Discovering ATCC Primary Immunology Cells - Model Systems to Study the Immune and Cardiovascular Systems

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Established partner to global researchers and scientists

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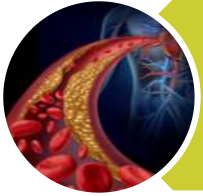
Outline



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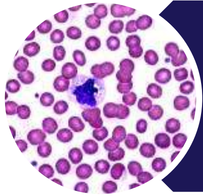
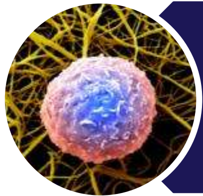
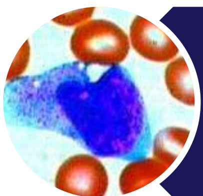


CD34+ hematopoietic stem & progenitor cells



Mononuclear cells



CD14+ monocytes

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Blood cells

Blood is comprised of a heterogeneous population of specialized cells

- **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

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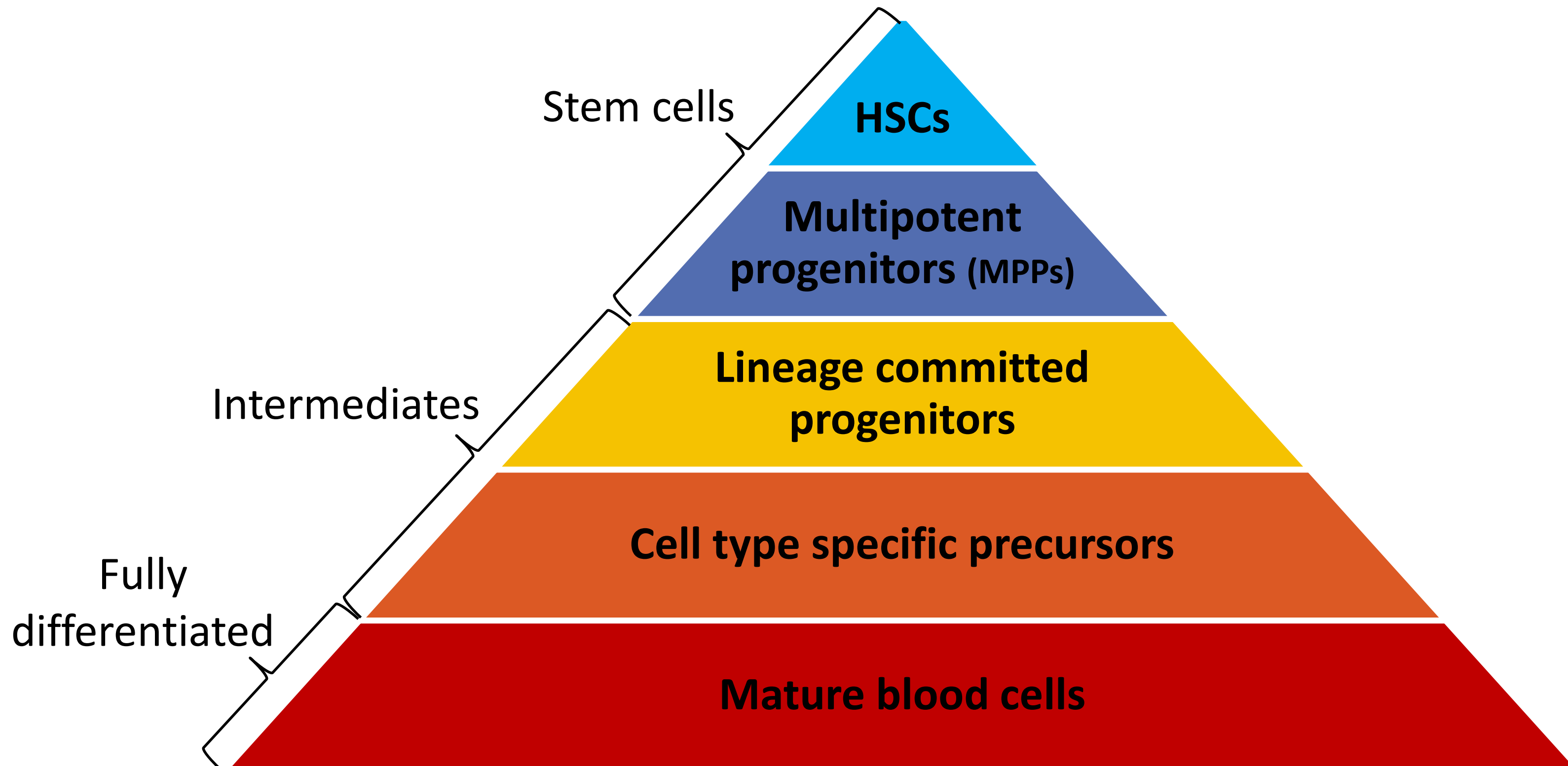
Hematopoiesis: A hierarchal system



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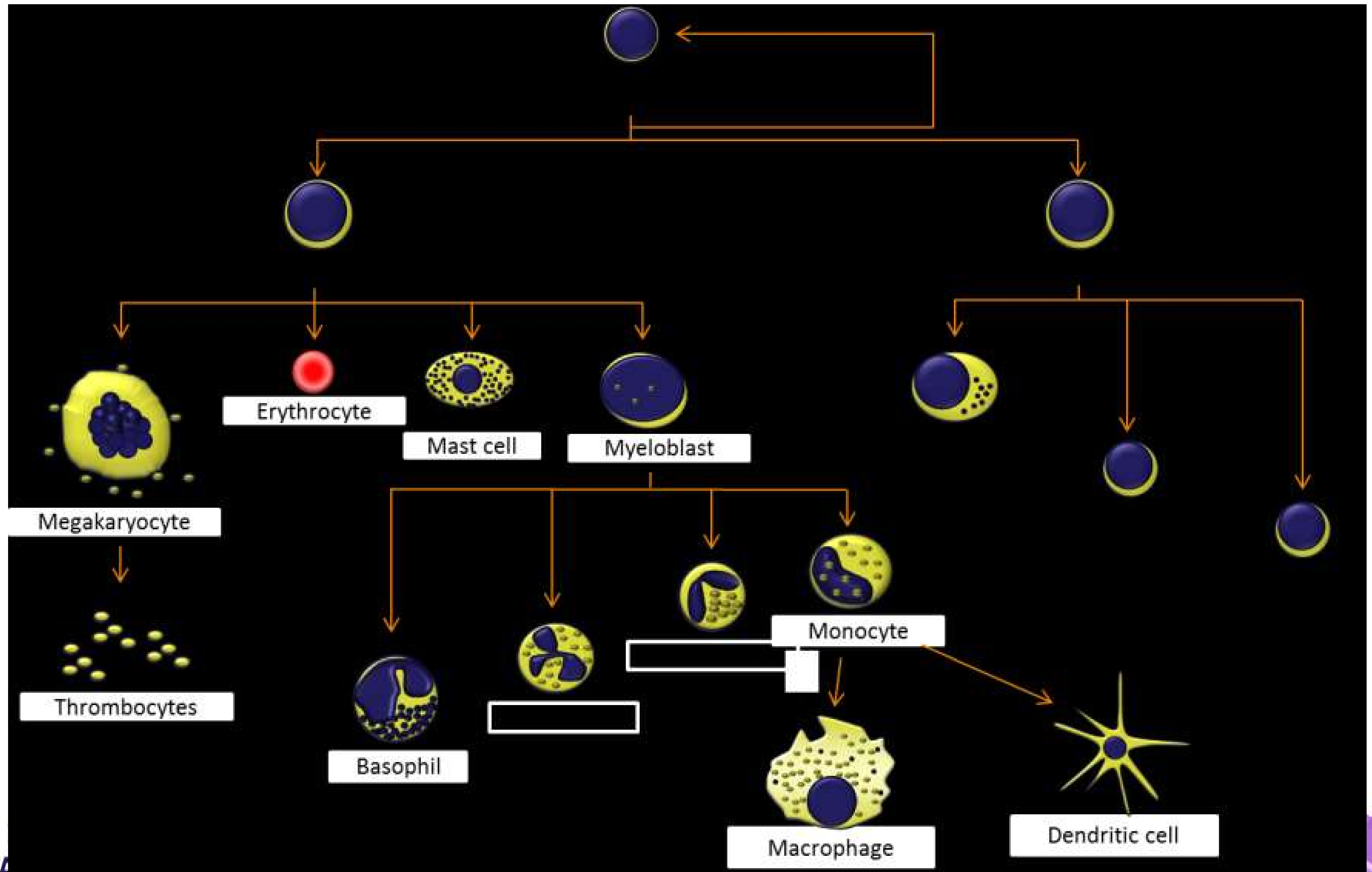
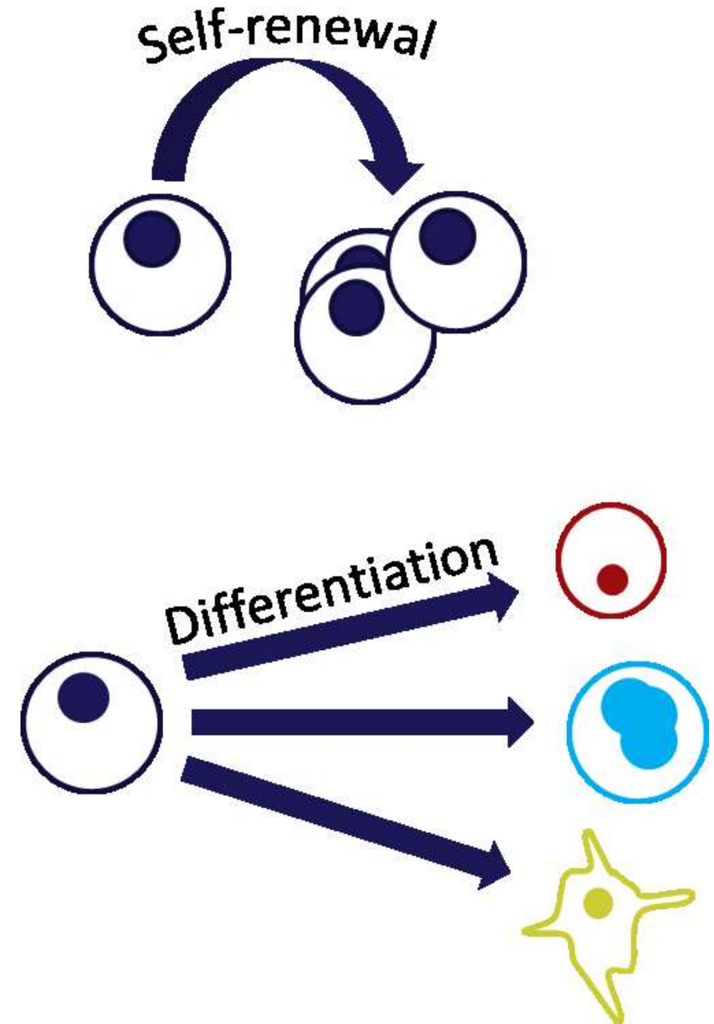
Hematopoietic cell fate and lineage



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



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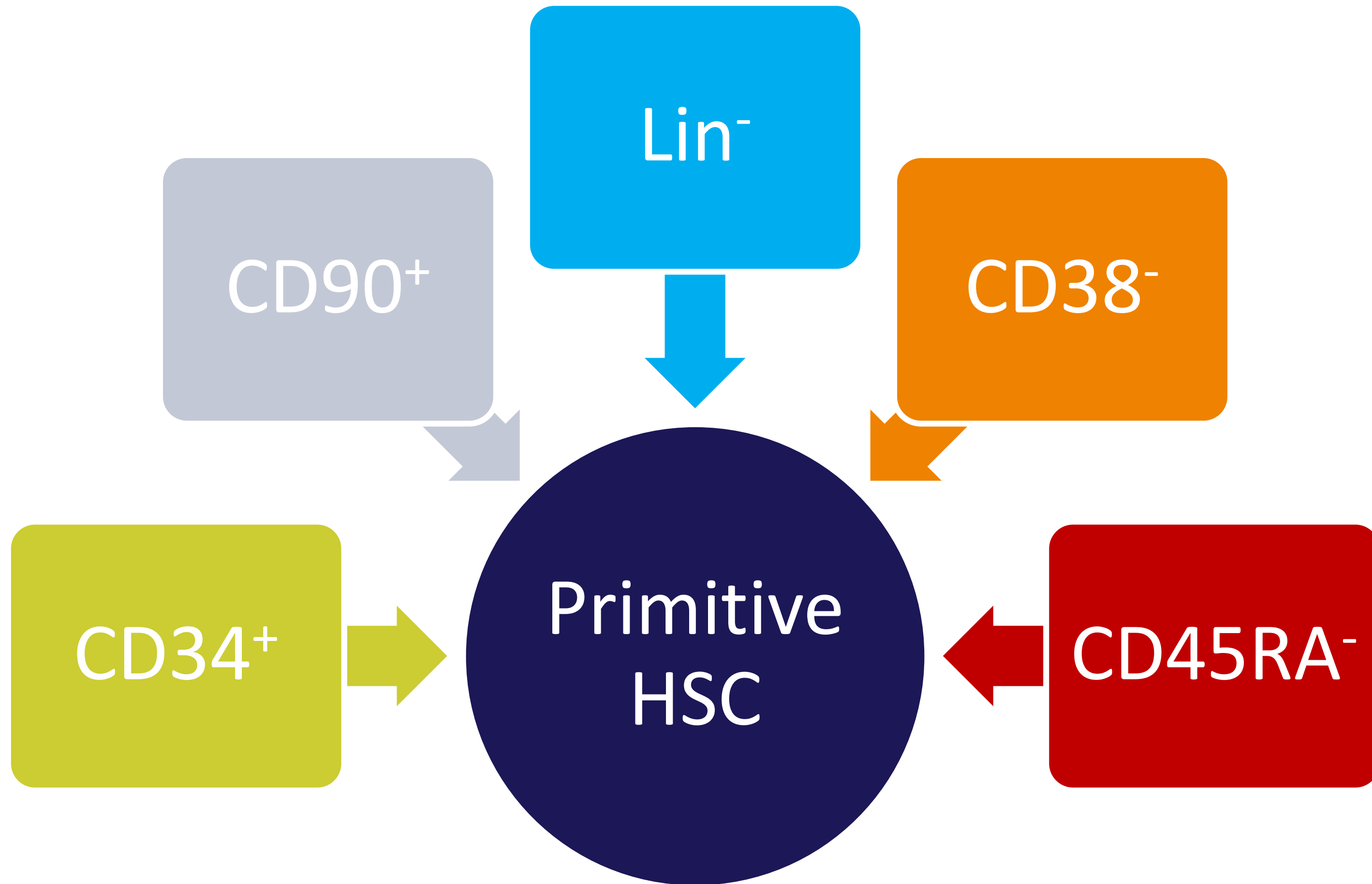
Hematopoietic stem cells: Markers



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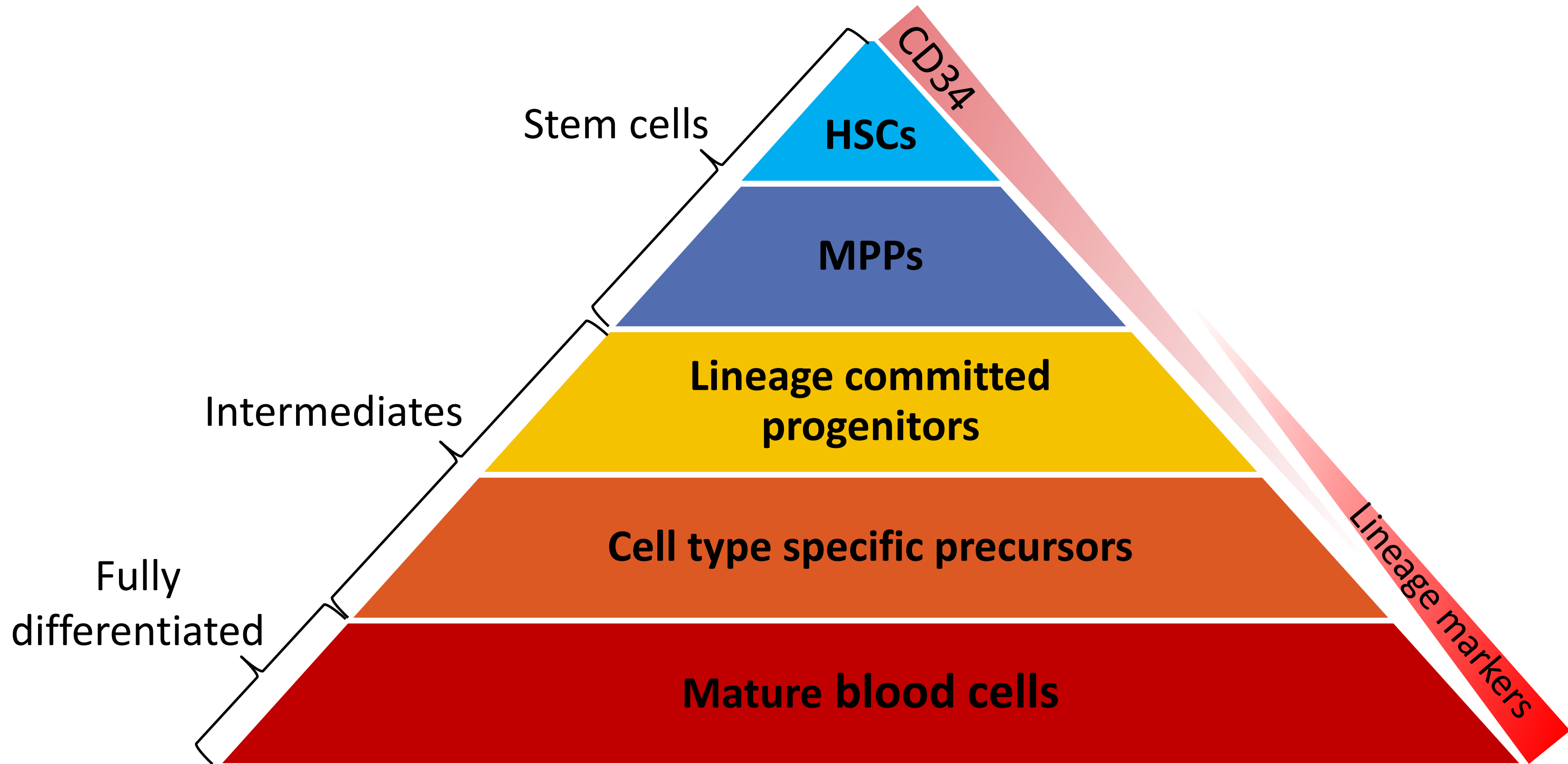
CD34+ cells are a mixed population of stem and progenitor cells



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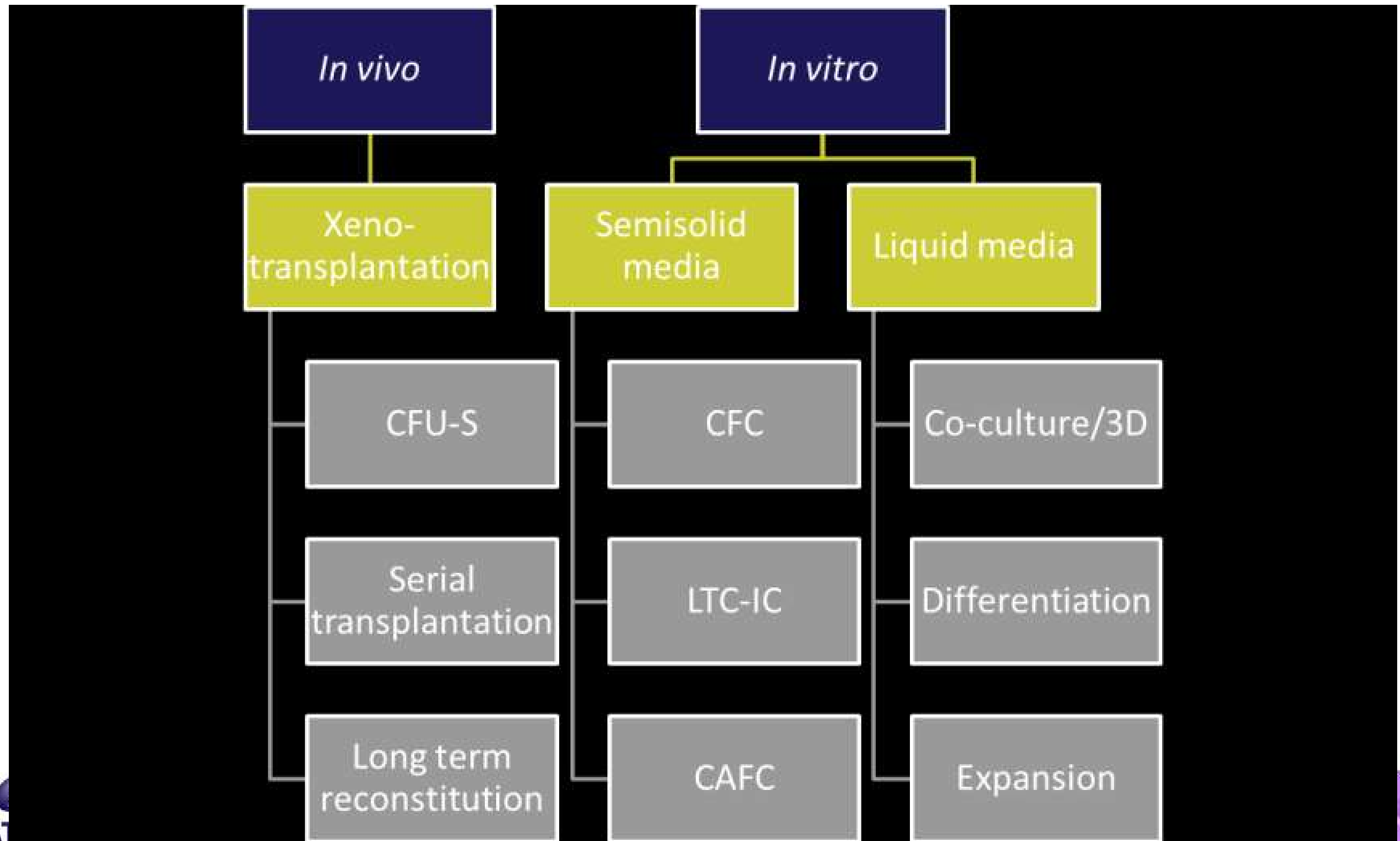
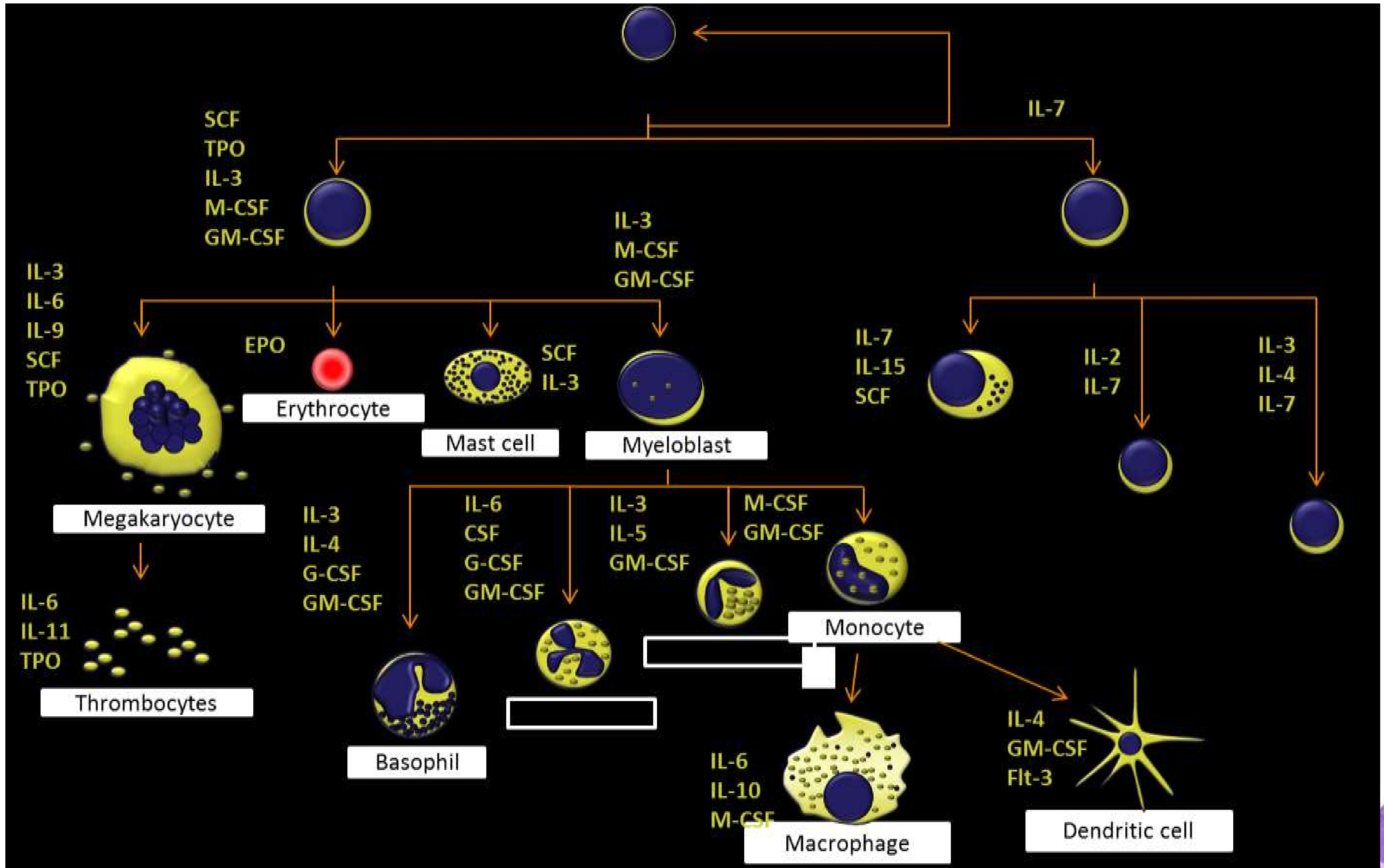
Assays to study hematopoietic stem and progenitor cells

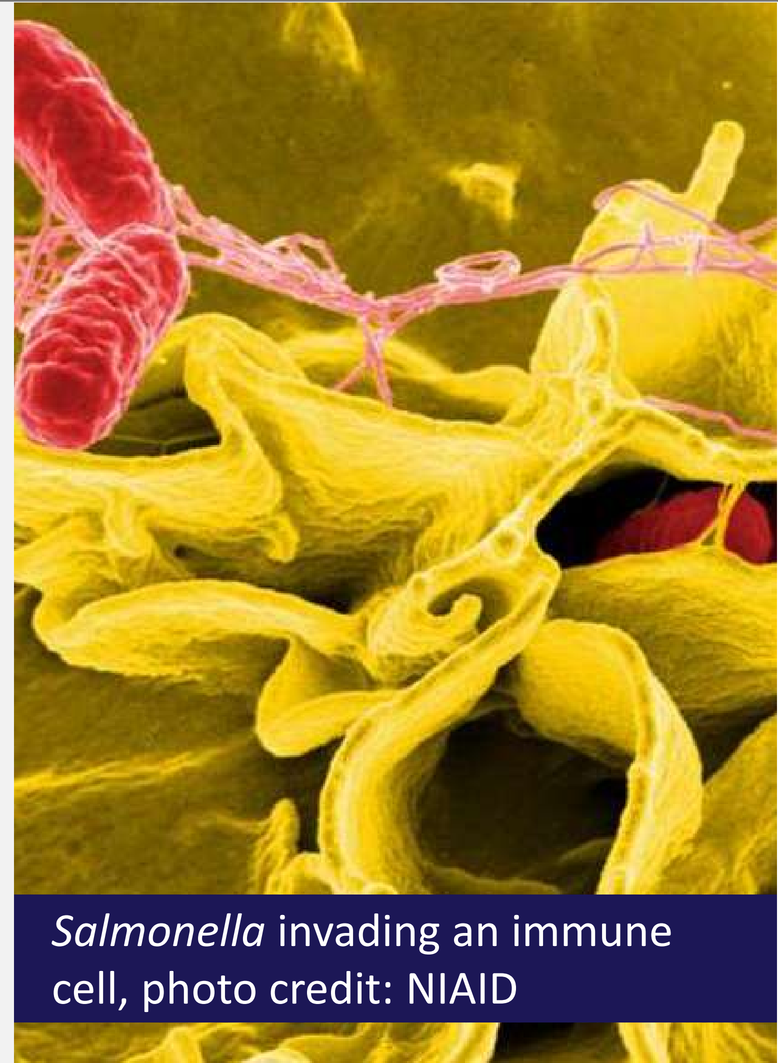


Cytokines influence cell fate and lineage *in vitro*



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



Salmonella invading an immune cell, photo credit: NIAID

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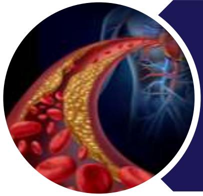
Outline



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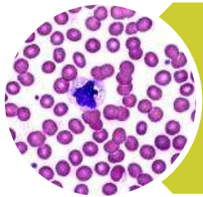
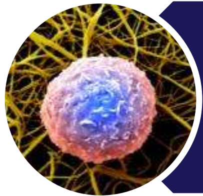
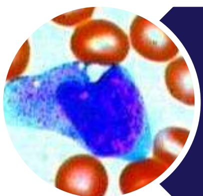


CD34+ hematopoietic stem & progenitor cells



Mononuclear cells



CD14+ monocytes

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Primary CD34+ HSPCs

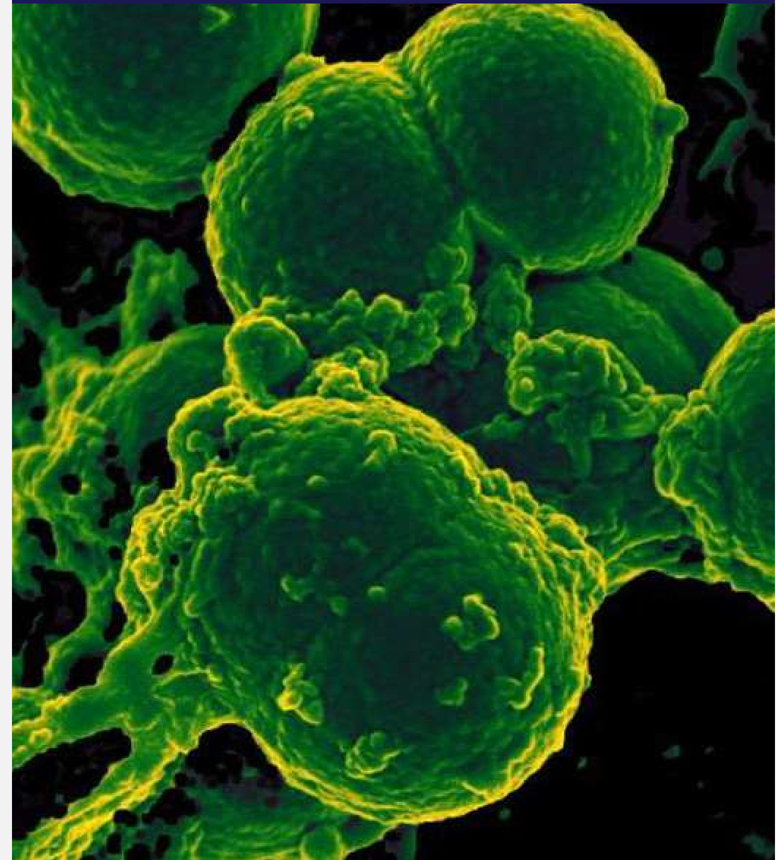
Applications

- *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

Lymphocytes ingesting bacteria, photo credit: National Institute of Allergy and Infectious Diseases, NIH



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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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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 xenogenic 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 8(7): e69572. doi:10.1371/journal.pone.0069572

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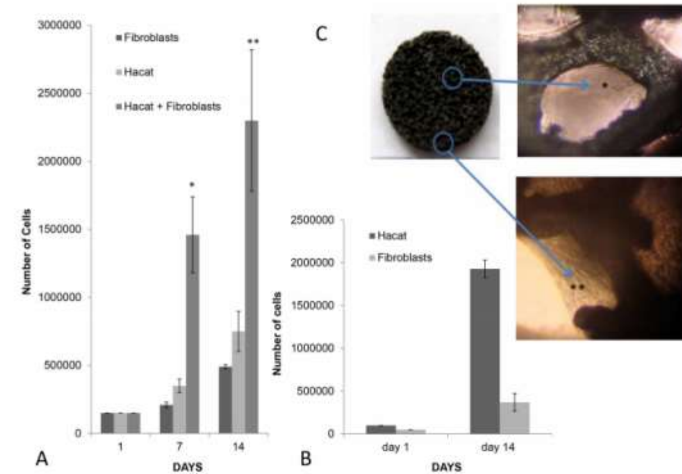
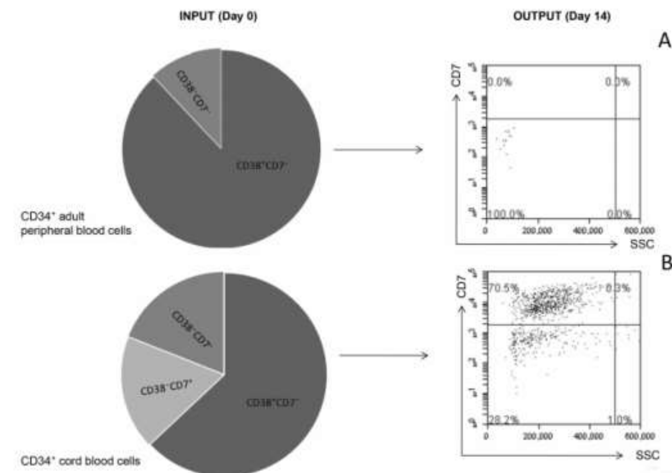
Introduction

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-DL1) [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 [6]. 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 [8].





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

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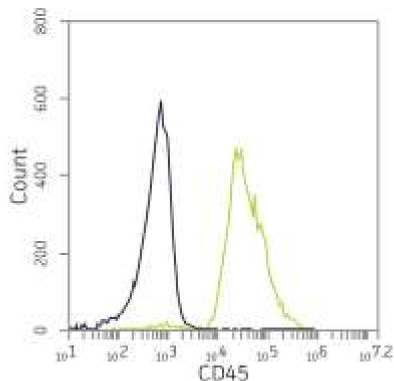
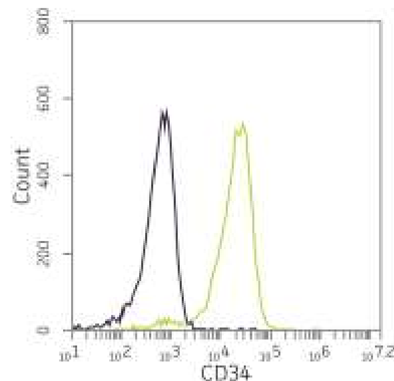
ATCC primary CD34+ HSPCs

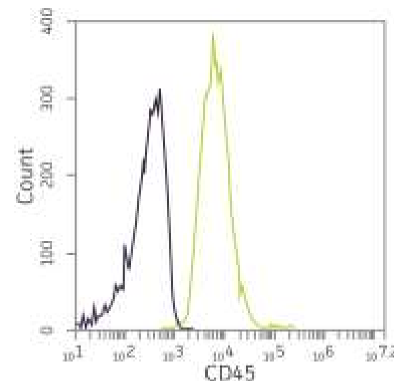
- Healthy human volunteer donors; IRB-approved informed consent
- Adult, non-pregnant (excluding cord blood)
- Cryopreserved at P0; Purity: ≥ 90% CD34+
- Age, gender, ethnicity, and blood type on CoA

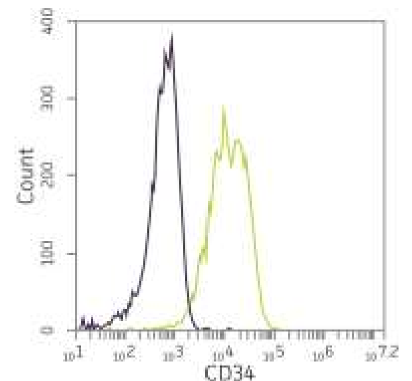
ATCC® No.	Tissue	Type	Size
PCS-800-012™	Bone marrow	Hematopoietic stem/progenitor cells (CD34+)	≥ 0.5 x 10^6
PCS-800-014™	Cord blood	Hematopoietic stem/progenitor cells (CD34+)	≥ 0.5 x 10^6





Bone marrow





Cord blood

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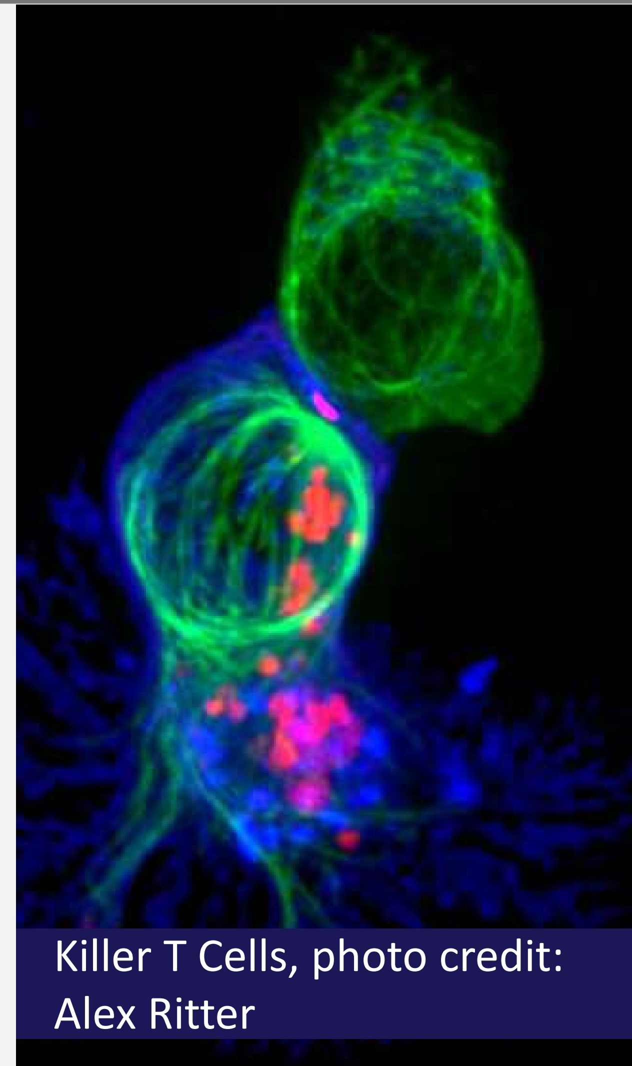
CD34+ HSPC lineage directed expansion and differentiation

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



Killer T Cells, photo credit: Alex Ritter

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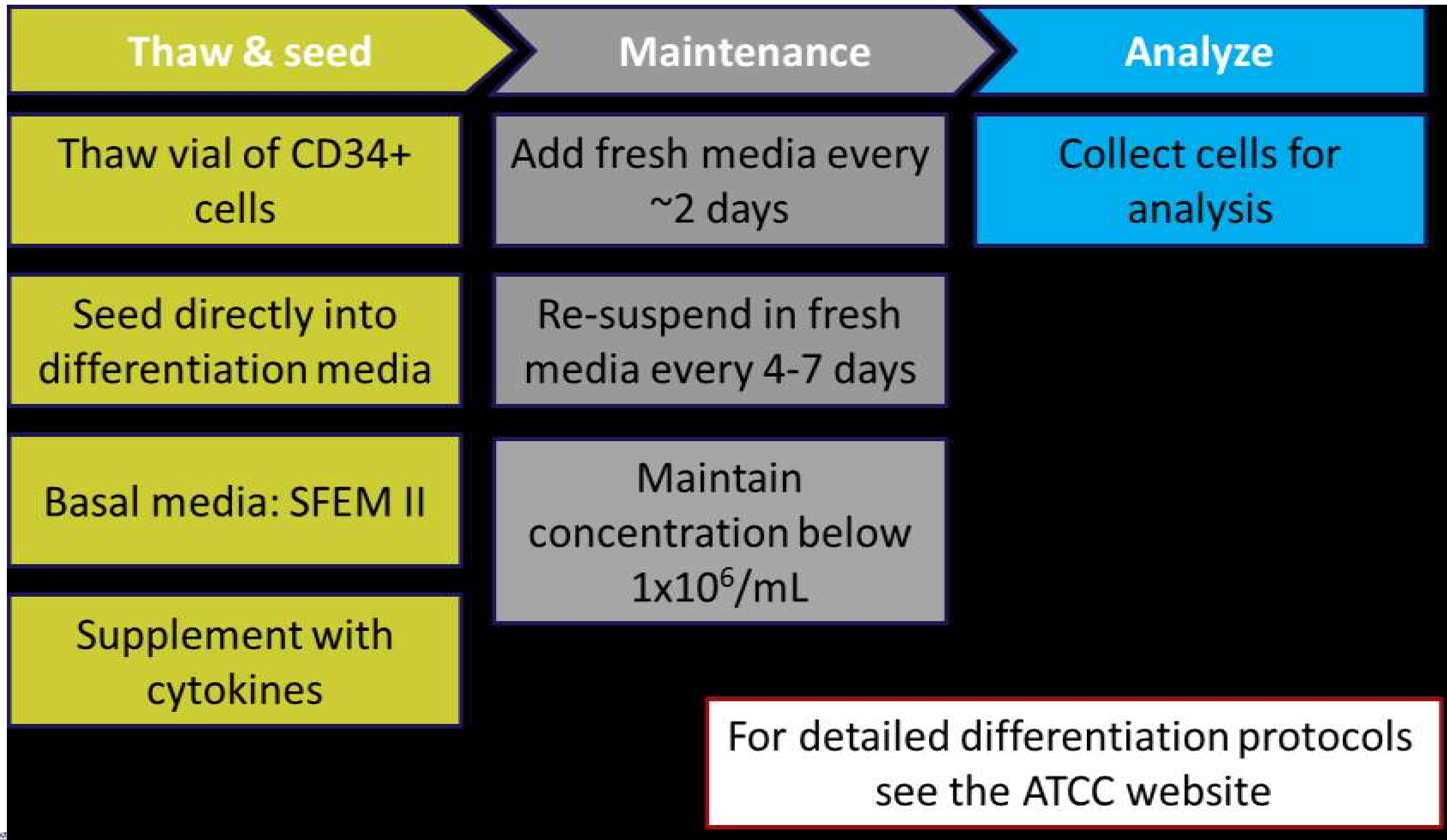
General differentiation protocol workflow



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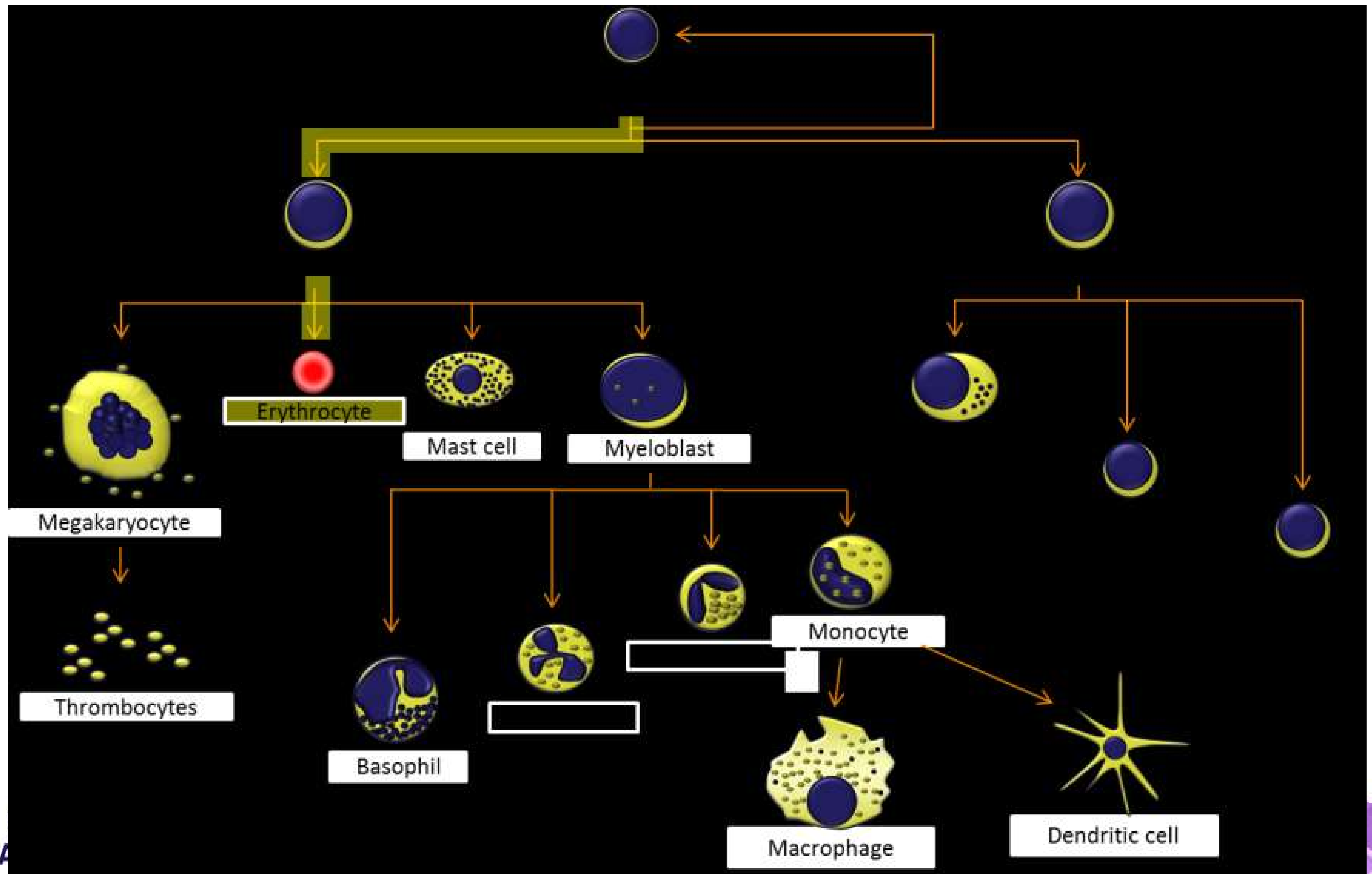
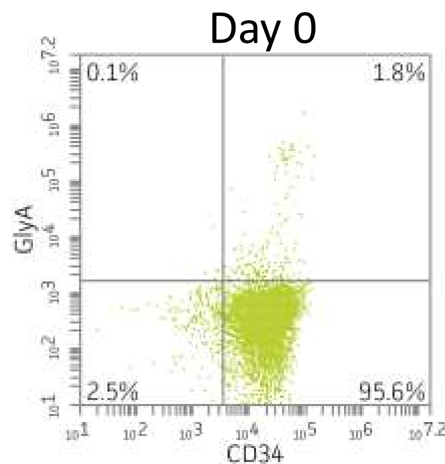
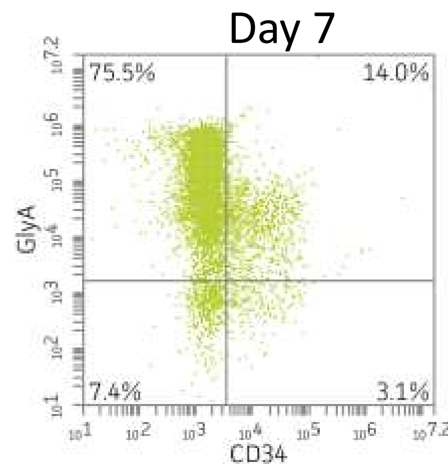
Erythroid differentiation and expansion

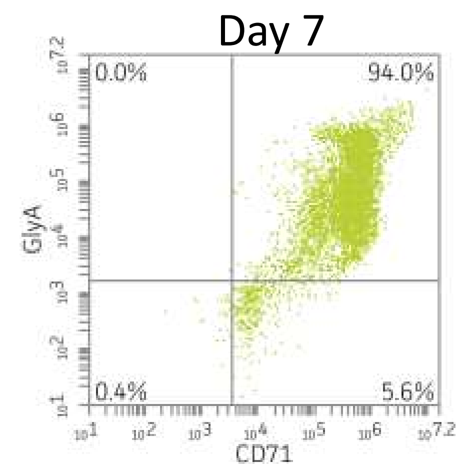


Expression of erythroid lineage markers on differentiated BM and CB CD34+ cells

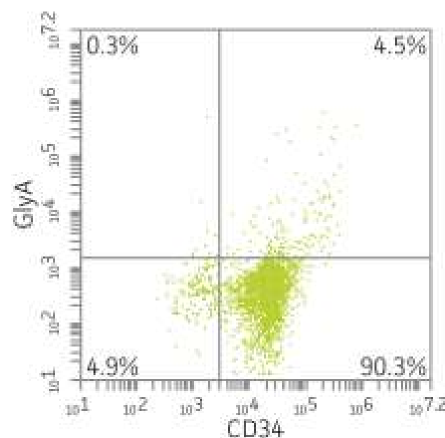
Bone Marrow CD34+ HSPCs

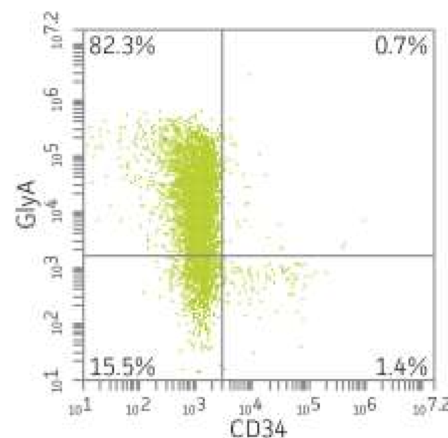


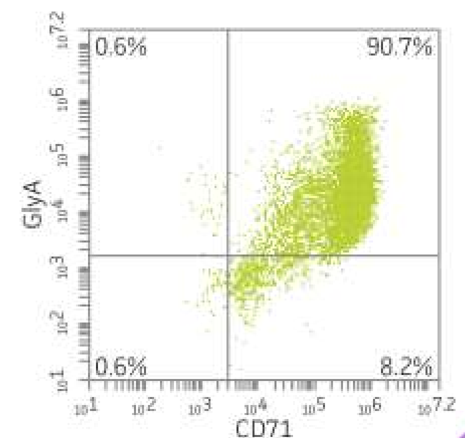




Cord Blood CD34+ HSPCs







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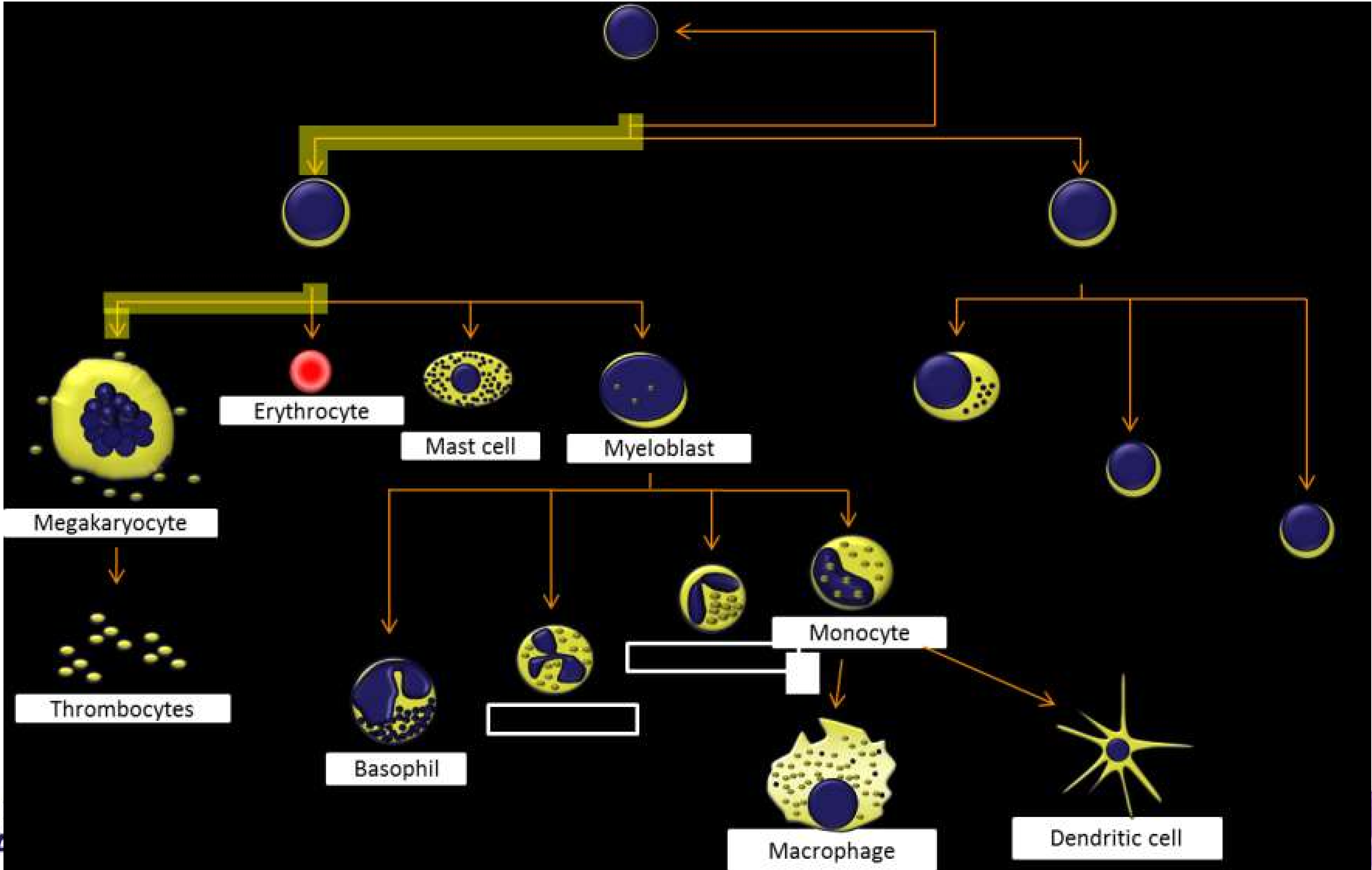
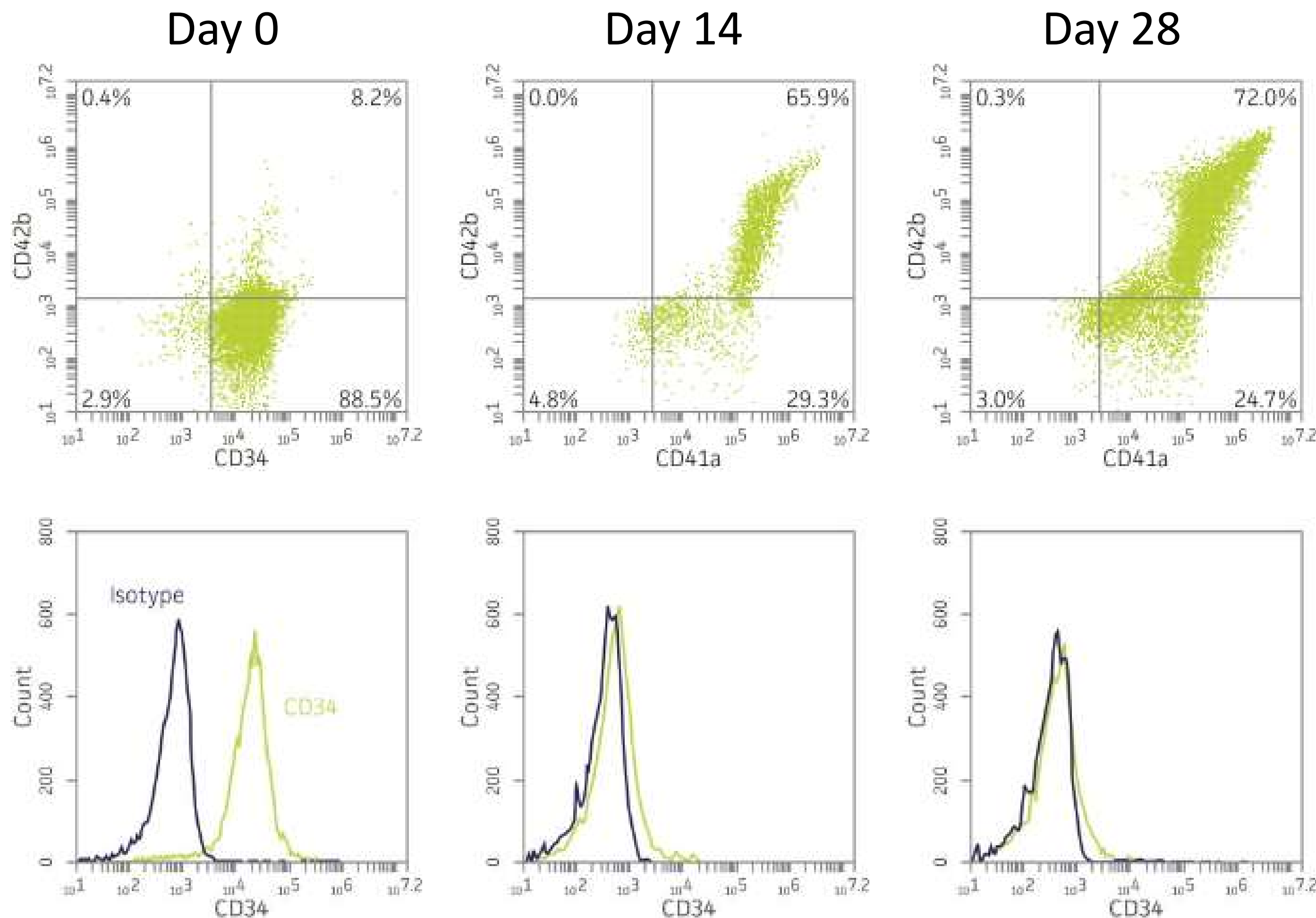
Megakaryocyte differentiation and expansion



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



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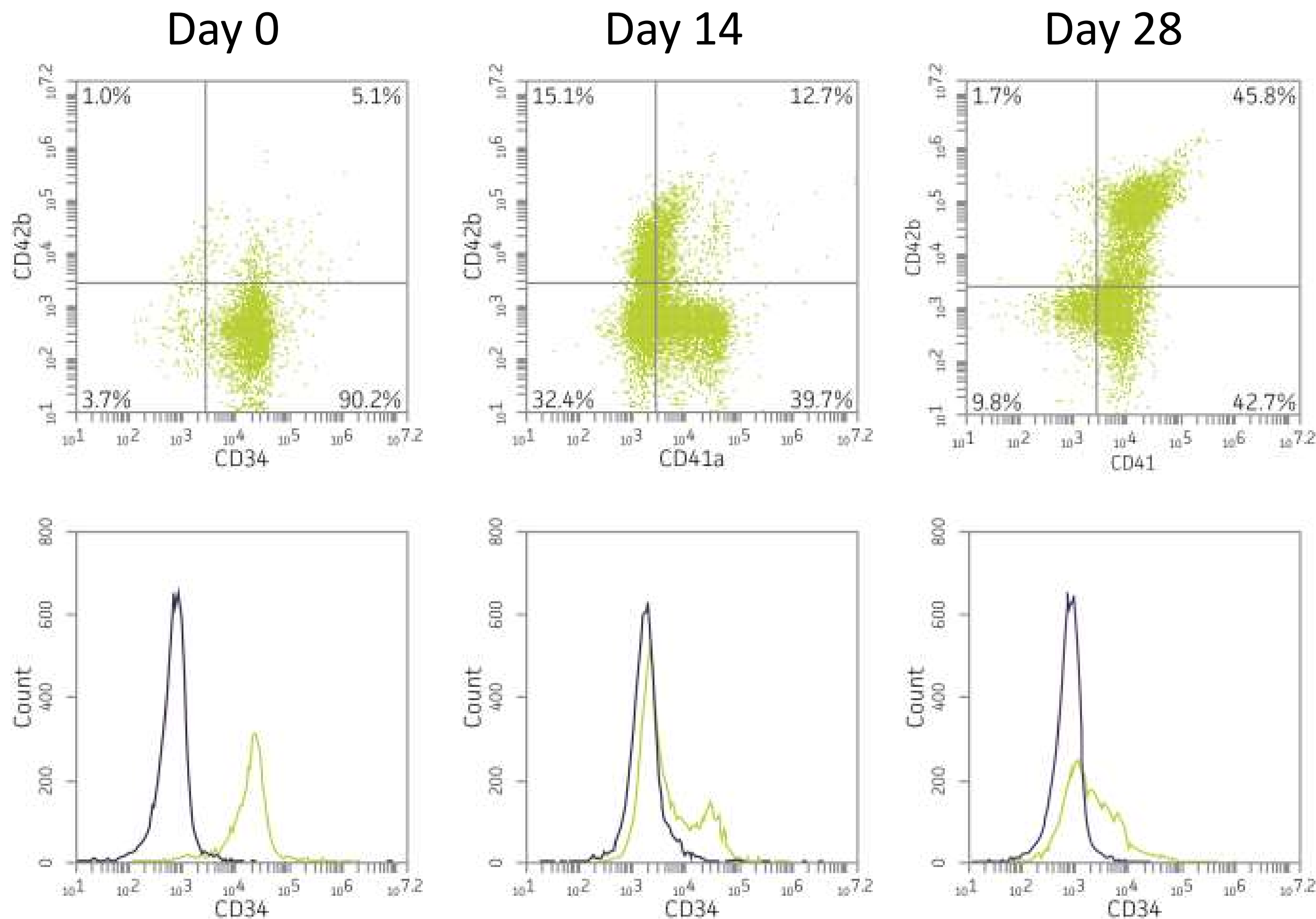
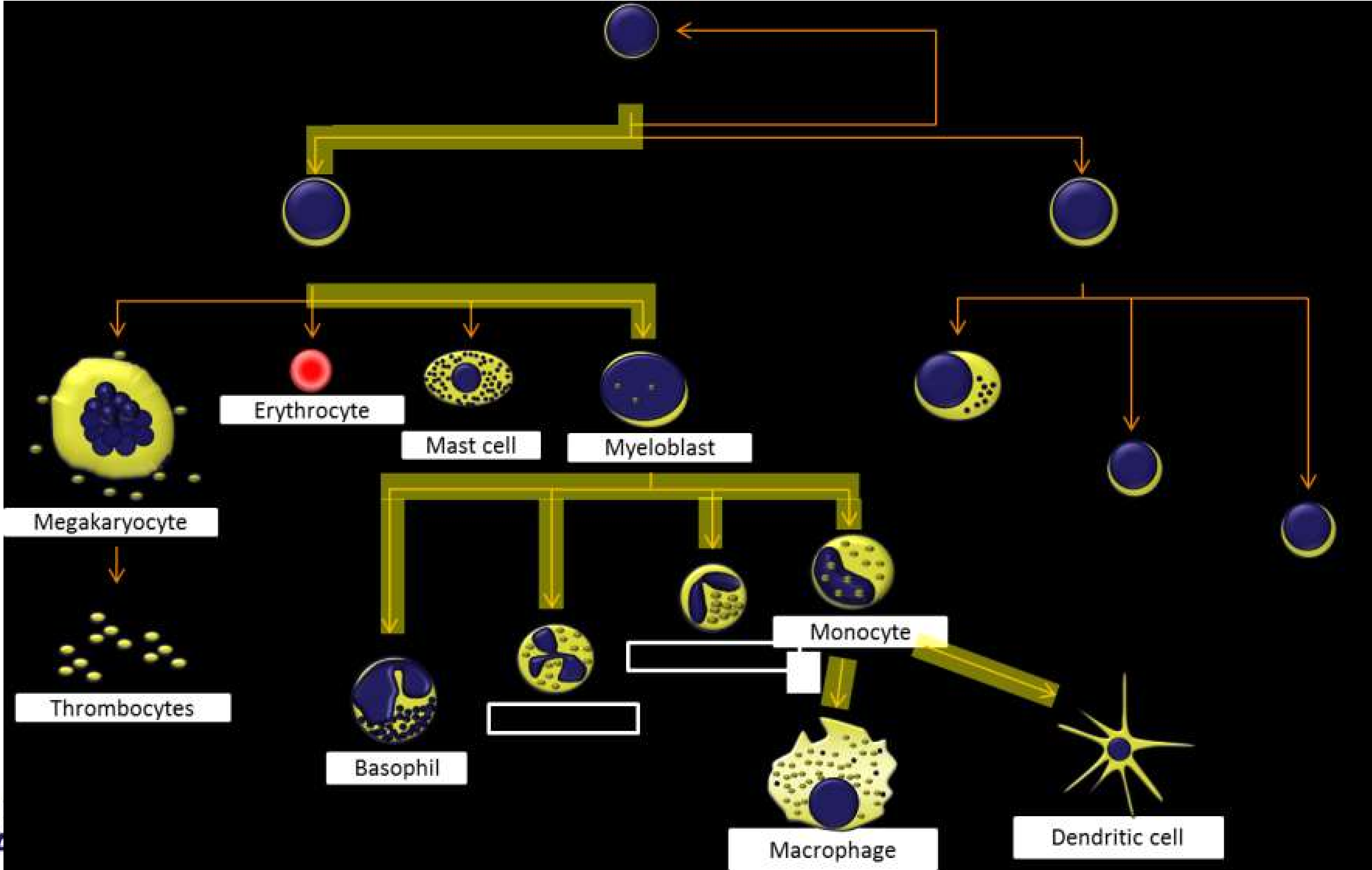
Expression of megakaryocyte lineage markers on differentiated cord blood CD34+ HPSCs



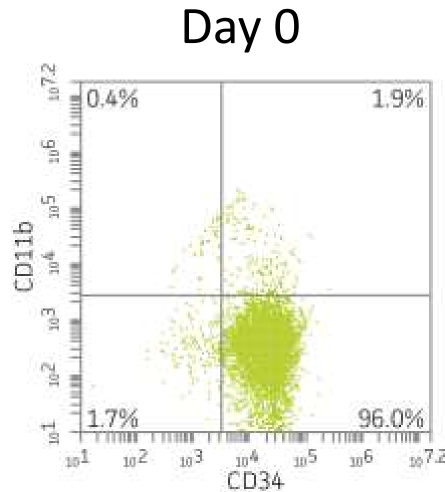
Pan-myeloid differentiation and expansion

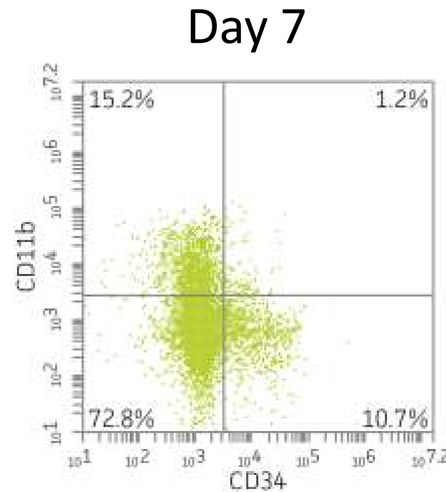


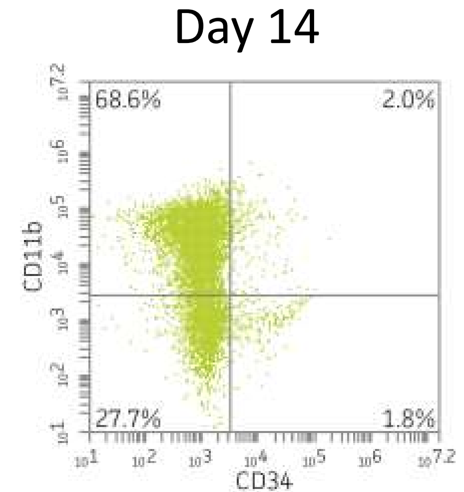
Expression of pan-myeloid lineage markers on differentiated BM and CB CD34+ HPSCs

Day 0 | Day 7 | Day 14

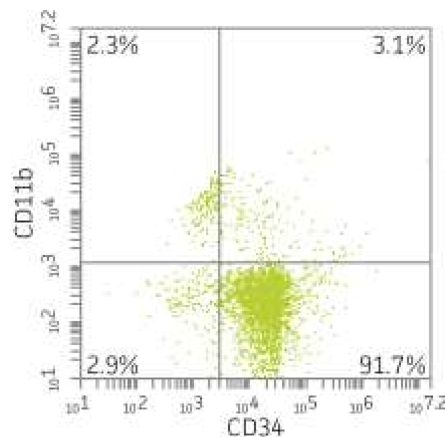
Bone Marrow CD34+ HSPCs

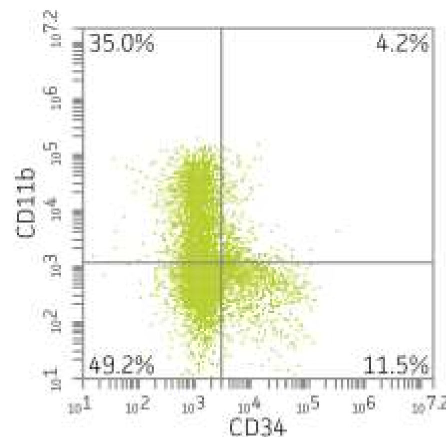


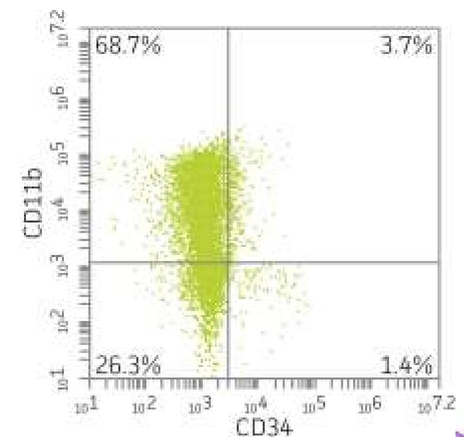




Cord Blood CD34+ HSPCs







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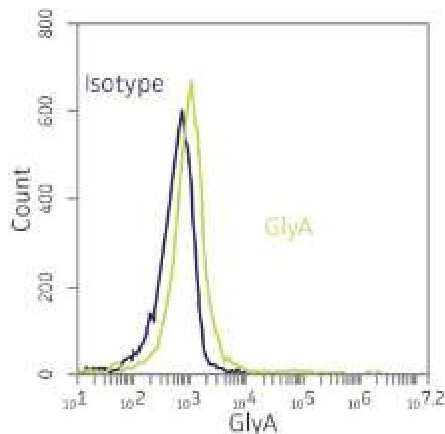
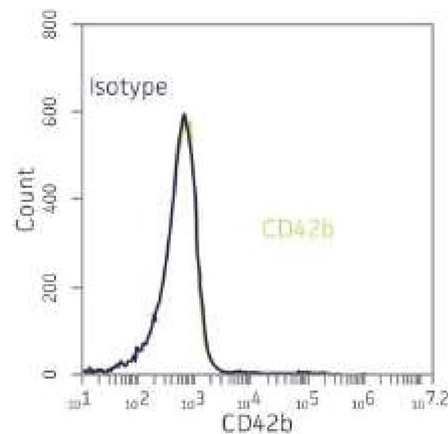
Specificity of pan-myeloid-directed differentiation of CD34+ HPSCs

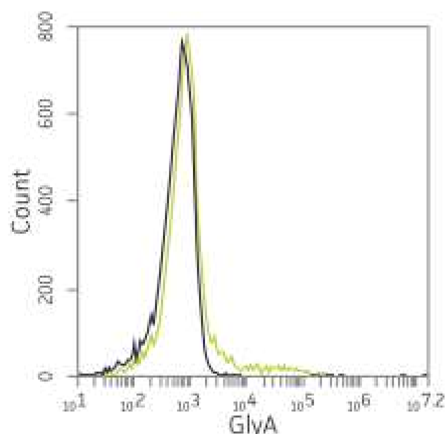
Day 14 | Day 14

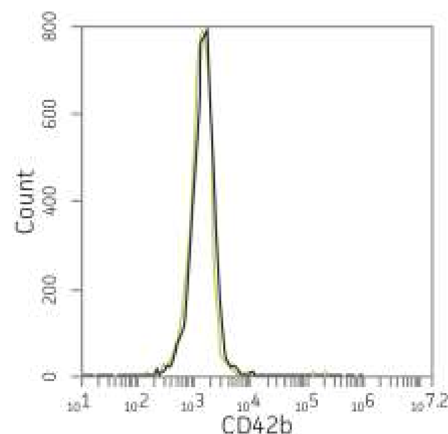
Bone Marrow CD34+ HSPCs





Cord Blood CD34+ HSPCs





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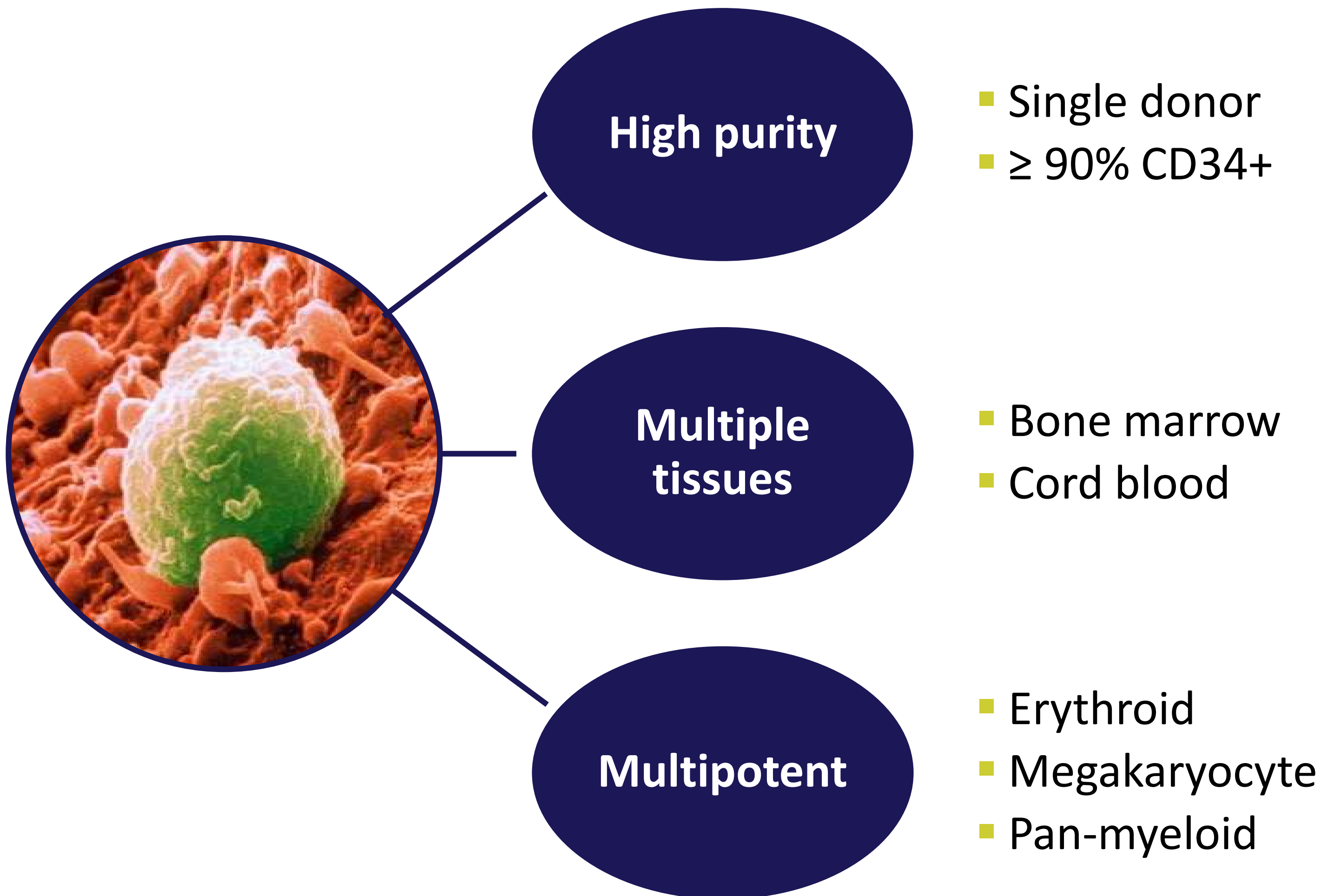
ATCC primary CD34+ HSPCs: Summary



- Single donor
- ≥ 90% CD34+

- Bone marrow
- Cord blood

- Erythroid
- Megakaryocyte
- Pan-myeloid

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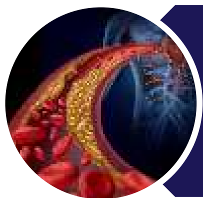
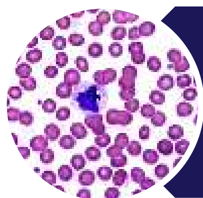
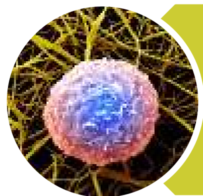
Outline



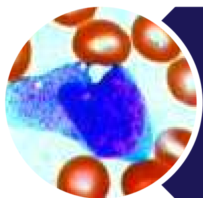
Background



CD34+ hematopoietic stem & progenitor cells



Mononuclear cells



CD14+ monocytes

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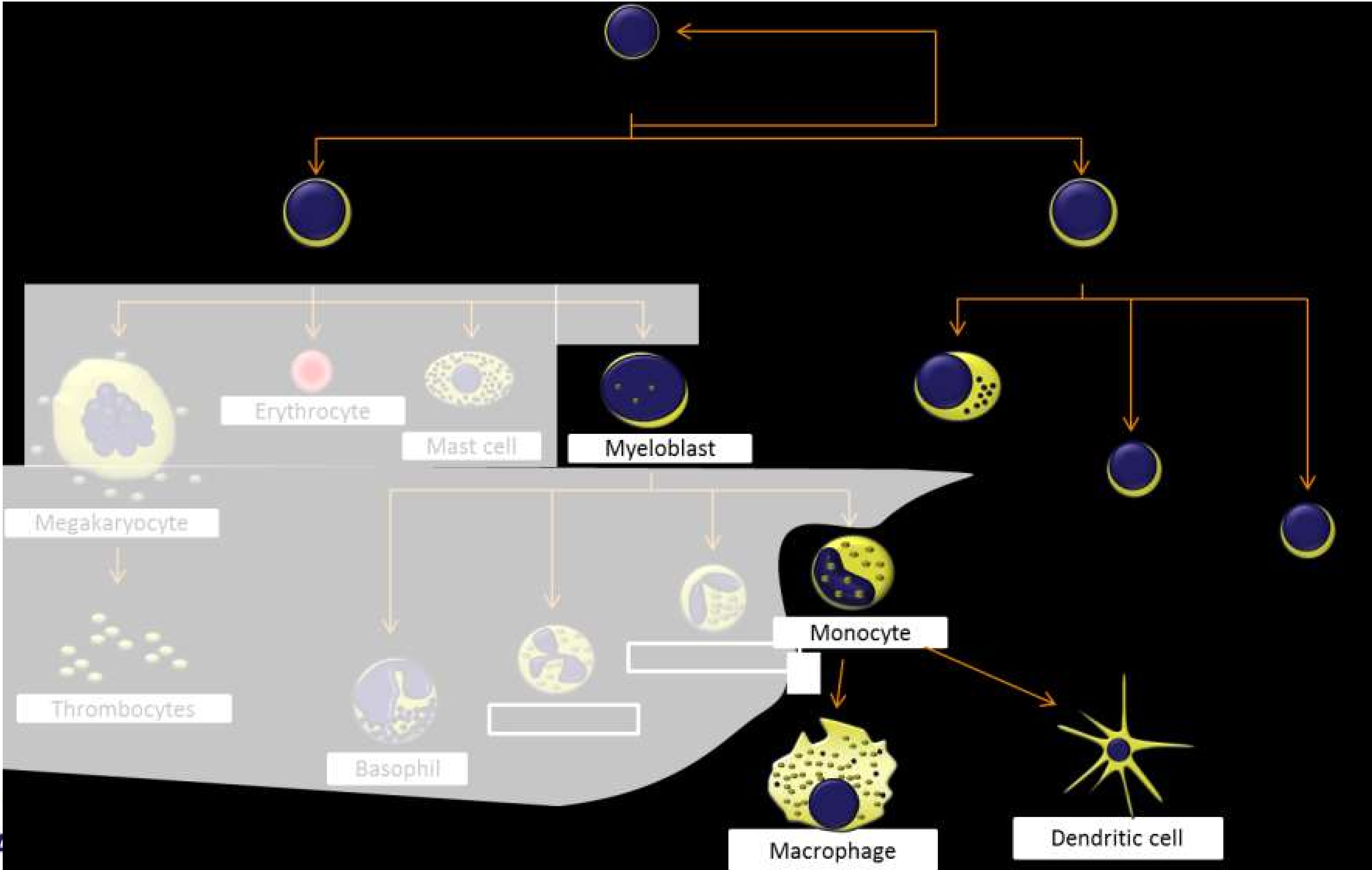
Mononuclear cells



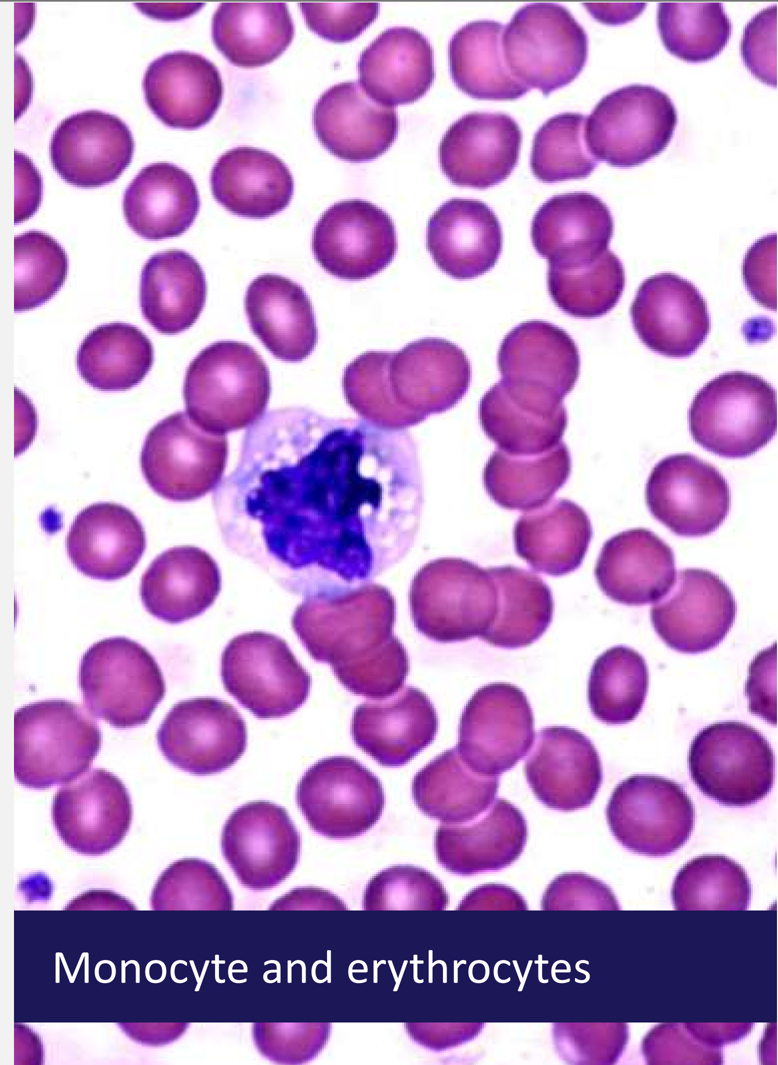
Primary mononuclear cells

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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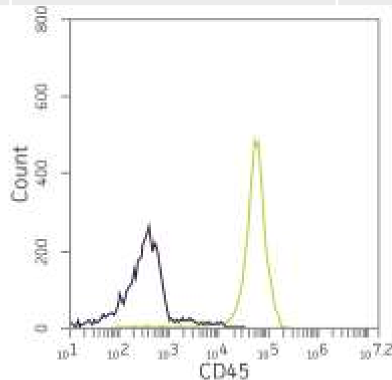
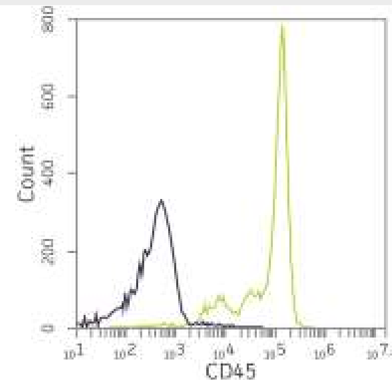
Primary mononuclear cells

- 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%

ATCC (American Type Culture Collection)
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or contact your local distributor

- Page 1 of 2 -

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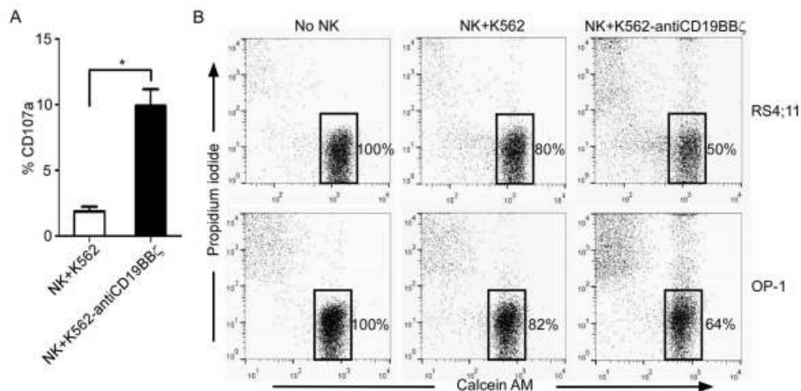
Non-viral method increases natural killer cells' anti-cancer cell cytotoxicity

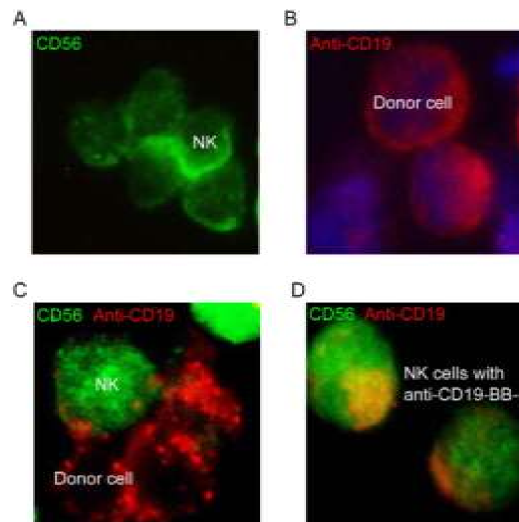
Enhanced Cytotoxicity of Natural Killer Cells following the Acquisition of Chimeric Antigen Receptors through Trogocytosis

Fu-Nan Cho, Tsung-Hsien Chang, Chih-Wen Shu, Ming-Chin Ko, Shuen-Kuei Liao, Kang-Hsi Wu, Ming-Sun Yu, Shyh-Jer Lin, Ying-Chung Hong, Chien-Hsun Chen, Chien-Hui Hung, Yu-Hsiang Chang

Abstract

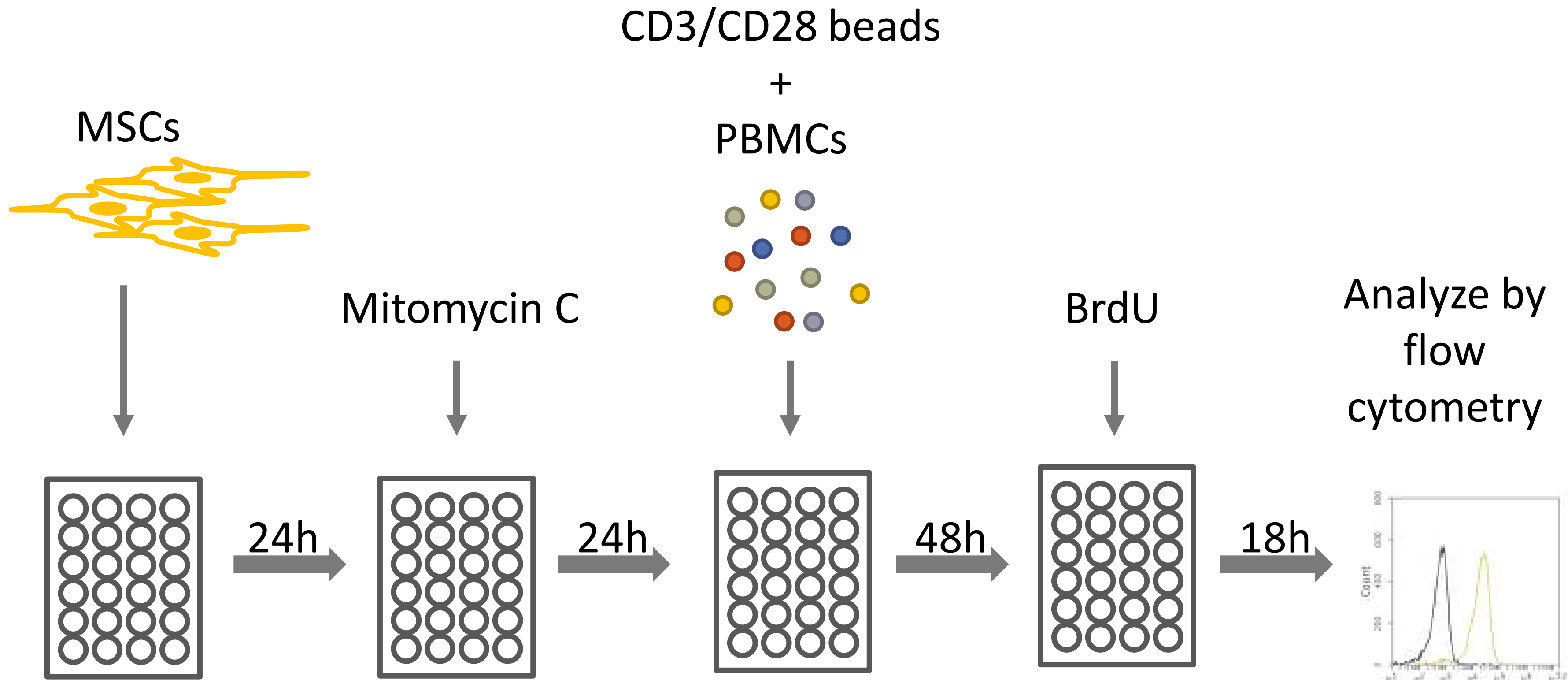
Introduction





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

Immunosuppressive assay using PBMCs



For detailed differentiation protocols see the ATCC website

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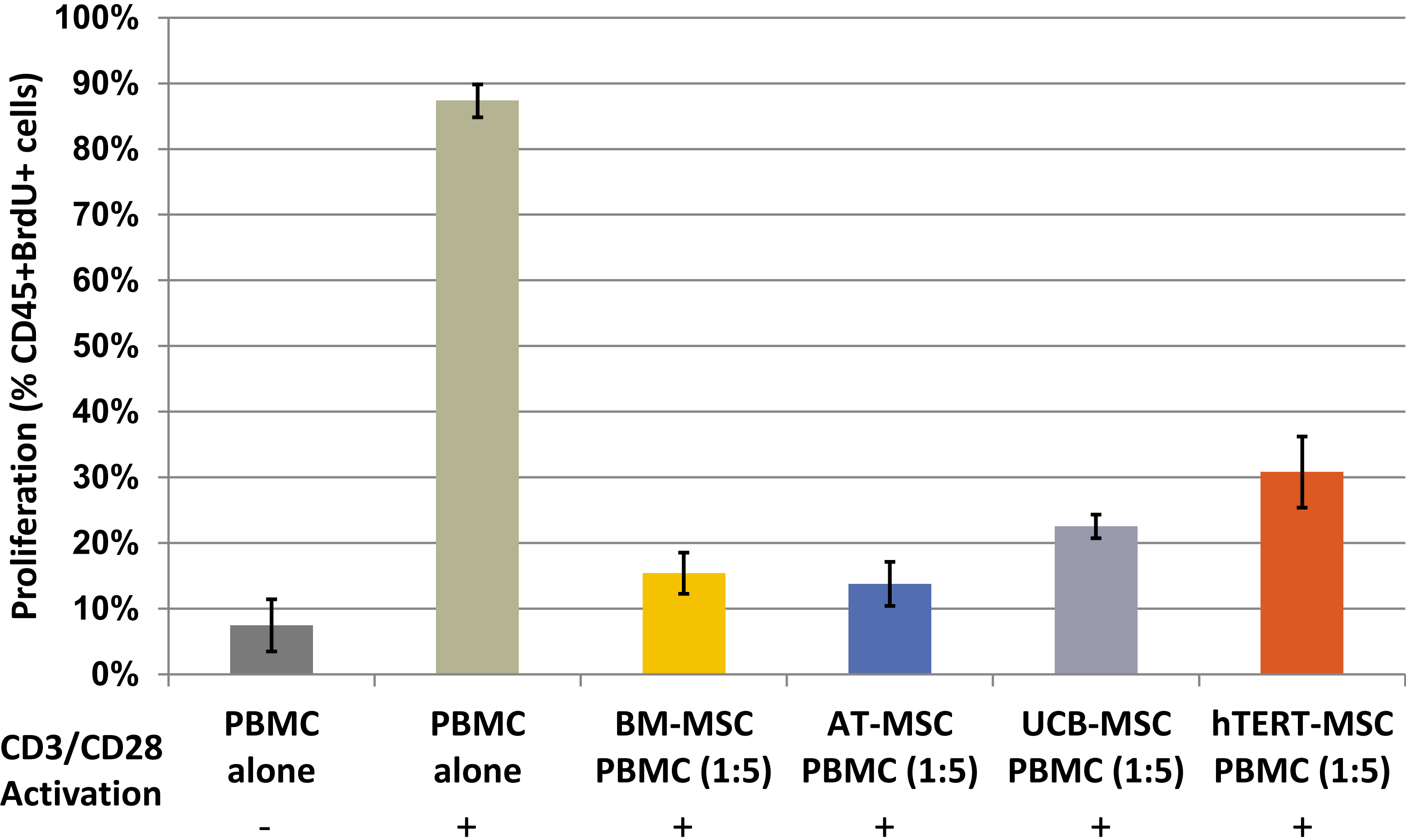
MSCs suppress activated T-cell proliferation



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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

Dezhong Yin, Ph.D., Joy A. Wells, James Clinton, Ph.D. and Chaozhong Zou, Ph.D.

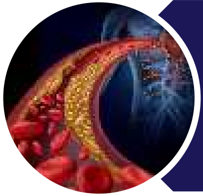
ATCC Cell Systems, 22 Firstfield Rd, Suite 180, Gaithersburg, MD 20878, USA

ISSCR Poster #: F-3115

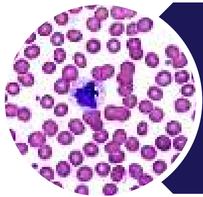
For more information on our MSC products: www.atcc.org/stemcells

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

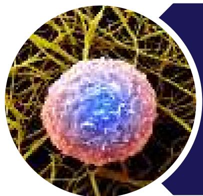
Outline



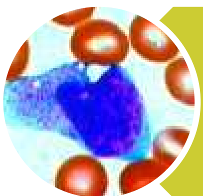
Background



CD34+ hematopoietic stem & progenitor cells



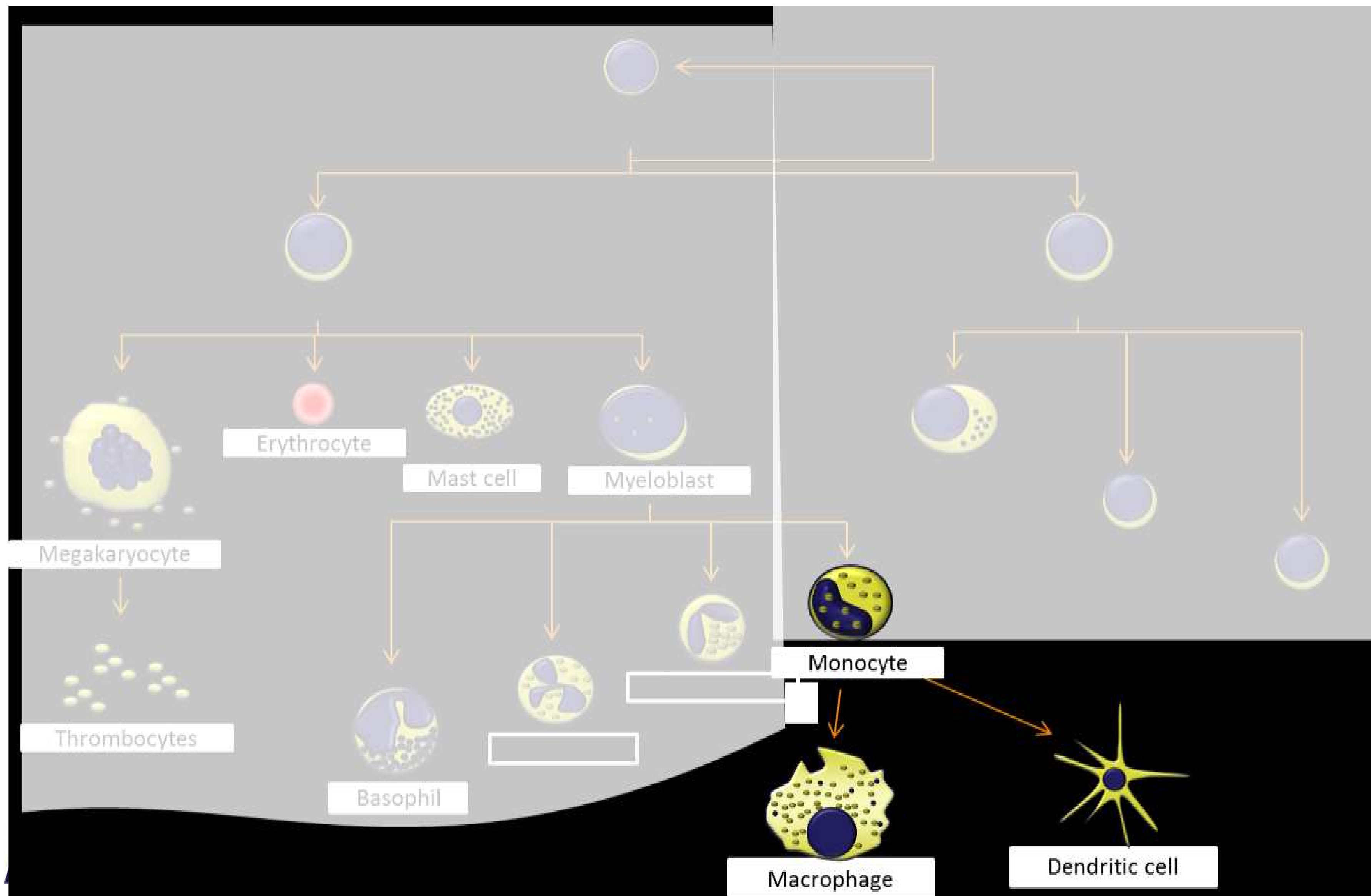
Mononuclear cells



CD14+ monocytes

ATCC

Primary monocytes (CD14+)



Primary CD14+ monocytes

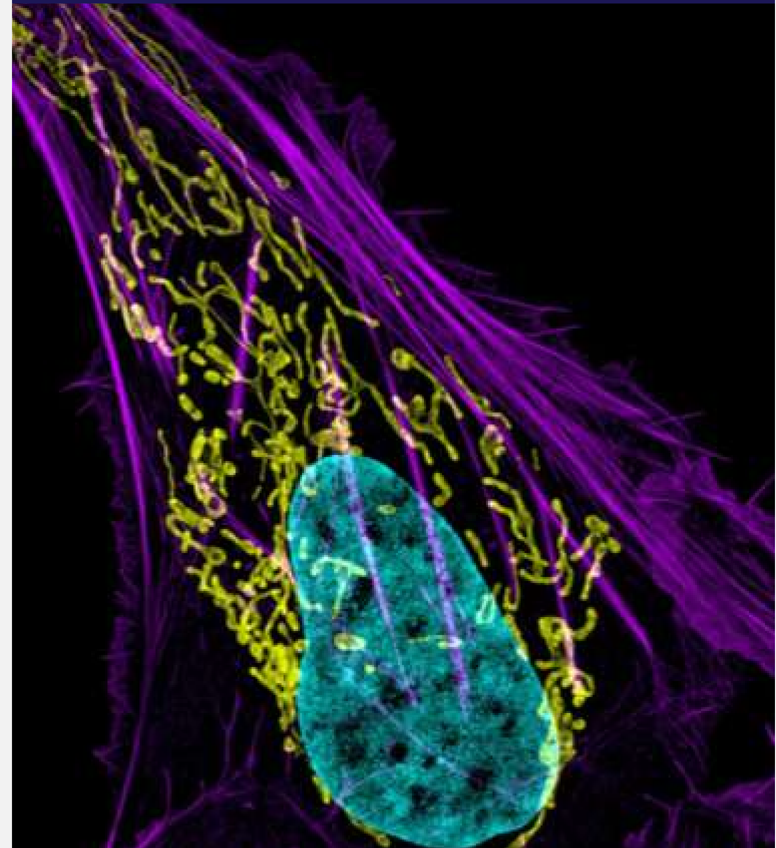
Applications

- 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, photo credit: Burnett Lippincott-Schwartz, National Cancer Institute



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

OPEN ACCESS Freely available online | PLOS ONE

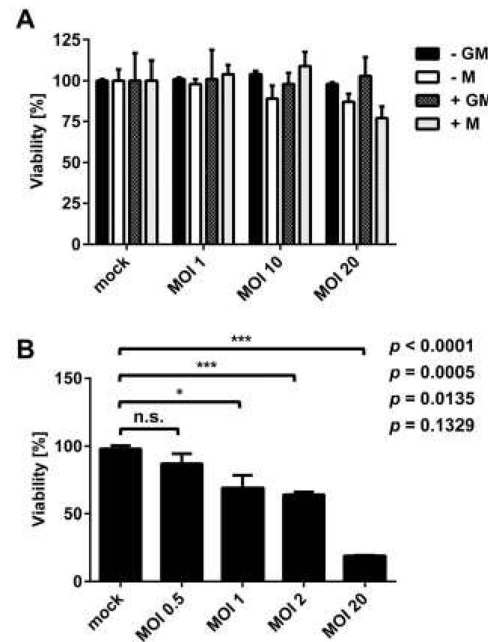
CD14-Dependent Monocyte Isolation Enhances Phagocytosis of *Listeria monocytogenes* by Proinflammatory, GM-CSF-Derived Macrophages

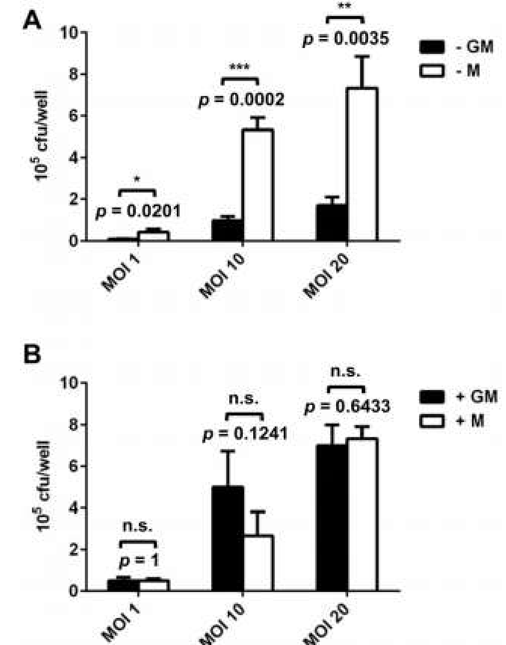
Carolina Neu[1], Anne Sedlag[1], Carina Bayer[2], Sabine Förster[3], Peter Crauwels[3], Jan-Hendrik Niess[4], Ger van Zandbergen[3], Giada Frascaroli[2], Christian U. Riedel[1]*

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.

Received March 7, 2013; Accepted May 13, 2013; Published June 11, 2013



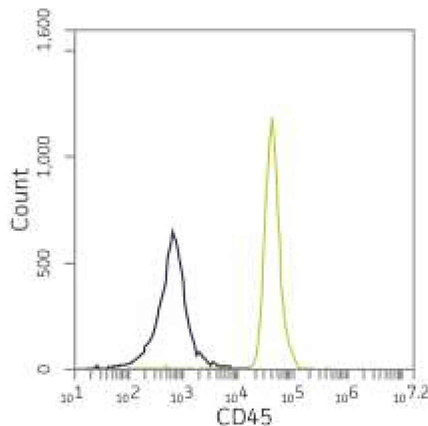


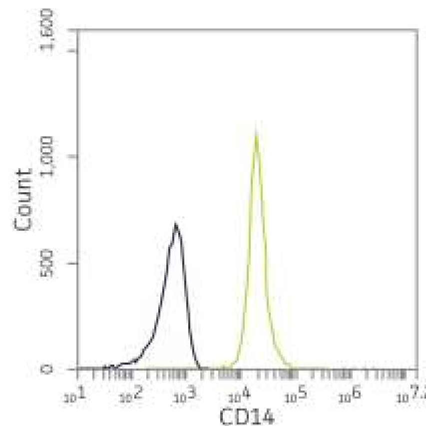
Neu C, *et al. PloS one* 8.6, 2013: e66898.

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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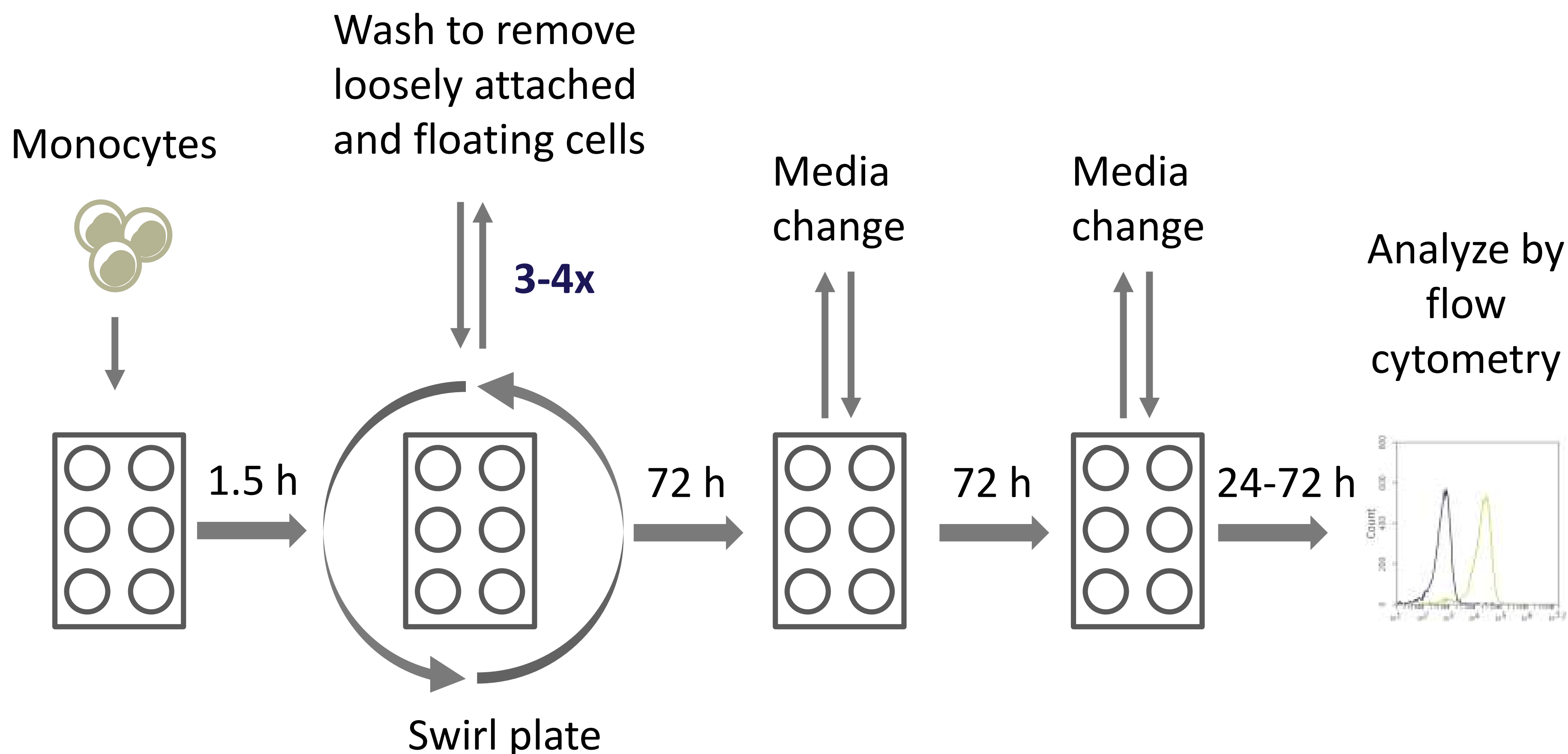
Macrophage differentiation protocol

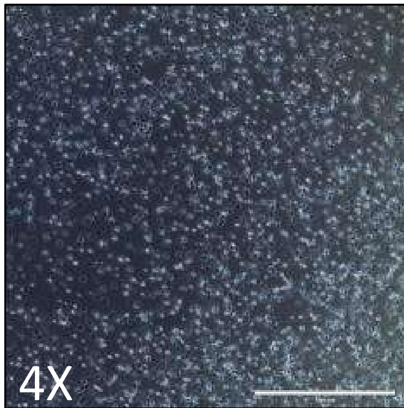


For detailed differentiation protocols see the ATCC website

ATCC

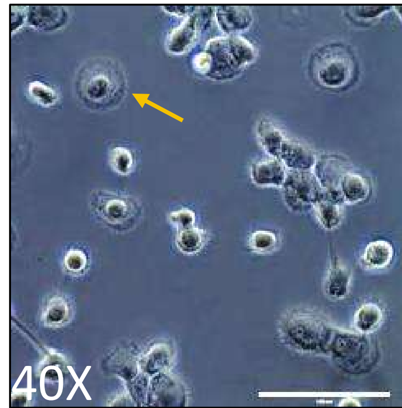
Generation of CD68+ macrophages from monocytes

Day 1

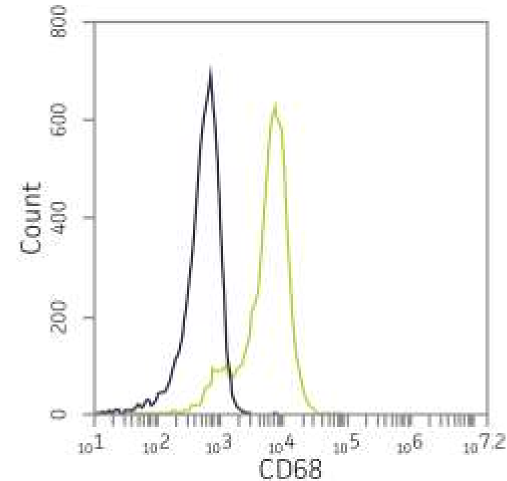


Monocytes

Day 9



Mature macrophage



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

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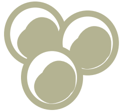
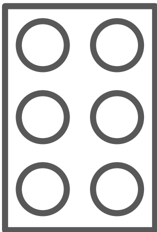
Dendritic cell differentiation protocol

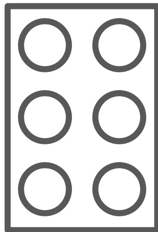
Monocytes





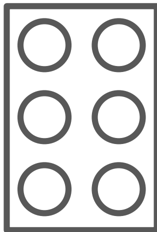
24 h

Media change. Retain non-adherent cells.



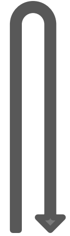
72 h

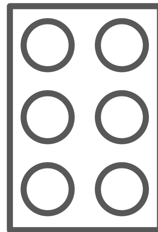
Media change. Retain non-adherent cells.



72 h

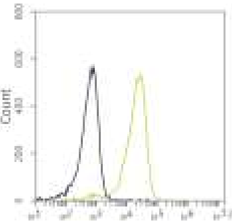
Media change. Retain non-adherent cells.





24-72 h

Analyze by flow cytometry

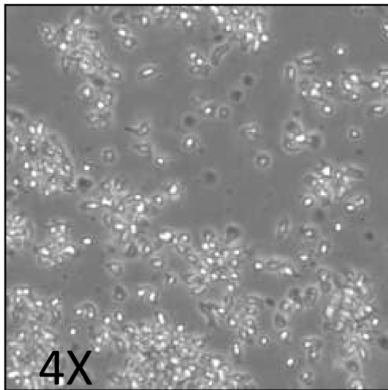


For detailed differentiation protocols see the ATCC website

ATCC

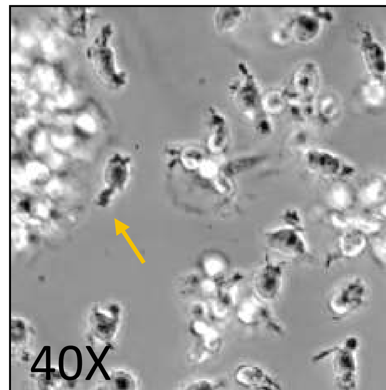
Generation of CD83+ DCs from monocytes

Day 1



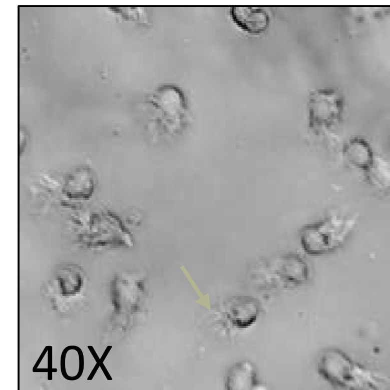
Monocytes

Day 6



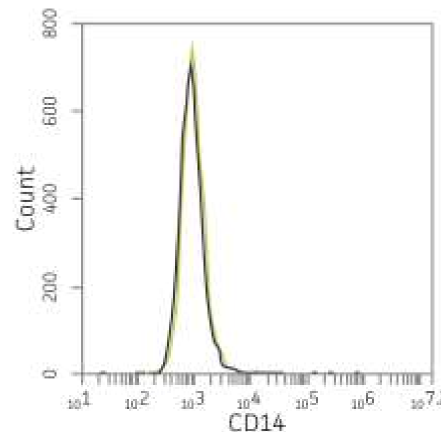
Immature DCs

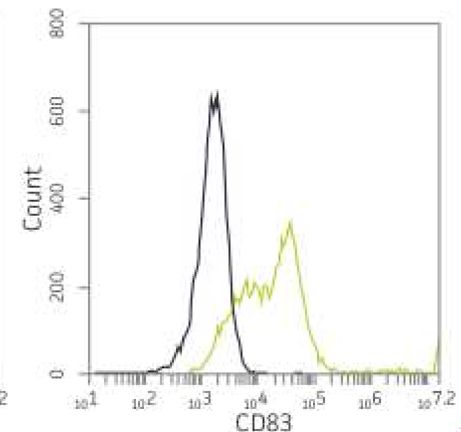
Day 8



Mature DCs

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





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Summary

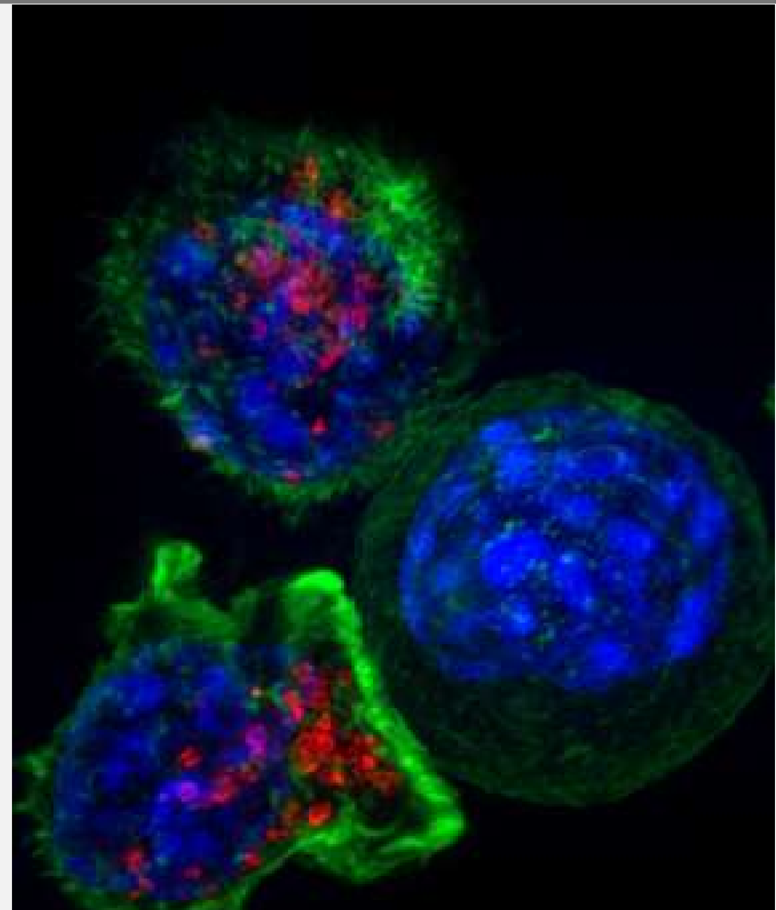
ATCC offers a variety of well-characterized and functionally validated primary hematopoietic cell types

- 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



Killer T Cells, photo credit: Alex Ritter

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- **July 28, 2016**
  12:00 PM EST
  Brian A. Shapiro, Ph.D., *Technical Writer,* ATCC
  Neural Progenitor Cells – Toxicological Models for the 21st Century