocytosis. PLoS ONE 9(10): e109352. doi:10.1371/journal.pone.0109352

Editor: Jacques Zimmer, Centre de Recherche Public de la Santé (CRP-Santé), Luxembourg

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Data Availability: The authors confirm that all data underlying the findings are fully available without restriction. All relevant data are within the paper.

Funding: This study was supported by grants from National Health Research Institutes (PS9808, http://english.nhri.org.tw/NHRI_WEB/nhriw001Action.do), and Kaohsiung Veterans General Hospital (VGHKS 102-104 and 103-123, http://www.vghks.gov.tw/English/) to YHC. This work is also supported in part by the Ministry of Health and Welfare of Taiwan (MOHW103-TD-B-111-01), http://www.mohw.gov.tw/EN/Ministry/Index.aspx) to SKL. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

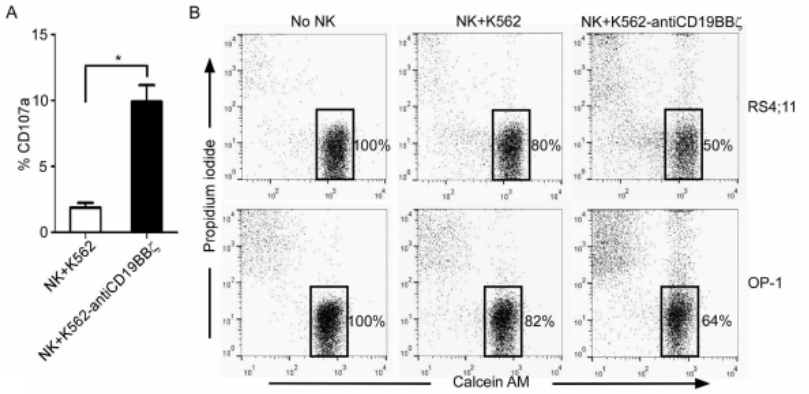
* Email: yhchang@vghks.gov.tw

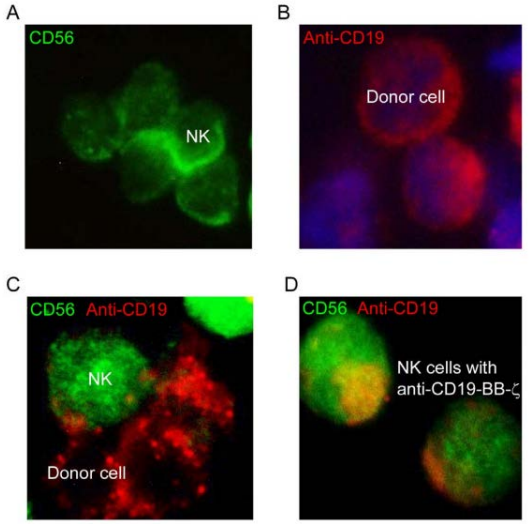
¶ These authors contributed equally to this work.

Introduction

Natural killer (NK) cells have the ability to recognize and eliminate tumor cells, making them ideal candidates for tumor immunotherapy [1,2]. NK cell activity is regulated by the cumulative effects of multiple activating and inhibitory signals that are transmitted through the receptors on the NK cell surface. We have previously genetically modified *in vitro* expanded NK cells to express DAP10 and the chimeric NKG2D receptor containing the CD3ζ signal domain, which altered the balance between the activating and inhibitory signals of NK cells and enhanced the cytotoxicity against NKG2D ligand-bearing tumors [3]. Further, expression of anti-CD19 chimeric antigen receptors (CARs) containing 41BB and CD3ζ signal domains on NK cells enhanced the activating signals originating from CD19 antigen engagement, leading to cytotoxicity specifically against B-cell leukemia [4].

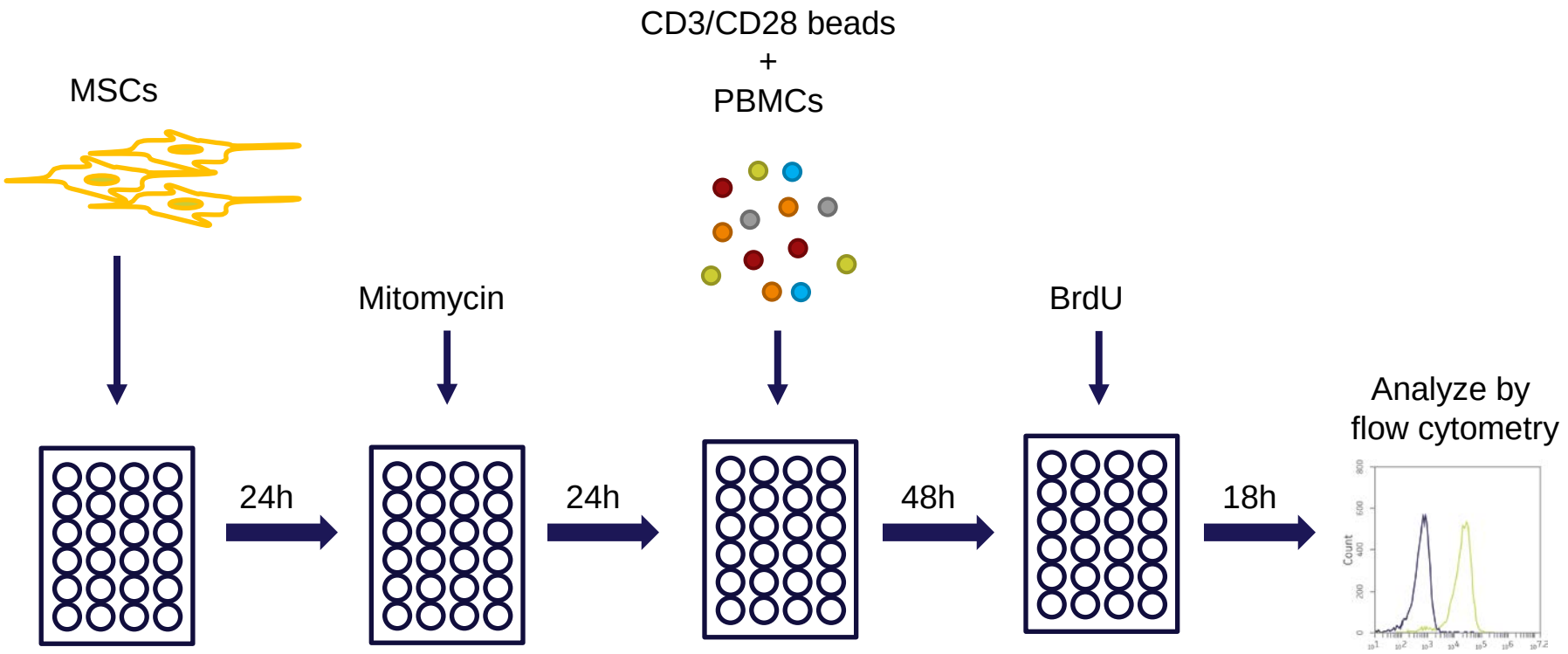
Trogocytosis is a process in which membrane patches are exchanged between target and immune cells [5–7]. When an NK cell interacts with a target cell, an immune synapse, which is strong enough to allow the transfer of small membrane patches from one cell to its partner cell, is formed [8,9]. Therefore, target cell surface molecules can be found on the surface of NK cells. The chemokine receptor CCR7 has been shown to be transferred from donor cells onto the surface of NK cells via trogocytosis, and this transfer stimulated NK cell migration, leading to enhanced lymph node homing [10,11]. Similarly, T cells captured NKG2D and NKp46 ligands on tumor cells through trogocytosis and promoted NK cell activity [12].





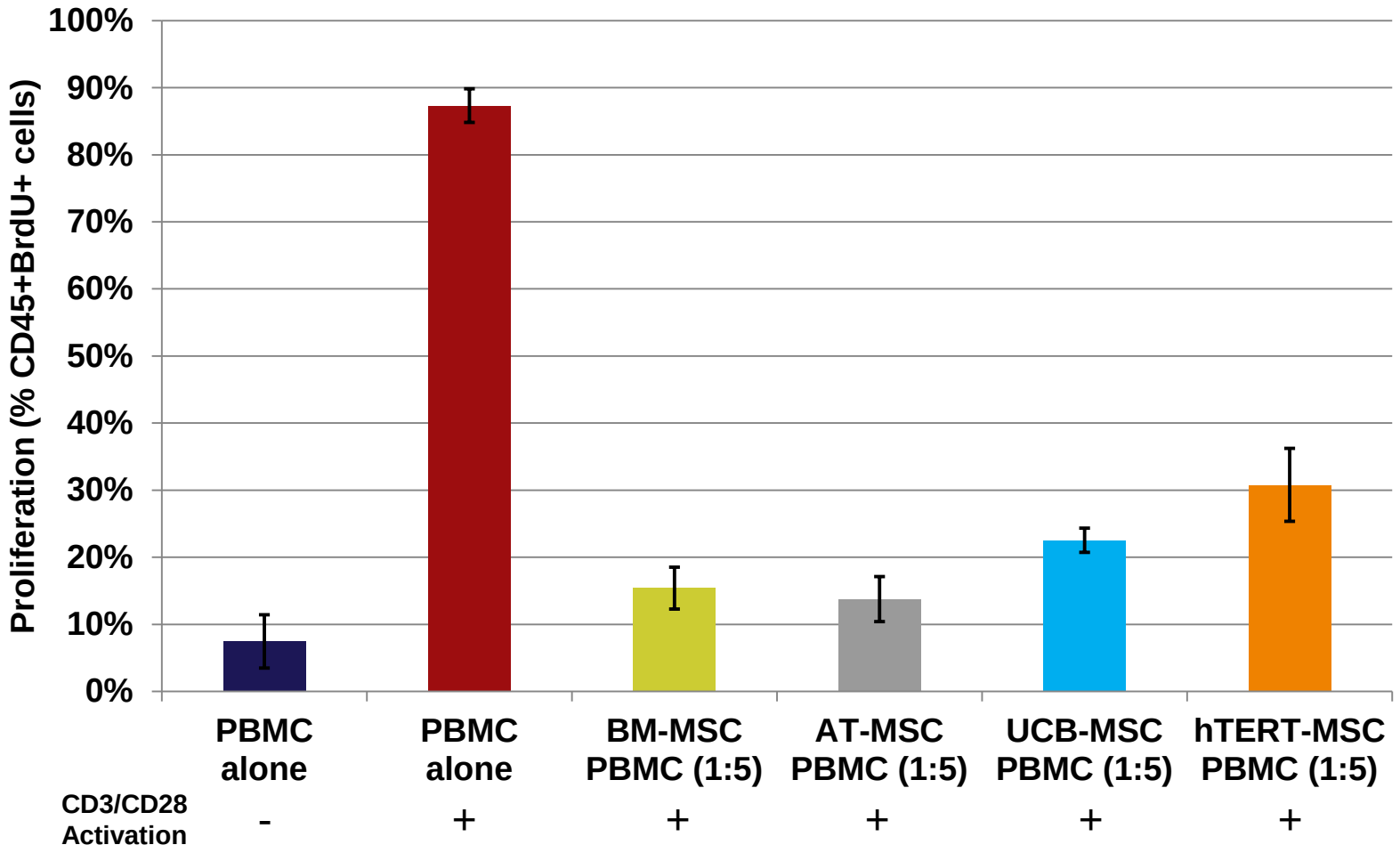
Cho, Fu-Nan, et al. *PloS one* 9.10 (2014): e109352.

Immunosuppressive assay using PBMCs



For detailed differentiation protocols see the ATCC website

MSCs suppress activated T-cell proliferation



ATCC Mesenchymal Stem Cells

The complete study, presented at ISSCR 2014, is available on the ATCC website:

Comparative analysis of cell proliferation, immunosuppressive action, and multi-lineage differentiation of immortalized MSC and MSC from bone marrow, adipose tissue, and umbilical cord blood

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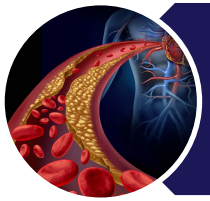
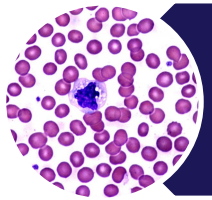
ATCC® No.	Type	Description
PCS-500-010	Primary	Umbilical Cord-derived Mesenchymal Stem Cells
PCS-500-011	Primary	Adipose-derived Mesenchymal Stem Cells
PCS-500-012	Primary	Bone Marrow-derived Mesenchymal Stem Cells
SCRC-4000	Immortalized	hTERT Immortalized Adipose-derived Mesenchymal Stem Cells

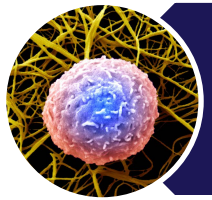
Outline



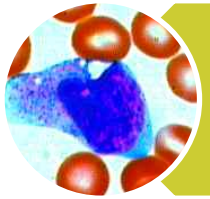
Background



CD34+ hematopoietic stem & progenitor cells



Mononuclear cells



CD14+ monocytes

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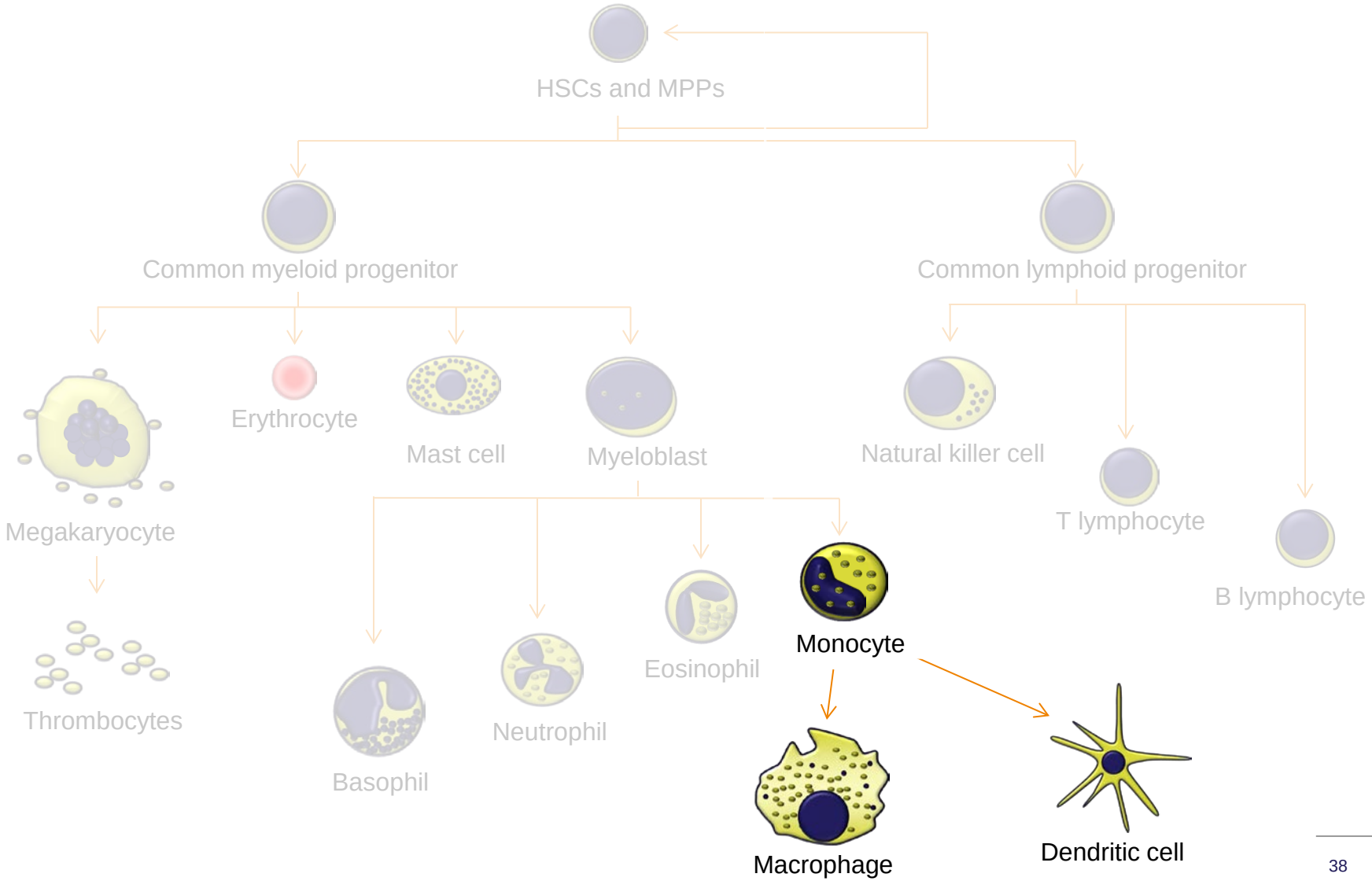
Primary monocytes (CD14+)



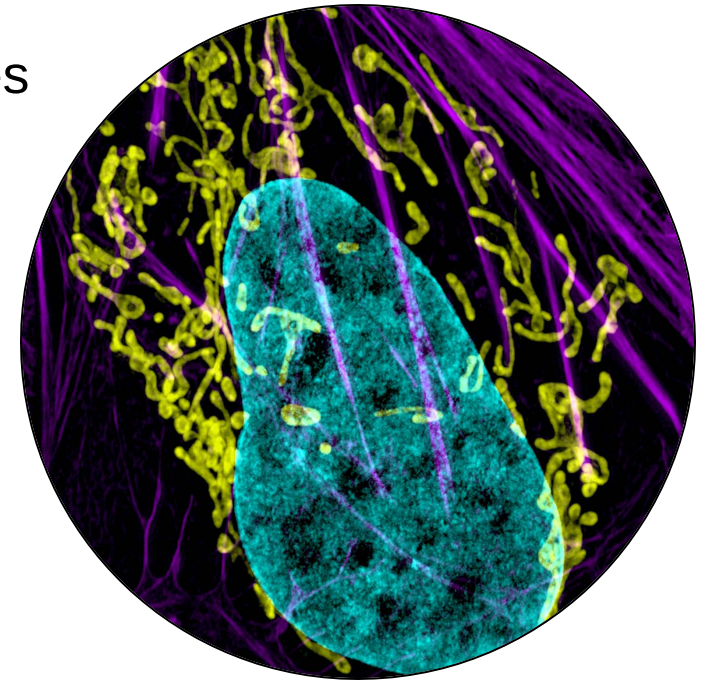
Primary CD14+ monocytes

Applications

- Isolation and study of monocyte subtypes
- Differentiation
- Phagocytosis
- Chemotaxis/migration assays

Key research areas

- Immunology
- Monocyte polarization
- Inflammation associated pathologies
- Infectious disease
- Cytokine release



Bone cancer cell, courtesy of Burnett Lippincott-Schwartz, National Cancer Institute

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Primary monocyte-derived macrophages are suitable for studying *Lm*, cell lines are not

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PLOS ONE

CD14-Dependent Monocyte Isolation Enhances Phagocytosis of *Listeria monocytogenes* by Proinflammatory, GM-CSF-Derived Macrophages

Caroline Neu[1], Anne Sedlag[1], Carina Bayer[2], Sabine Förster[3], Peter Crauwels[3], Jan-Hendrik Niess[4], Ger van Zandbergen[3], Giada Frascaroli[2], Christian U. Riedel[1]*

1 Institute of Microbiology and Biotechnology, University of Ulm, Ulm, Germany, 2 Institute of Virology, University Medical Center Ulm, Ulm, Germany, 3 Division of Immunology, Paul-Ehrlich-Institute, Federal Institute for Vaccines and Biomedicines, Langen, Germany, 4 Department of Visceral Medicine and Surgery, Inselspital, Bern, Switzerland

Abstract

Macrophages are an important line of defence against invading pathogens. Human macrophages derived by different methods were tested for their suitability as models to investigate *Listeria monocytogenes (Lm)* infection and compared to macrophage-like THP-1 cells. Human primary monocytes were isolated by either positive or negative immunomagnetic selection and differentiated in the presence of granulocyte macrophage colony-stimulating factor (GM-CSF) or macrophage colony-stimulating factor (M-CSF) into pro- or anti-inflammatory macrophages, respectively. Regardless of the isolation method, GM-CSF-derived macrophages (GM-Mφ) stained positive for CD206 and M-CSF-derived macrophages (M-Mφ) for CD163. THP-1 cells did not express CD206 or CD163 following incubation with PMA, M- or GM-CSF alone or in combination. Upon infection with *Lm*, all primary macrophages showed good survival at high multiplicities of infection whereas viability of THP-1 was severely reduced even at lower bacterial numbers. M-Mφ generally showed high phagocytosis of *Lm*. Strikingly, phagocytosis of Lm by GM-Mφ was markedly influenced by the method used for isolation of monocytes. GM-Mφ derived from negatively isolated monocytes showed low phagocytosis of *Lm* whereas GM-Mφ generated from positively selected monocytes displayed high phagocytosis of *Lm*. Moreover, incubation with CD14 antibody was sufficient to enhance phagocytosis of *Lm* by GM-Mφ generated from negatively isolated monocytes. By contrast, non-specific phagocytosis of latex beads by GM-Mφ was not influenced by treatment with CD14 antibody. Furthermore, phagocytosis of *Lactococcus lactis*, *Escherichia coli*, human cytomegalovirus and the protozoan parasite *Leishmania major* by GM-Mφ was not enhanced upon treatment with CD14 antibody indicating that this effect is specific for *Lm*. Based on these observations, we propose macrophages derived by *ex vivo* differentiation of negatively selected human primary monocytes as the most suitable model to study *Lm* infection of macrophages.

Citation: Neu C, Sedlag A, Bayer C, Förster S, Crauwels P, et al. (2013) CD14-Dependent Monocyte Isolation Enhances Phagocytosis of *Listeria monocytogenes* by Proinflammatory, GM-CSF-Derived Macrophages. PLoS ONE 8(6): e66898. doi:10.1371/journal.pone.0066898

Editor: Jörn Coers, Duke University Medical Center, United States of America

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Competing Interests: The authors have declared that no competing interests exist.

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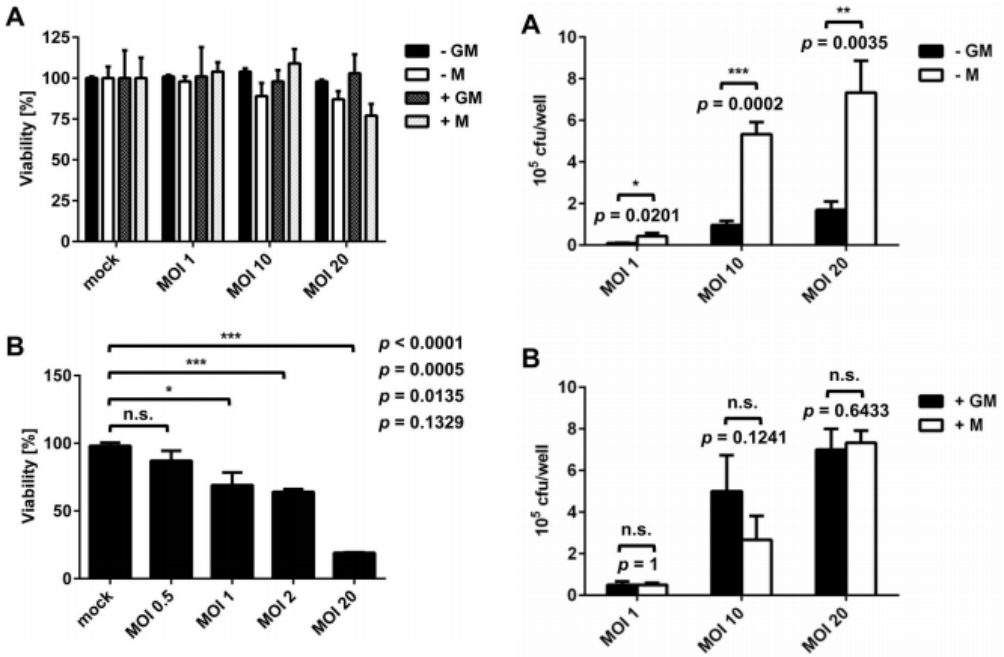
Introduction

Listeria monocytogenes (*Lm*) is a food-borne Gram-positive obligate intracellular pathogen that is able to cope with a wide range of environmental conditions and is thus found in various of habitats [1]. In humans, the disease caused by *Lm* is termed listeriosis and manifests primarily in immunocompromised individuals, pregnant women, new-borns, and elderly patients with a mortality of 20–30% in these at risk groups [2]. Infections with *Lm* are usually acquired upon consumption of contaminated food products and thus the first habitat inside the host is the gastrointestinal tract [3]. *Lm* is able to cross the intestinal barrier, subsequently enters the blood and lymph stream, and finally colonizes liver and spleen where it is primarily phagocytosed by resident macrophages [4]. [illegible] the secretion of two phospholipases, PlcA and PlcB, and the pore-forming toxin listeriolysin O (LLO) [5]. This results in the release of *Lm* into the cytoplasm where it starts to replicate and spread from one cell to another by hijacking the host cell actin cytoskeleton [6].

Macrophages play a central role in activating and finely balancing the pro- and anti-inflammatory pathways of the host immune system to mount effective host responses against invading pathogens. *In vivo*, macrophage differentiation is driven by GM- and M-CSF [7,8]. High levels of GM-CSF induce a pro-inflammatory phenotype resulting in high IL-12 secretion. These pro-inflammatory cells are also termed M1 macrophages. By contrast, M-CSF polarizes macrophages to an anti-inflammatory phenotype characterized by IL-10 secretion, which is referred to as [illegible]

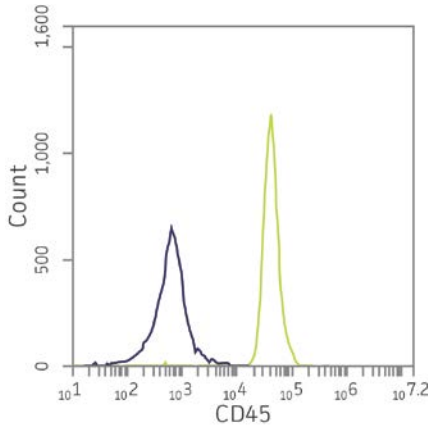


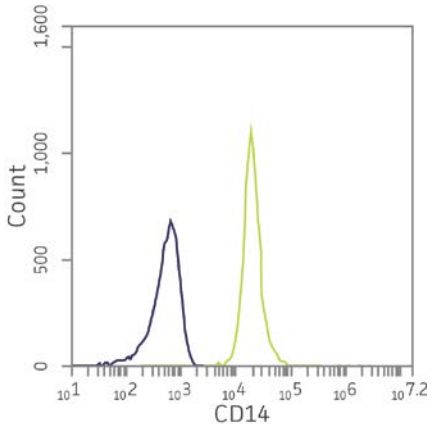
Neu C, *et al. PloS one* 8.6, 2013: e66898.

ATCC primary CD14+ monocytes

- Healthy human volunteer donors; IRB-approved informed consent
- Adult, non-pregnant
- Cryopreserved at P0; Purity: ≥ 90% CD14+
- Age, gender, ethnicity, and blood type on CoA

ATCC® No.	Tissue	Type	Size
PCS-800-010	Peripheral blood	Monocytes (CD14+)	≥ 50 x 10^6





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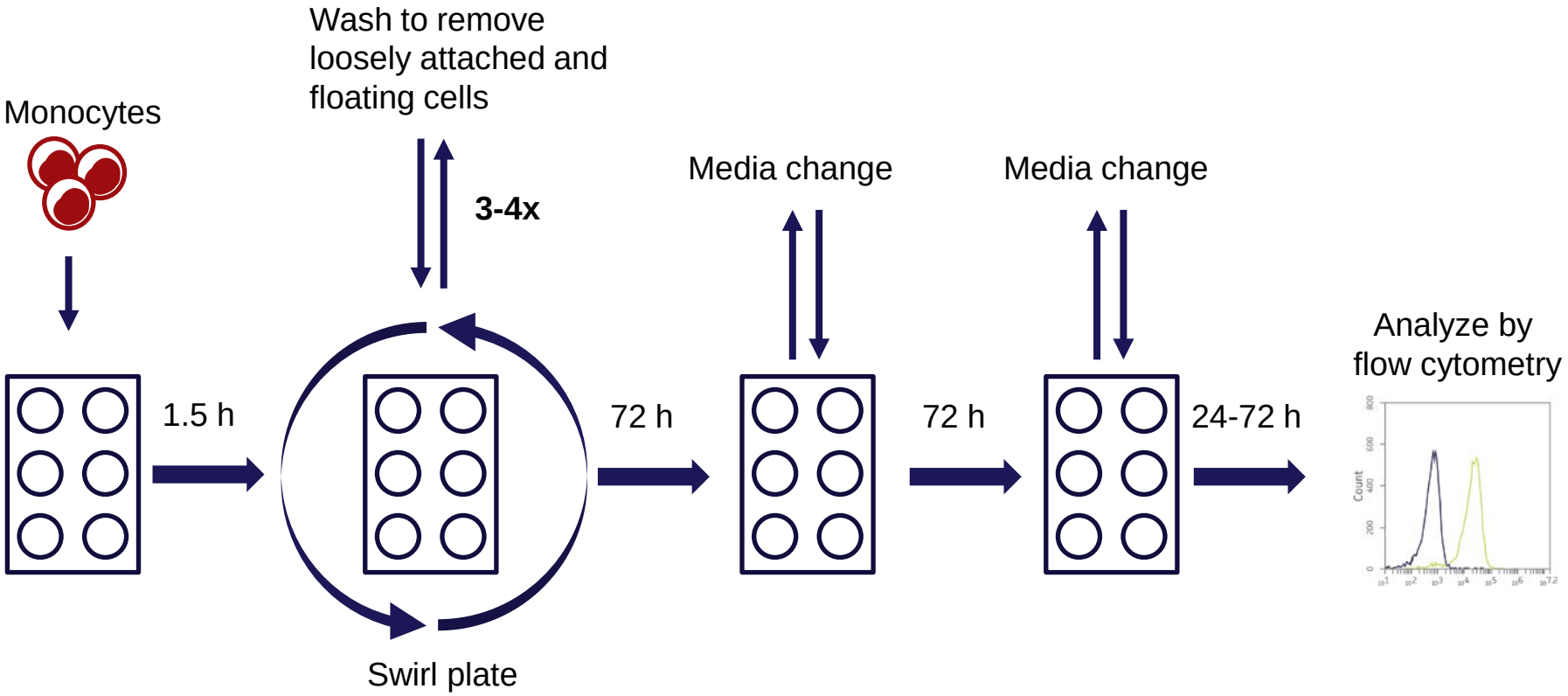
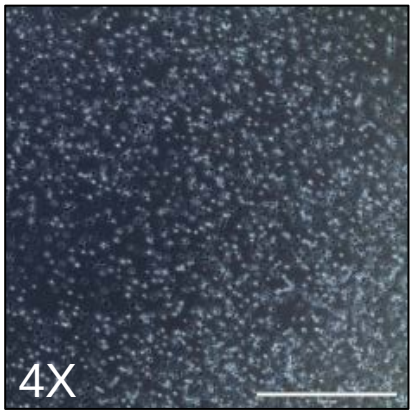
Macrophage differentiation protocol



For detailed differentiation protocols see the ATCC website

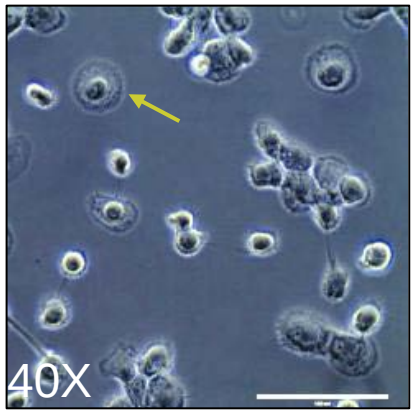
Generation of CD68+ macrophages from monocytes

Day 1

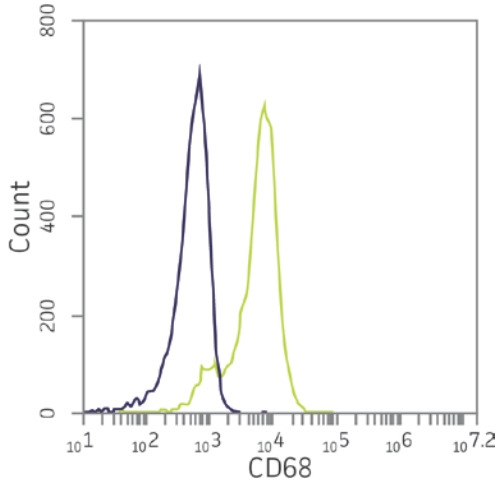


Monocytes

Day 9

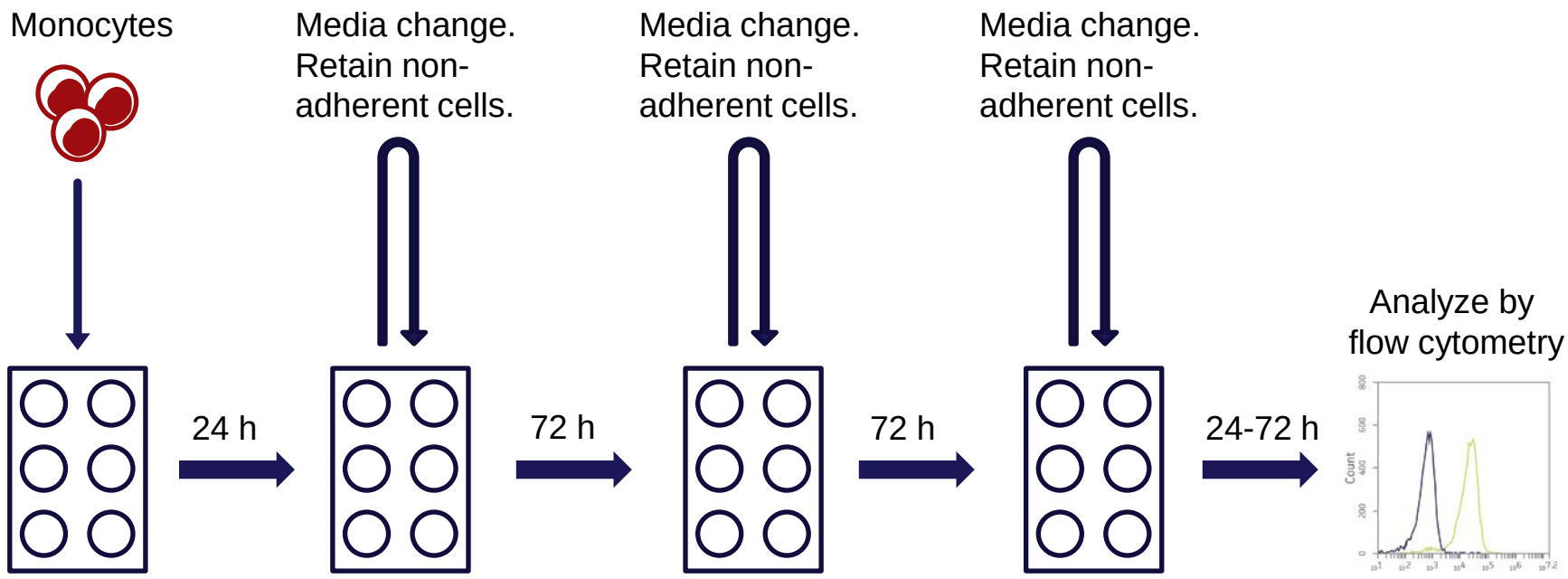


Mature macrophage



- Macrophages differentiated from CD14+ monocytes for 9 days.
- Morphology characteristic of type M1-polarized macrophages.
- > 80% of cells were CD68+

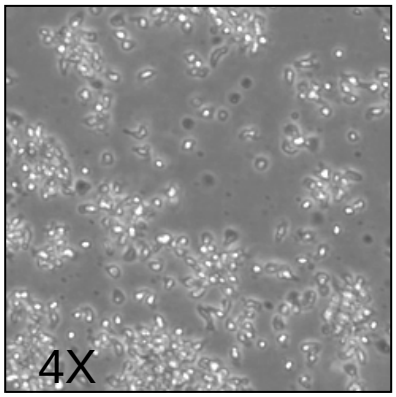
Dendritic cell differentiation protocol



For detailed differentiation protocols see the ATCC website

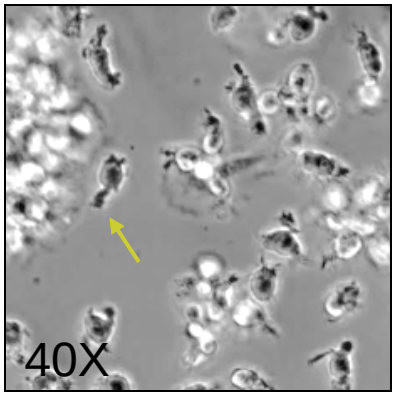
Generation of CD83+ DCs from monocytes

Day 1



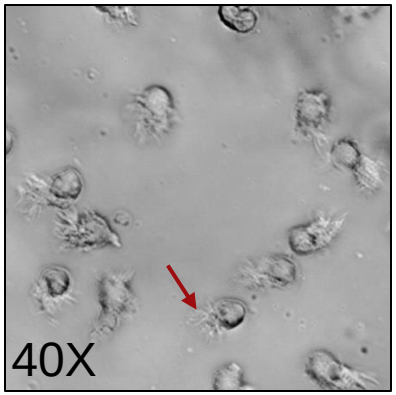
Monocytes

Day 6



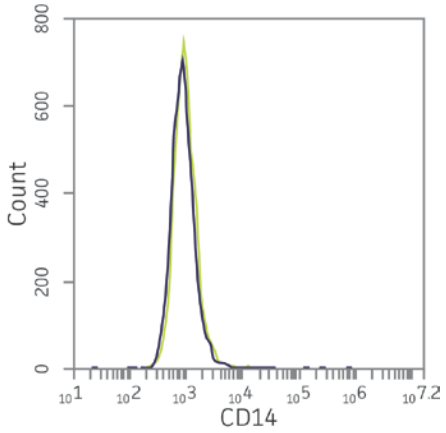
Immature DCs

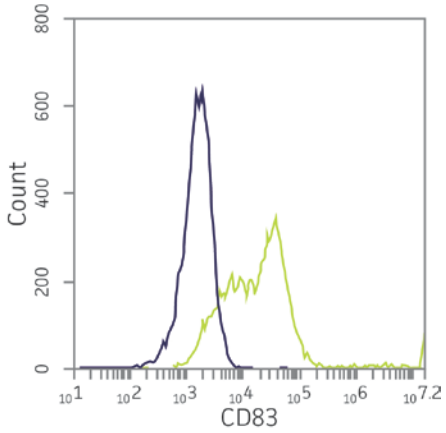
Day 8



Mature DCs

- After 8 days differentiation 95% of non-adherent cells were CD14-
- >70% of cells were CD83+





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Summary

ATCC offers a variety of well-characterized and functionally validated primary hematopoietic cell types

- CD34+ HSPCs
- BMMCs and PBMCs
- CD14+ monocytes

ATCC provides hematopoietic lineage-specific differentiation protocols

- Erythroid
- Megakaryocyte
- Non-specific myeloid
- Dendritic
- Macrophage

ATCC hematopoietic cells are useful in numerous areas of research

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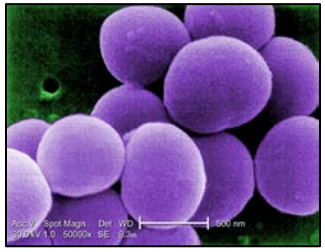
March 5, 2015
10:00 AM, 3:00 PM ET
David Clawson, *Lead Biologist*, ATCC
Enhancing vector-borne research with biological and molecular standards



March 19, 2015
10:00 AM, 3:00 PM ET
Scott Sutton, Ph.D., *Principal*, Microbiology Network and
Tracy Vandenbroek, *Product Line Business Manager*, ATCC
Microbiology quality control as described in the Compendia

Thank you for joining today!
Please send additional questions to tech@atcc.org

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