Comparison of iPSC-derived Microglia-like Differentiation Protocols

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iPSC NeuroHub Journal Club
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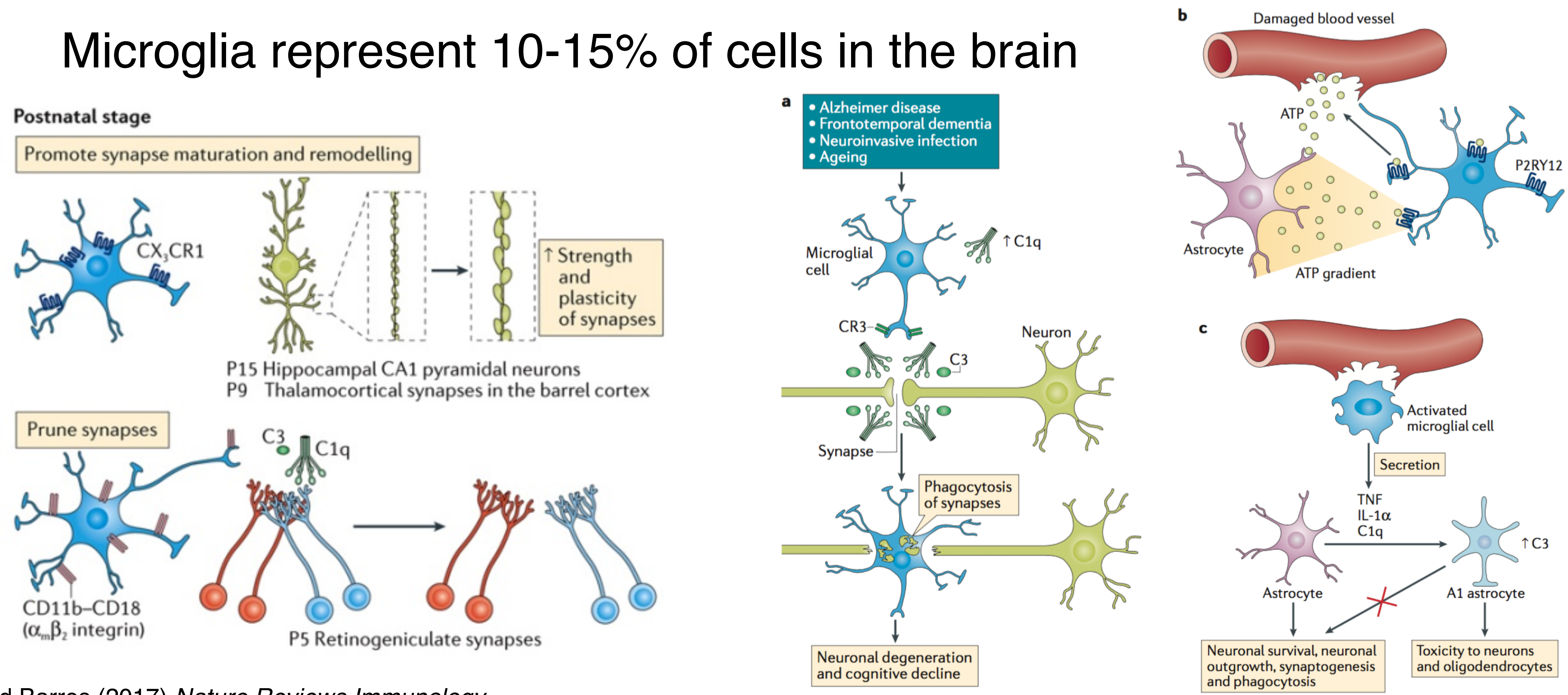
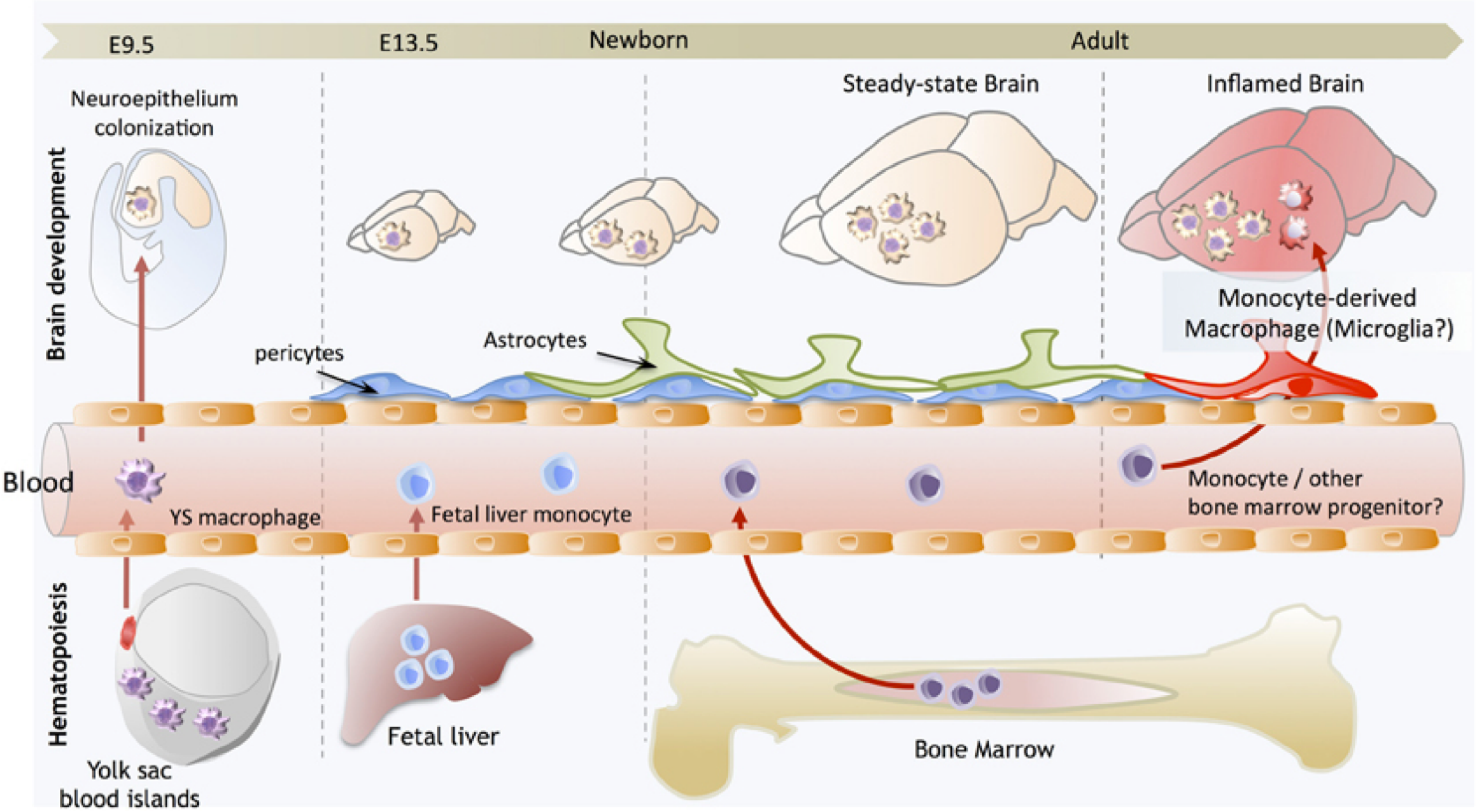
Microglia are the resident macrophages of the brain

Microglia represent 10-15% of cells in the brain



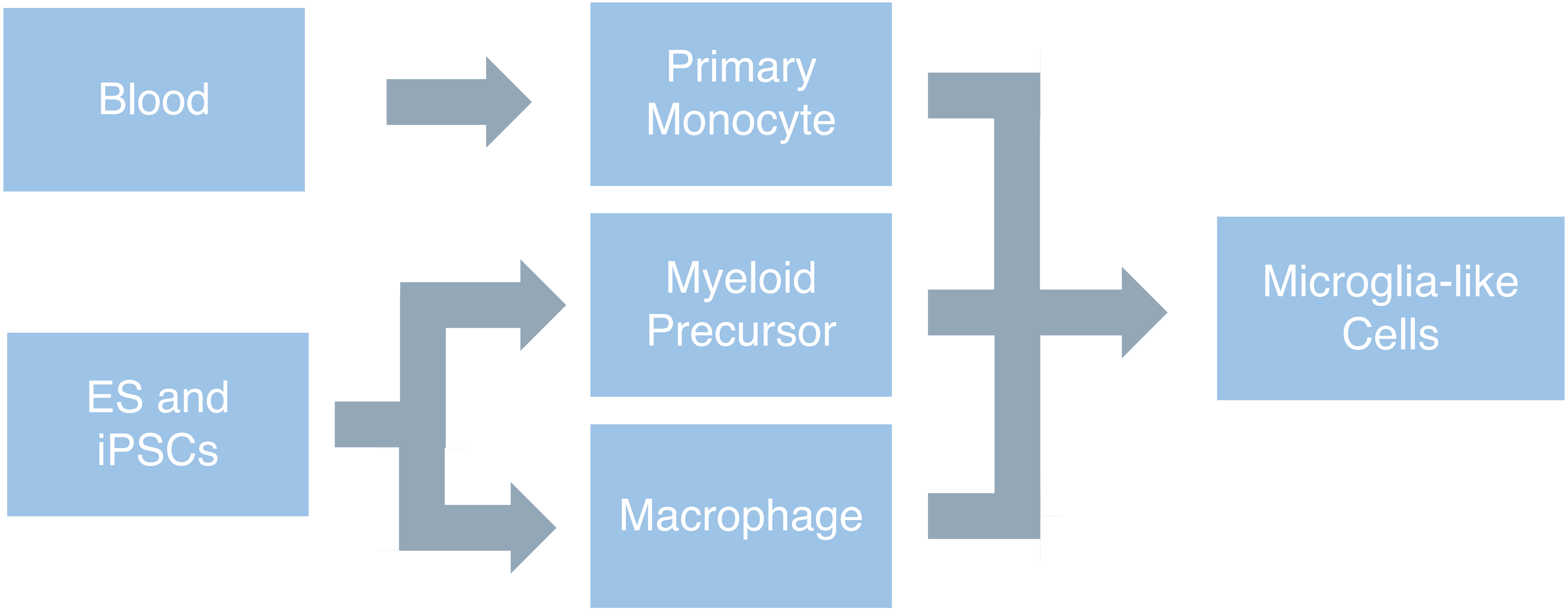
Li and Barres (2017) *Nature Reviews Immunology*

Microglia develop from yolk sac myeloid progenitors



Ginhoux et al (2013) *Frontiers in Cellular Neuroscience*

Microglia Differentiation Approaches



Microglia Differentiation Protocols

Muffat et al (2016) *Nature Medicine*

Efficient derivation of microglia-like cells from human pluripotent stem cells

Julien Muffat[1,8], Yun Li[1,8], Bingbing Yuan[1], Maisam Mitalipova[1], Attya Omer[1,2], Sean Corcoran[1,3], Grisilda Bakiasi[1,4], Li-Huei Tsai[5], Patrick Aubourg[2,6], Richard M Ransohoff[7] & Rudolf Jaenisch[1,3]

Pandaya et al (2017) *Nature Neuroscience*

Differentiation of human and murine induced pluripotent stem cells to microglia-like cells

Hetal Pandya[1], Michael J Shen[1], David M Ichikawa[1], Andrea B Sedlock[1], Yong Choi[1], Kory R Johnson[1], Gloria Kim[1], Mason A Brown[1], Abdel G Elkahloun[2], Dragan Maric[1], Colin L Sweeney[3], Selamawit Gossa[1], Harry L Malech[3], Dorian B McGavern[1] & John K Park[1,4]

Abud et al (2017) *Neuron*

iPSC-Derived Human Microglia-like Cells to Study Neurological Diseases

Edsel M. Abud,[1,2,3] Ricardo N. Ramirez,[4] Eric S. Martinez,[1,2,3] Luke M. Healy,[5] Cecilia H.H. Nguyen,[1,2,3] Sean A. Newman,[2] Andriy V. Yeromin,[6] Vanessa M. Scarfone,[2] Samuel E. Marsh,[2,3] Cristhian Fimbres,[3] Chad A. Caraway,[3] Gianna M. Fote,[1,2,3] Abdullah M. Madany,[11] Anshu Agrawal,[7] Rakez Kayed,[8] Karen H. Gylys,[9] Michael D. Cahalan,[6] Brian J. Cummings,[2,3,10] Jack P. Antel,[5] Ali Mortazavi,[4] Monica J. Carson,[11] Wayne W. Poon,[3,*] and Mathew Blurton-Jones[1,2,3,12,*]

Dourvaras et al (2017) *Stem Cell Reports*

Directed Differentiation of Human Pluripotent Stem Cells to Microglia

Panagiotis Douvaras,[1,*] Bruce Sun,[1] Minghui Wang,[2] Ilya Kruglikov,[1] Gregory Lallos,[1] Matthew Zimmer,[1] Cecile Terrenoire,[1] Bin Zhang,[2] Sam Gandy,[3] Eric Schadt,[2] Donald O. Freytes,[4] Scott Noggle,[1,5] and Valentina Fossati[1,5]

Microglia Differentiation Protocols

Haenseler et al (2017) *Stem Cell Reports*

A Highly Efficient Human Pluripotent Stem Cell Microglia Model Displays a Neuronal-Co-culture-Specific Expression Profile and Inflammatory Response

Walther Haenseler,[1,11] Stephen N. Sansom,[2,11] Julian Buchrieser,[1] Sarah E. Newey,[3] Craig S. Moore,[4] Francesca J. Nicholls,[5] Satyan Chintawar,[6] Christian Schnell,[7] Jack P. Antel,[8] Nicholas D. Allen,[7] M. Zameel Cader,[6] Richard Wade-Martins,[9,10] William S. James,[1] and Sally A. Cowley[1,10,*]

Takata et al (2017) *Immunity*

Induced-Pluripotent-Stem-Cell-Derived Primitive Macrophages Provide a Platform for Modeling Tissue-Resident Macrophage Differentiation and Function

Kazuyuki Takata,[1,2,11] Tatsuya Kozaki,[1,11] Christopher Zhe Wei Lee,[1,12] Morgane Sonia Thion,[3,12] Masayuki Otsuka,[1] Shawn Lim,[1] Kagistia Hana Utami,[4] Kerem Fidan,[5] Dong Shin Park,[1] Benoit Malleret,[1,6] Svetoslav Chakarov,[1] Peter See,[1] Donovan Low,[1] Gillian Low,[1] Marta Garcia-Miralles,[4] Ruizhu Zeng,[4] Jinqiu Zhang,[4] Chi Ching Goh,[1] Ahmet Gul,[7] Sandra Hubert,[1] Bernett Lee,[1] Jinmiao Chen,[1] Ivy Low,[1] Nurhidaya Binte Shadan,[1] Josephine Lum,[1] Tay Seok Wei,[1] Esther Mok,[1] Shohei Kawanishi,[2] Yoshihisa Kitamura,[2,13] Anis Larbi,[1] Michael Poidinger,[1] Laurent Renia,[1] Lai Guan Ng,[1] Yochai Wolf,[8] Steffen Jung,[8] Tamer Önder,[5] Evan Newell,[1] Tara Huber,[9] Eishi Ashihara,[2] Sonia Garel,[3] Mahmoud A. Pouladi,[4,10] and Florent Ginhoux[1,14,*]

Ryan et al (2017) *Science Translational Med*

A human microglia-like cellular model for assessing the effects of neurodegenerative disease gene variants

Katie J. Ryan,[1,2,3,4] Charles C. White,[1,2,4] Kruti Patel,[1,2,3,4] Jishu Xu,[1,2,4] Marta Olah,[4,5] Joseph M. Replogle,[1,2,3,4*] Michael Frangieh,[1,2,4] Maria Cimpean,[1,2,4†] Phoebe Winn,[1,2,4] Allison McHenry,[1,2,4] Belinda J. Kaskow,[1,2,3,4] Gail Chan,[1,2,3,4] Nicole Cuerdon,[1,2,3,4] David A. Bennett,[6] Justin D. Boyd,[1,3] Jaime Imitola,[7] Wassim Elyaman,[4,5] Philip L. De Jager,[4,5] Elizabeth M. Bradshaw[4,5‡]

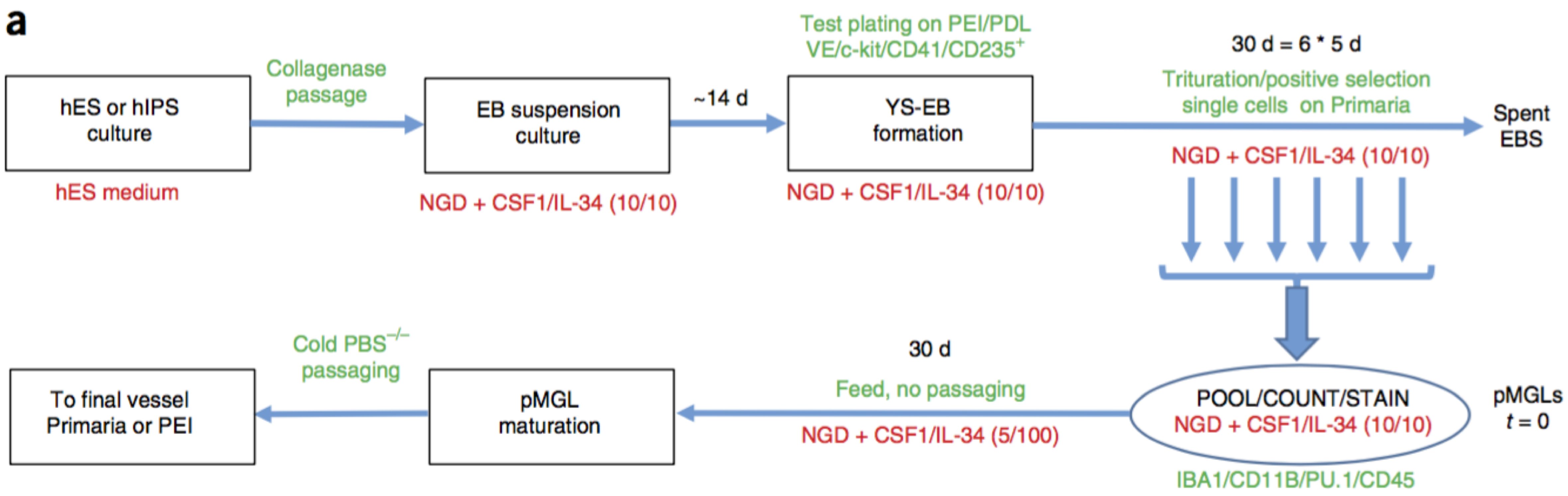
Brownjohn et al (2018) *Stem Cell Reports*

Functional Studies of Missense TREM2 Mutations in Human Stem Cell-Derived Microglia

Philip W. Brownjohn,[1] James Smith,[1] Ravi Solanki,[1] Ebba Lohmann,[2,3] Henry Houlden,[4] John Hardy,[4] Sabine Dietmann,[5] and Frederick J. Livesey[1,*]

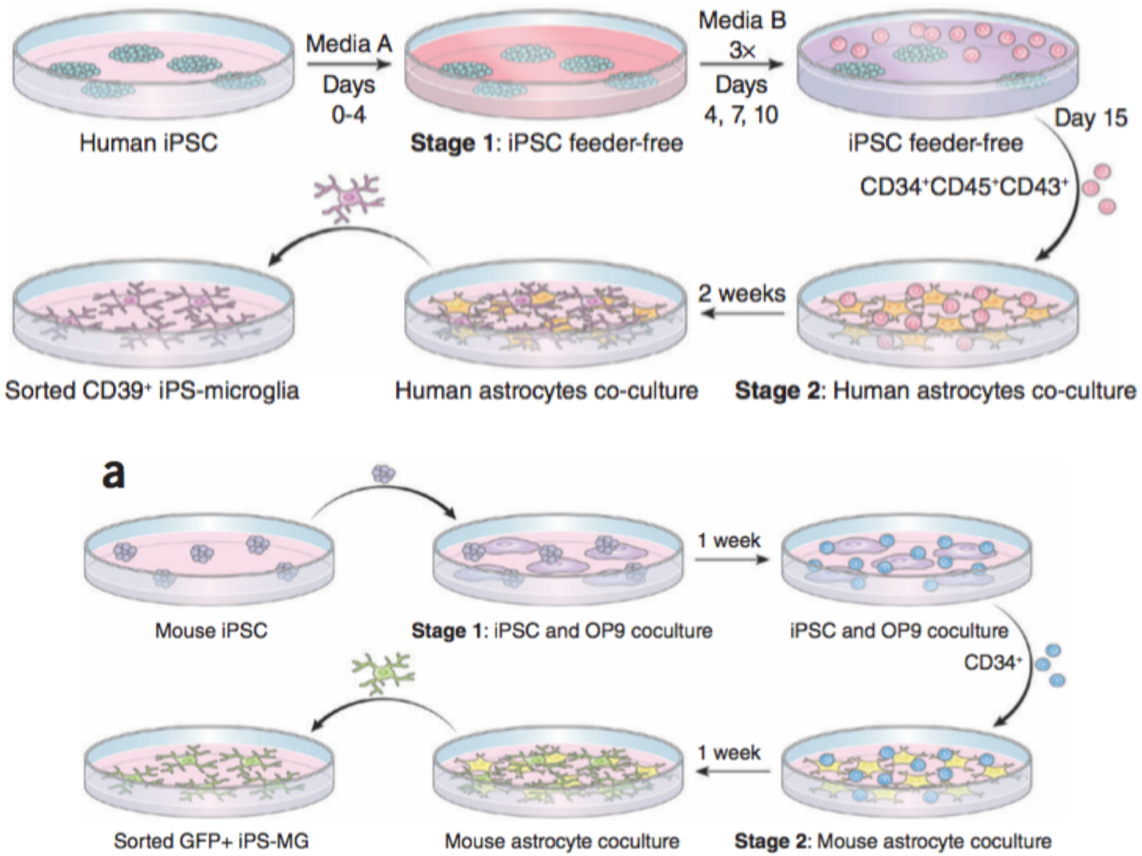
Differentiation Timelines

Muffat et al (2016) *Nature Medicine*



Differentiation Timeline

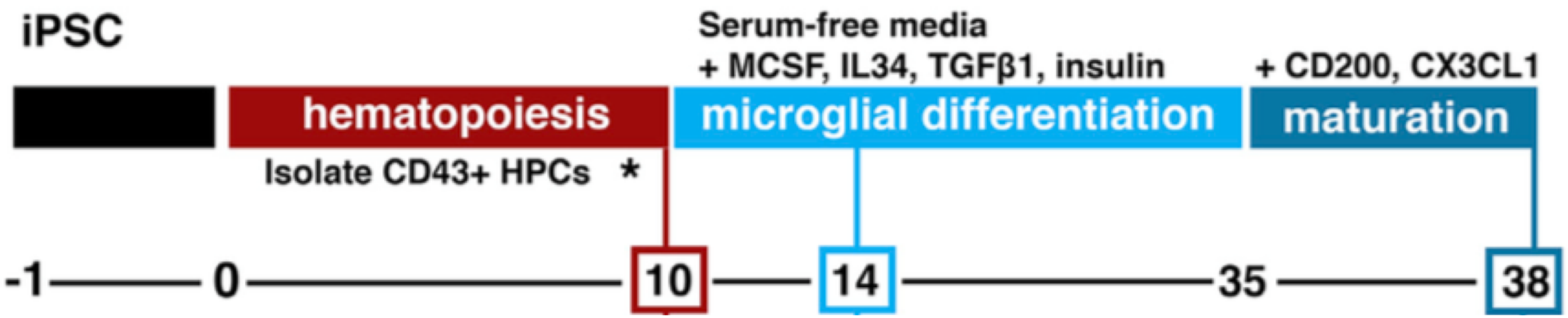
Pandaya et al (2017) *Nature Neuroscience*



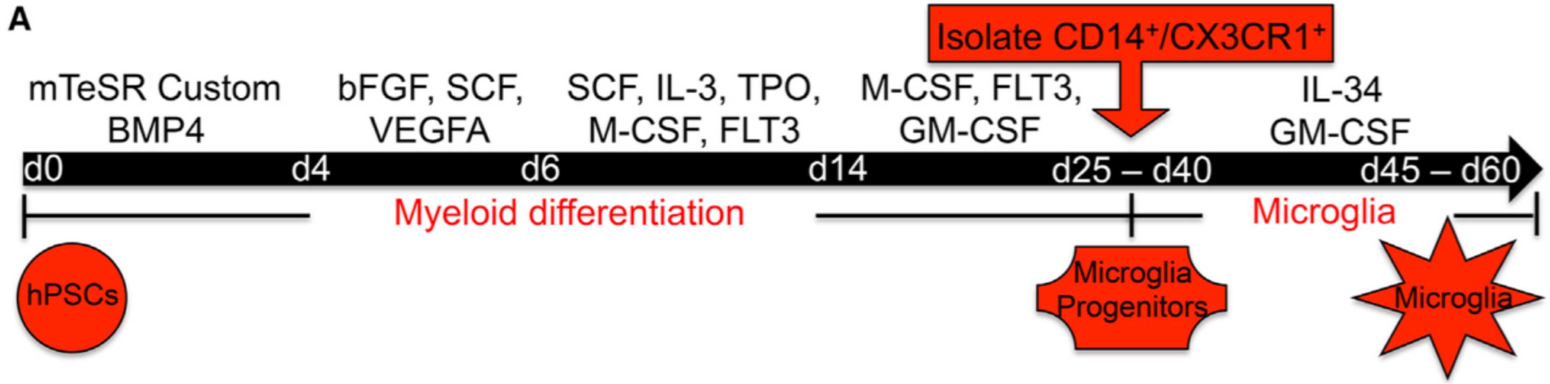
- Two-step Differentiation
  - **Step 1**: Generation of hematopoietic progenitor-like intermediate cell
  - **Step 2**: Astrocyte Co-Culture
- Feeder free and OP9

Differentiation Timeline

Abud et al (2017) *Neuron*



Dourvaras et al (2017) *Stem Cell Reports*



Protocol	Starting Cells	Important Intermediates	Major differentiation factors	Feeder layer or co-culture	Yield (range)	Time (weeks)	Advantages
Muffat et al (2017) Nat Med	ES cells and iPSCs	YS embryoid bodies	IL-34 and CSF1	MEFs for human ES cell and iPSC propagation	0.5-4	8	Compatible with 3D culture
Pandya et al (2017) Nat Neuro	iPSCs	Haematopoeitic progenitor-like cells	GM-CSF, CSF1 and IL-3	OP9 murine stromal feeder layer Astrocyte-co-culture	2-3	2-4	Also works for iPSCs
Abud et al (2017) Neuron	iPSCs	Haematopoietic progenitors	MCSF, IL-34, TGFß-1, CD200, CX_3CL1	Rat hippocampal neurons during maturation	30-40	5	High purity; high yield; graftable to mouse brains
Douvaras et al (2017) Stem Cell Reports	ES cells and iPSCs	Myeloid progenitors	IL-34 and GM-CSF	No	2-3	8.5	Highly pure; can start with fewer iPSCs (10^5)

Adapted from Li and Barres (2017) *Nature Reviews Immunology*

Microglia Markers and Functional

Markers	Functional
• CX_3CR1 • CD11b • SALL1 • $CD45^{low}$ • MHC Class II^{low} • **P2RY12** • **TMEM119**	• Cytokine secretion • Responsive to LPS • Phagocytosis • Responsive to ADP • Environmental response

Protocol	Markers	Cytokine secretion	Phagocytosis	ADP response	Co-Culture
Muffat et al (2017) *Nat Med*	TMEM119 IBA1 CD45 P2RY12 (some)	**Yes** Unstimulated: IL-8, CSCL1, CCL2 Simulated with INFy and LPS: CSCL10, CCL3, IL-6, TNFa	**Yes** pH-rodo tagged *E. coli* particles	**N/A**	3D culture with neurons and astrocytes from the same iPSCs
Pandya et al (2017) *Nat Neuro*	CD11b IBA1 CX3CR1 CD45 TREM2	**Yes** Production of ROS and cytokines	**Yes** pH-rodo tagged *E. coli* particles	**N/A**	Co-culture with astrocytes during differentiation, implanted into mice with gliomas
Abud et al (2017) *Neuron*	CX3CR1 TREM2 TMEM119 (after transplantation)	**Yes** Responsive to LPS and IL-1ß stimulation	**Yes** Human synaptosomes, tau, ß-amyloid	**Yes**	3D organoid co-culture, Co-culture with rat hippocampal neurons, transplanted into mice
Douvaras et al (2017) *Stem Cell Reports*	CD11c TMEM119 P2RY12 IBA1 CX3CR1	**Yes** Secretion, did not assay for stimulation	**Yes** Fluorescently-labeled latex microbeads	**Yes** Responsiveness to ADP with Ca^{2+} transients	None