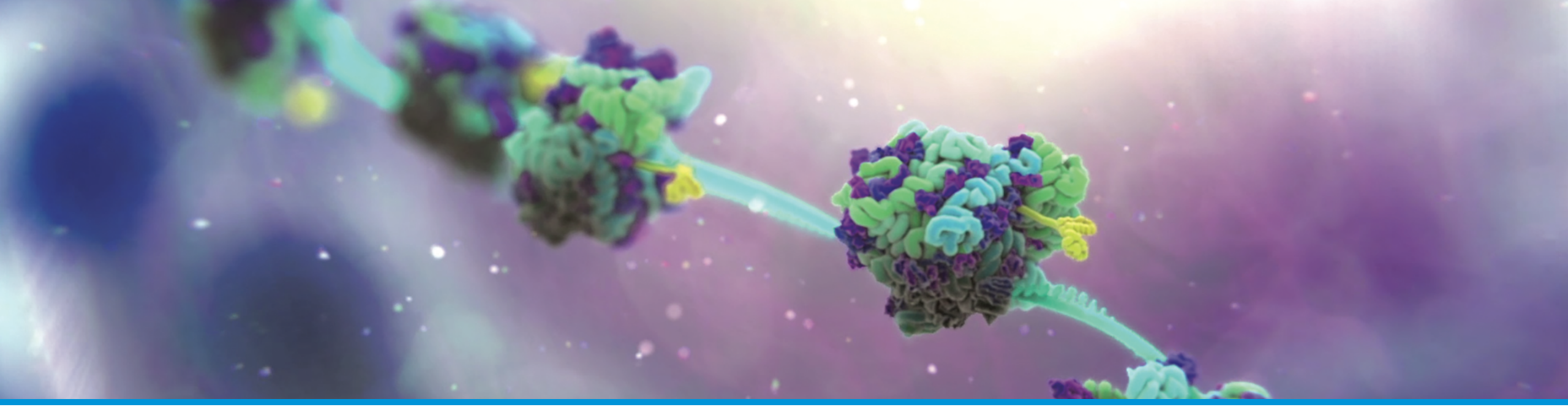

iPSC-Derived Monocytes Generate Functional M1 and M2 Macrophages with Enhanced Cytokine Secretion and Tumor Cell-Killing Activity

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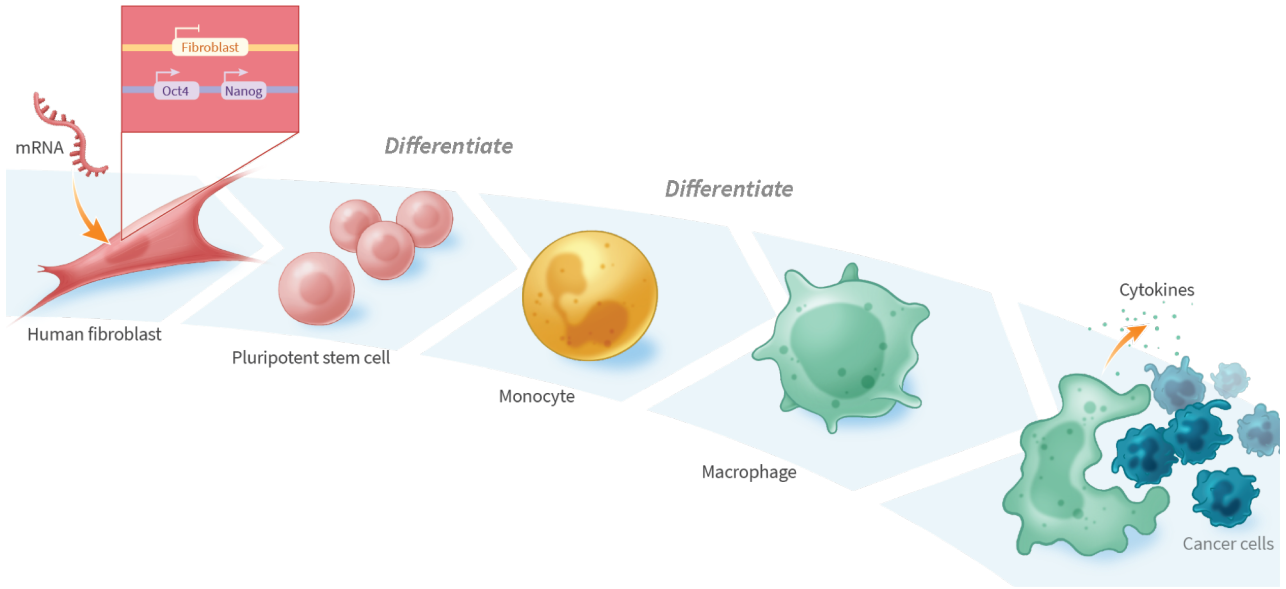
Introduction and Study Goal

- We have previously demonstrated iPSCs can be generated using an mRNA reprogramming process (Harris *et al*., Mol Ther, Vol 28 No 4S1, 2020)
- Monocytes and monocyte-derived macrophages possess innate functionality in tumor development; sourcing cells from PBMCs has proven a challenge in research and clinical development
- The goal of this study was to investigate these iPSCs' potential to differentiate into monocytes and monocyte-derived macrophages



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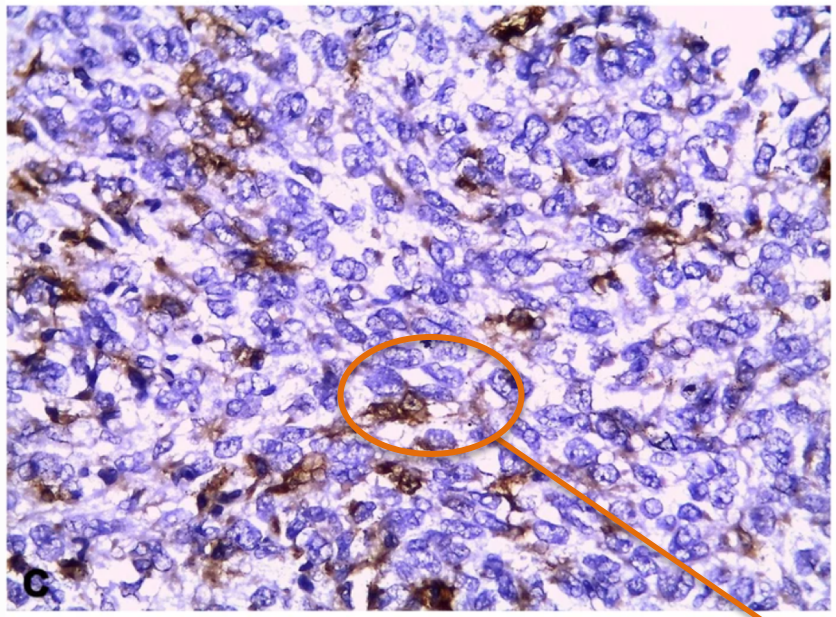
Macrophages Evident Role in Various Cancers

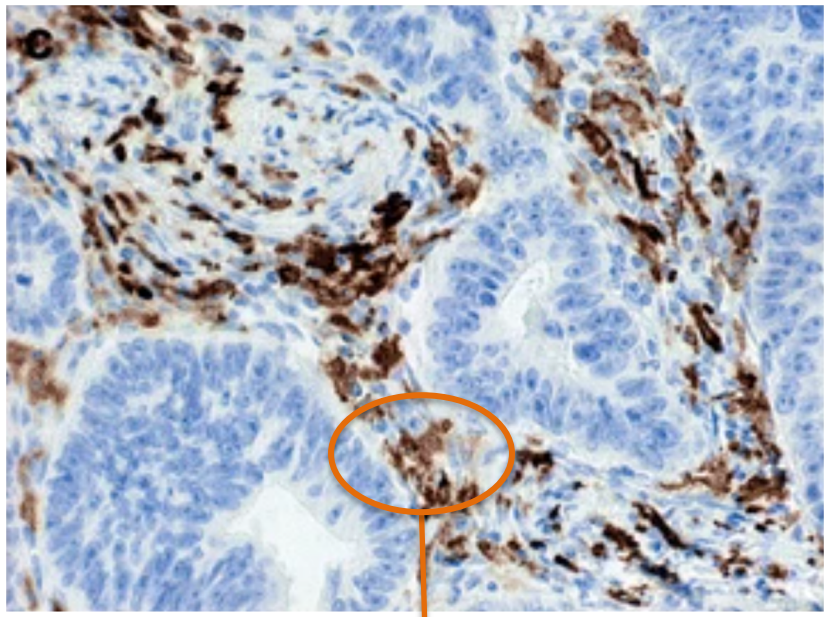
CD68+ CD163+



Breast Carcinoma

Mwafy, S.E., et al. *J Egypt Natl Canc Inst* **32,** 6 (2020).

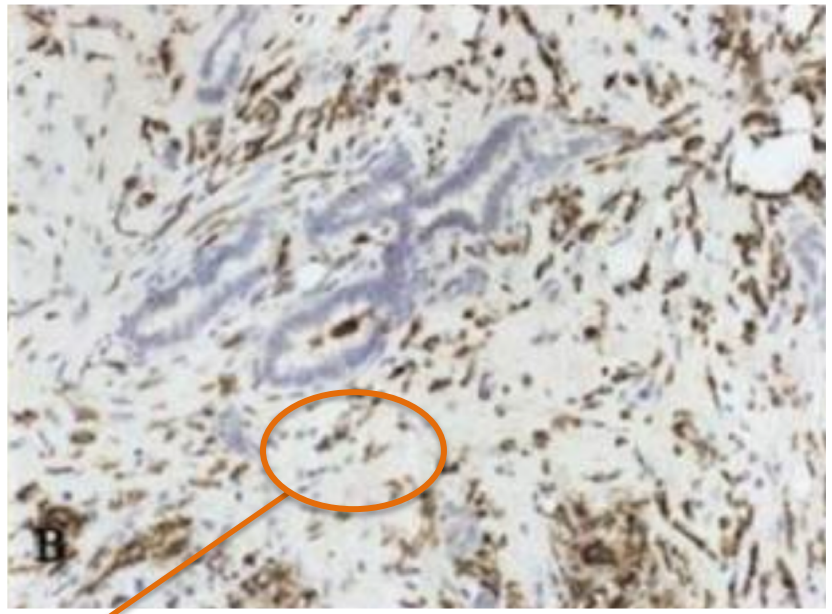
CD163+



Colorectal Carcinoma

Shabo, I., et al. *Cancer Microenvionment* **7** 61-69 (2014)

CD68+



Pancreatic Carcinoma

Gardian, K., et al. *J Cancer* **3** 285-291 (2012)

Macrophages

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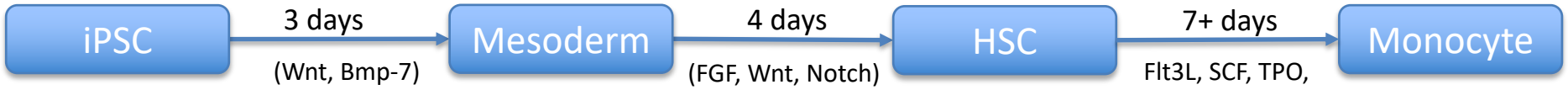
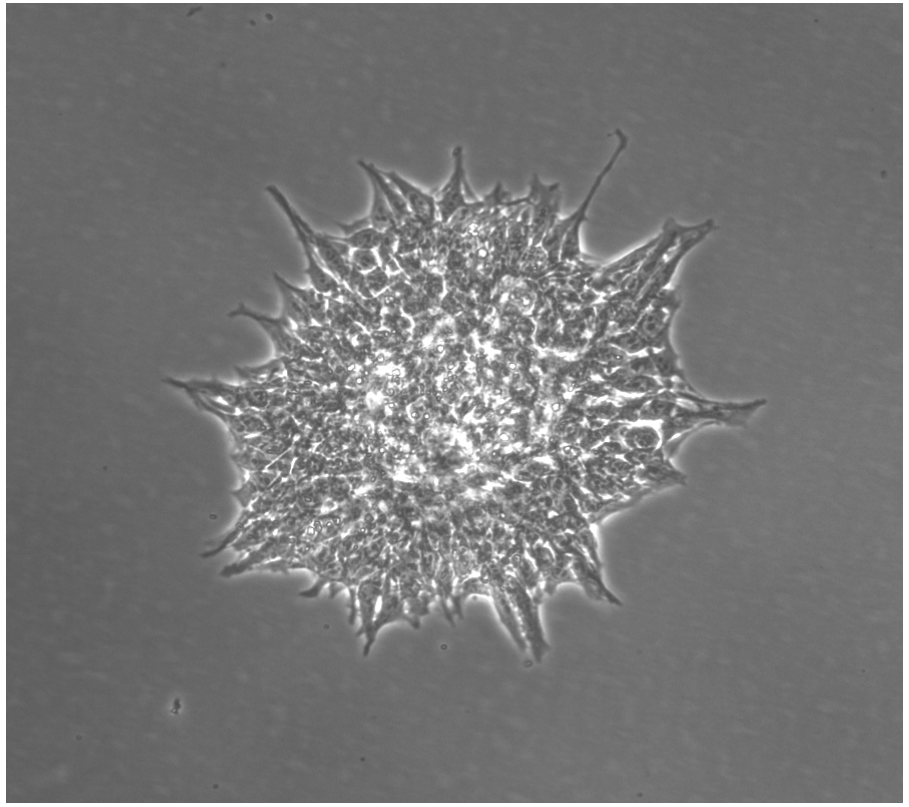
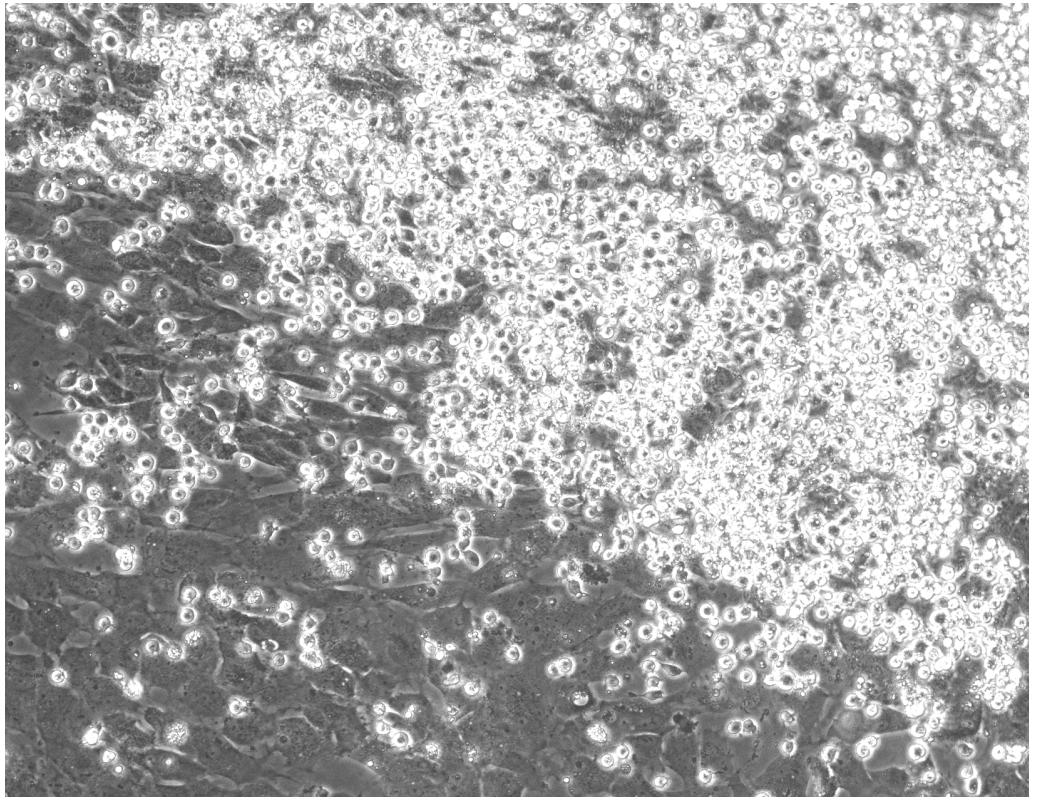
iPSC-Derived Monocytes in 14 days





Day 0 – iPSC colony



Day 14 – CD14+ cells in suspension

- 14-day differentiation protocol yielded CD14+ cells in suspension with large nucleus and round morphology

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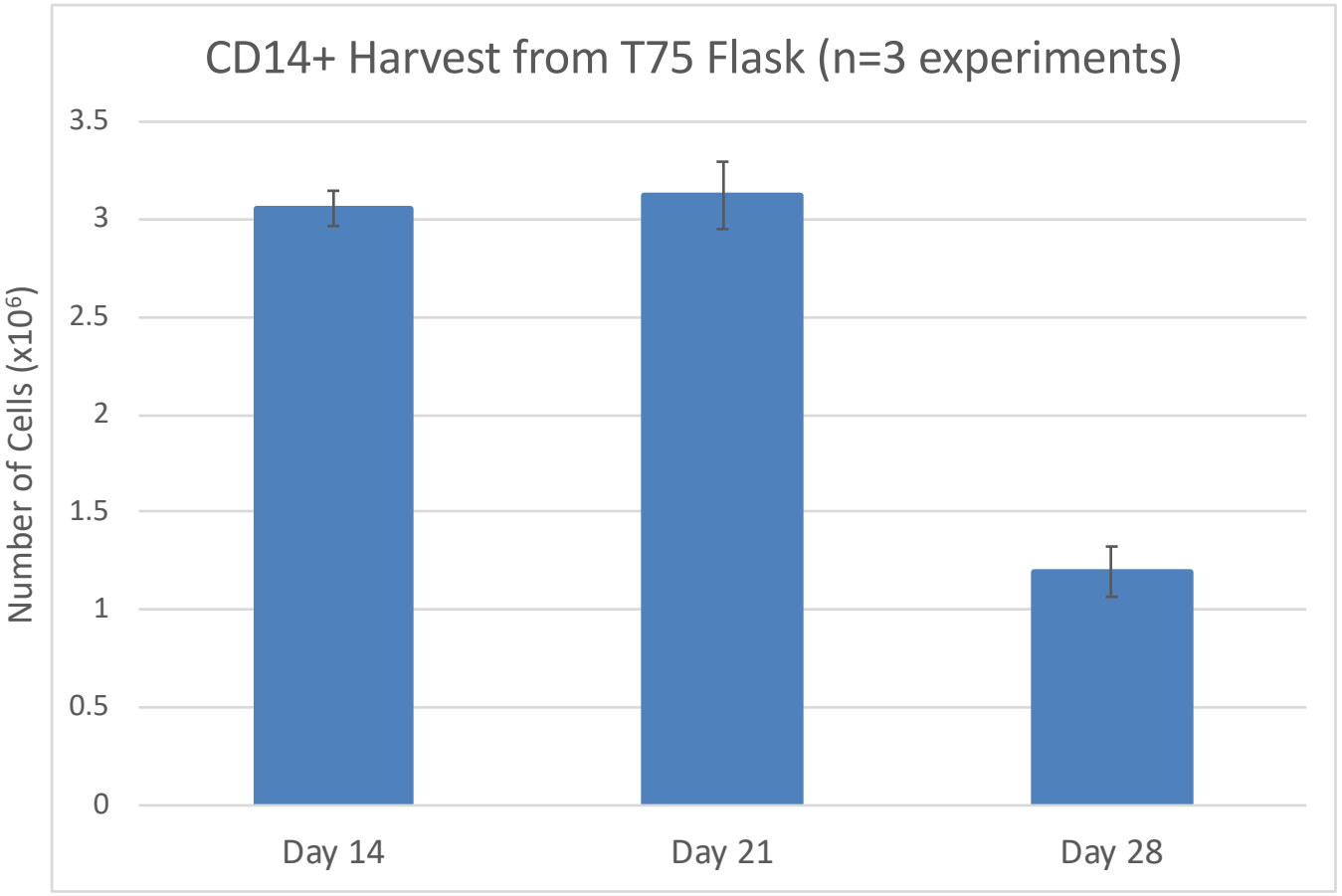
Yield of CD14+ Monocytes



- 14-day differentiation protocol
- Thawed and plated 10^6 iPSCs
- Seeded single cell suspension to grow small homogeneous colonies
- Harvest window of ~2 weeks

- Average yield per cm^2: $4.1x10^4$ cells
- Average yield per iPSC: 7.1 cells

- iPSCs generated CD14+ monocytes within 18 days from frozen to harvest

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Surface Marker Characterization of Monocytes

	Day 0	Day 14
Tra-1-60	97%	4.1%
Tra-1-81	88%	0.4%

Flow Cytometry of Pluripotency Markers

- Harvested suspension cells negative for pluripotent markers Tra-1-60 and Tra-1-81
- iPSC-Monocytes displayed similar expression to CD14+ PBMCs of key monocyte markers: CD11b, CD13, CD33, CD45

	PBMC-Monocytes	iPSC-Monocytes
CD11b	81%	93%
CD13	99%	99%
CD33	99%	82%
CD45	99%	98%
CD68	0.0%	6.5%
CD80	0.0%	99%
CD206	0.8%	93%
SIRPa	99%	62%

Flow Cytometry Characterization of CD14+ Populations

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Cryopreservation and Recovery of Monocytes

	PBMC-Monocytes	iPSC-Monocytes
Live/Dead	99%/1.1%	94%/5.8%
CD14	83%	79%
CD45	99%	95%
CD163	13%	3.0%

Flow Cytometry of PBMC- and iPSC-monocytes directly after thawing vials of $5x10^6$ cells/mL in 20% DMSO / 80% RPMI1640 frozen for 1 month

- iPSC-Monocytes capable of cryopreservation with similar results compared to PBMC-Monocytes

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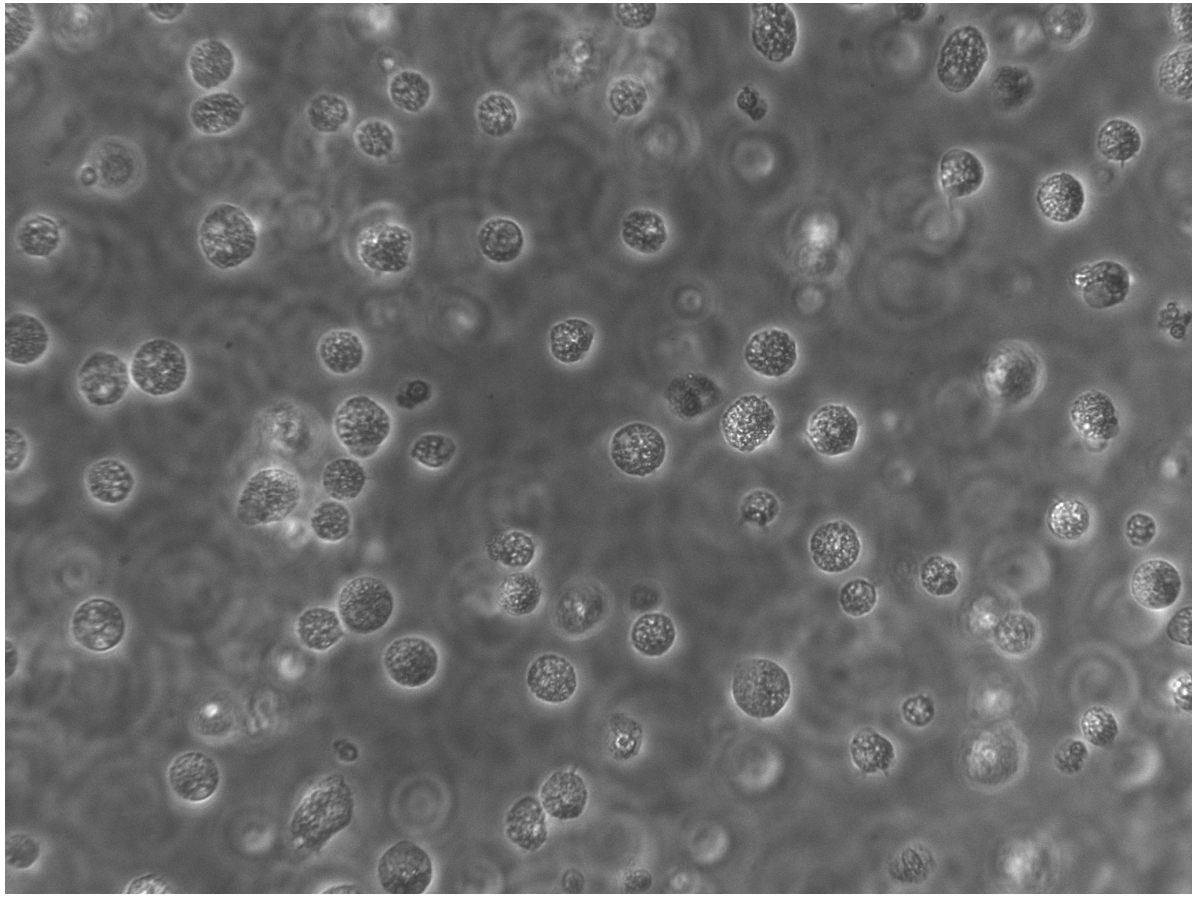
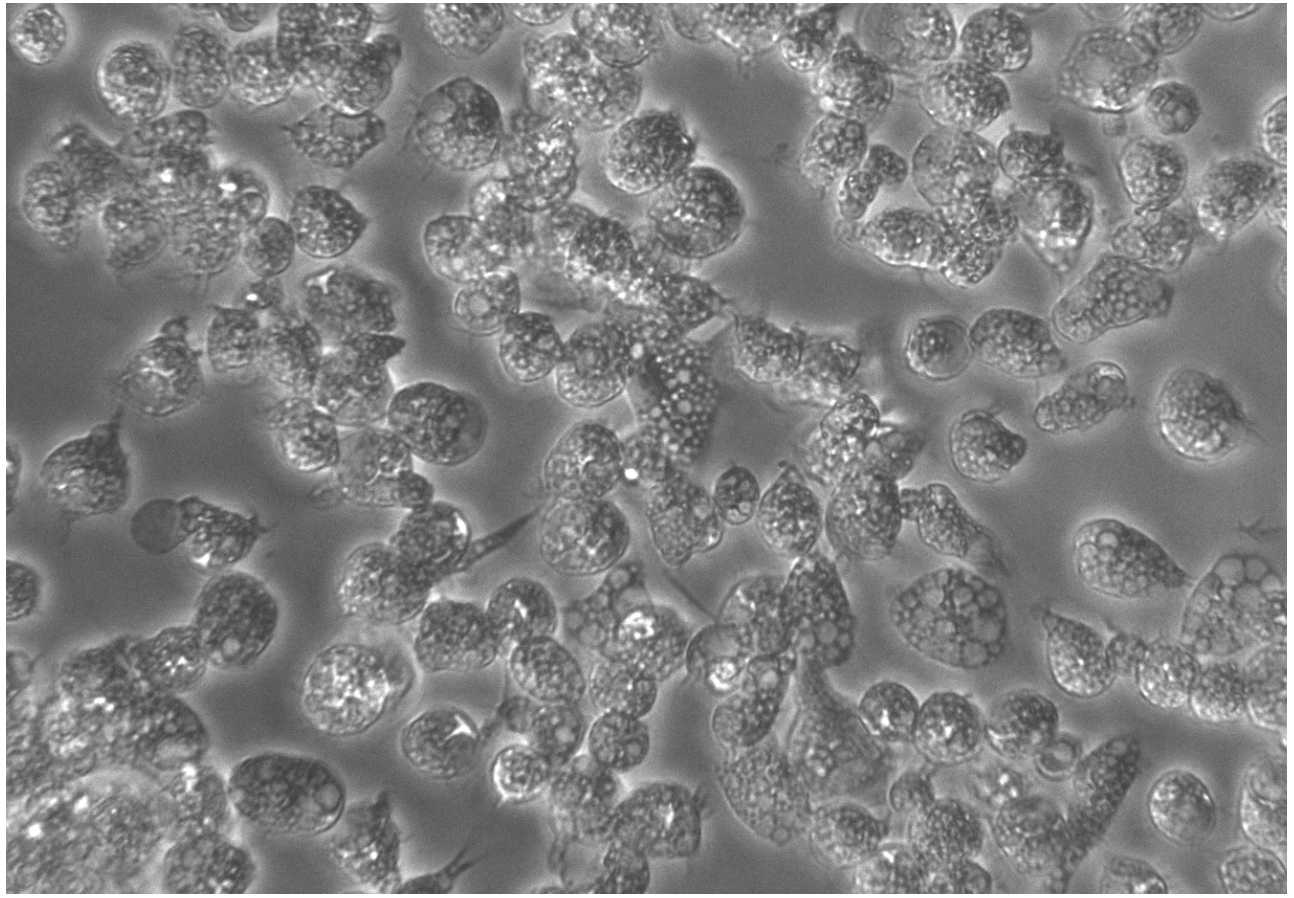
iPSC-Monocyte-Derived Macrophages



Day 0



Day 4

- Over 4 days with 50 ng/mL M-CSF, >90% of CD14+ cells adhered, and morphology resembled macrophage shape with prominent vacuoles

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iPSC-Monocytes Capable of Differentiating with High Yield

Polarization	Stimulant
M1	50 ng/mL IFNy + 10 ng/mL LPS
M2a	10 ng/mL IL-4

Fresh iPSC-Monocytes	Harvest/well	Yield
M0 Stimulation	$4.17x10^5$	83.4%
M1 Stimulation	$5.04x10^5$	100.8%
M2a Stimulation	$5.43x10^5$	108.6%

Cryopreserved Monocytes	Harvest/well	Yield
iPSC-Monocytes (M0)	$0.94x10^5$	18.8%
PBMC-Monocytes (M0)	$0.56x10^5$	11.2%

Macrophage yields from plating 500k CD14+ cells per well with 50 ng/mL of M-CSF in a 24-well plate for 4 days

- iPSC-monocytes capable of differentiating into macrophages with high yield
- iPSC-monocytes recover from cryopreservation and differentiate into macrophages with yields comparable to PBMC-monocytes

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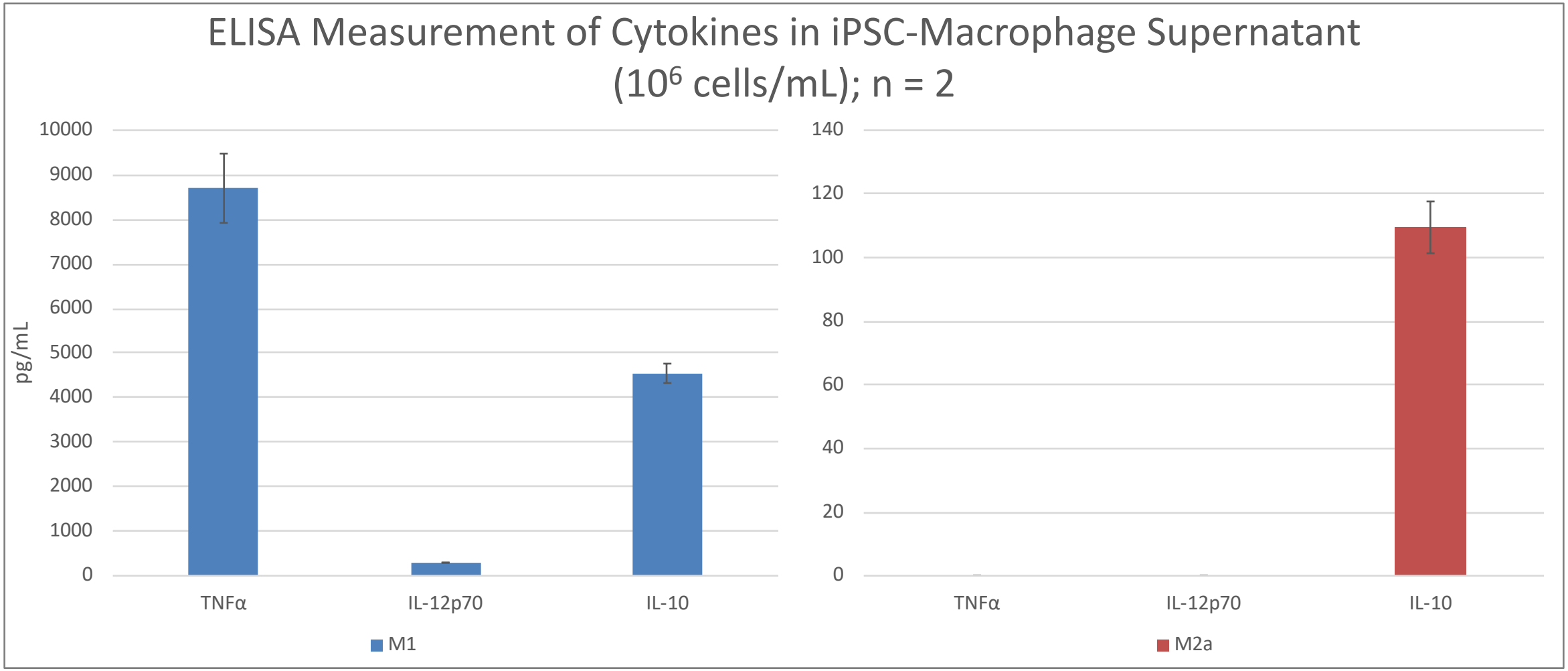
Cytokine Secretion from iPSC-Derived Macrophages (M1 and M2a)



- iPSC-Macrophages capable of enhanced cytokine secretion after stimulation into both pro- and anti-inflammatory phenotypes

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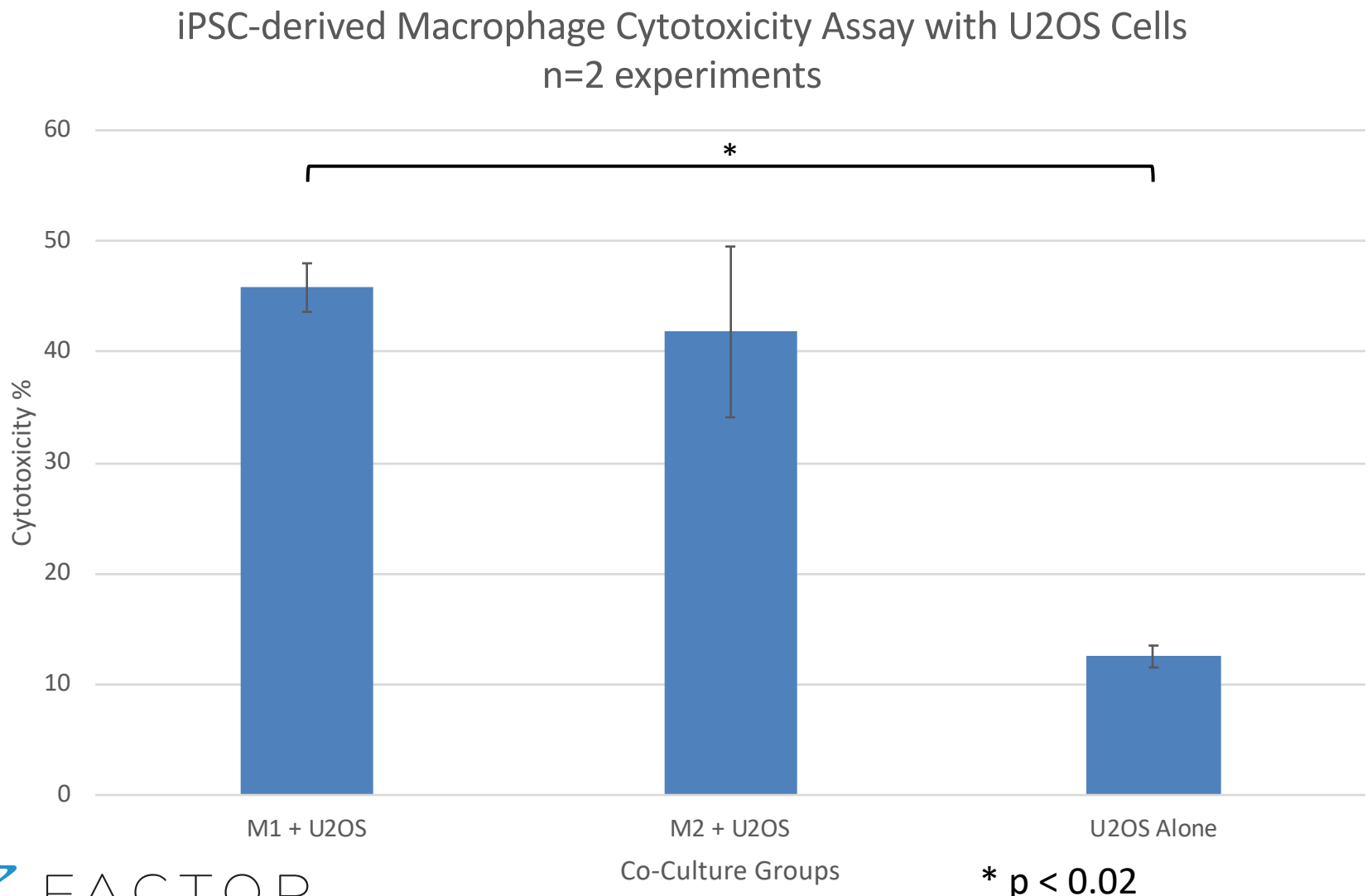
iPSC-Derived Macrophages Kill U2OS Osteosarcoma Cells *in Vitro*



* $p < 0.02$

- CFSE cytotoxicity assay
- iPSC-Macrophages capable of killing 45% of U2OS cancer cells on average when co-cultured at 5:1 E:T ratio

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Summary

- First reported differentiation and characterization of mRNA-reprogrammed iPSCs into monocyte-derived macrophages
- iPSC-monocytes capable of cryopreservation and recovery with similar yields compared to PBMC alternatives
- iPSC-monocytes able to differentiate into macrophages and polarize in presence of stimulants; cytotoxic towards U2OS cancer cells
- mRNA iPSCs show promise as a perpetual source of therapeutic macrophages

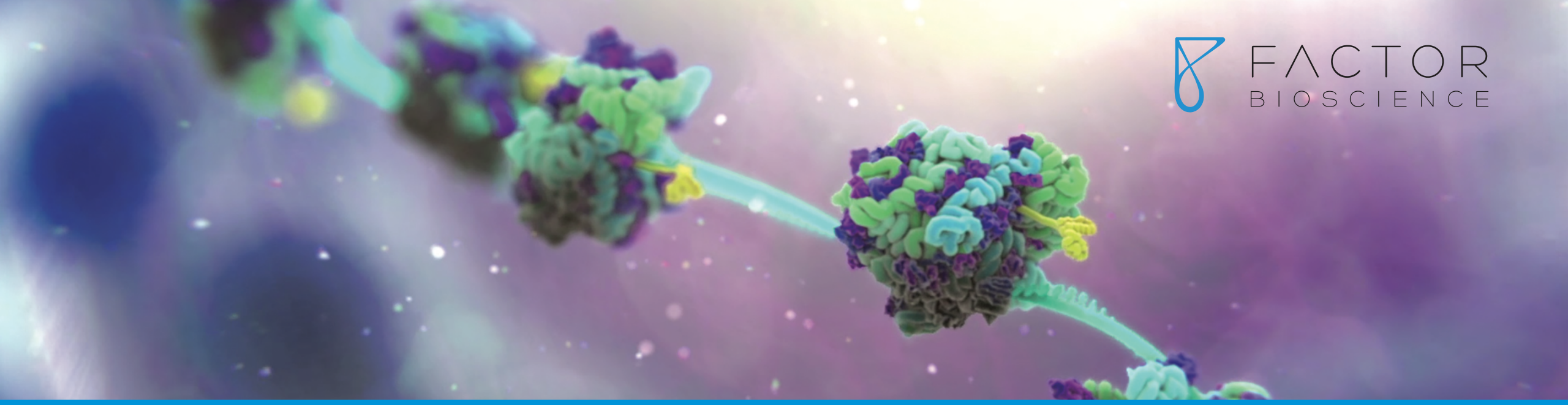
Future Directions

- Optimization of monocyte cryopreservation and recovery
- Characterization of gene expression in polarized macrophages
- Functional assessment of phagocytosis, antigen presentation (T cell activation), tumor infiltration
- Scaling up differentiation process to support pre-clinical and clinical development
- Investigating gene editing for targeting molecules (e.g. chimeric antigen receptors), cytokine expression cassettes, etc.

Author Disclosures

I.H., C.R. and M.A. are employees of Factor Bioscience Inc.

I.H., C.R. and M.A. are inventors on patents assigned to Factor Bioscience Inc.



Thank you!

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