CD34+ Hematopoietic Stem Cell Differentiation Platform

Oncology & Immunology Unit, WuXi Biology

2023.06

Outline

- **Stem cell biology**
- **CD34$^+$ hematopoietic stem cell differentiation platform**
  - Monocyte/Macrophage differentiation and polarization
  - Neutrophil differentiation
  - Eosinophil differentiation
  - Mast cell differentiation and degranulation assay
  - Megakaryocyte and Platelet differentiation
  - Erythroid differentiation

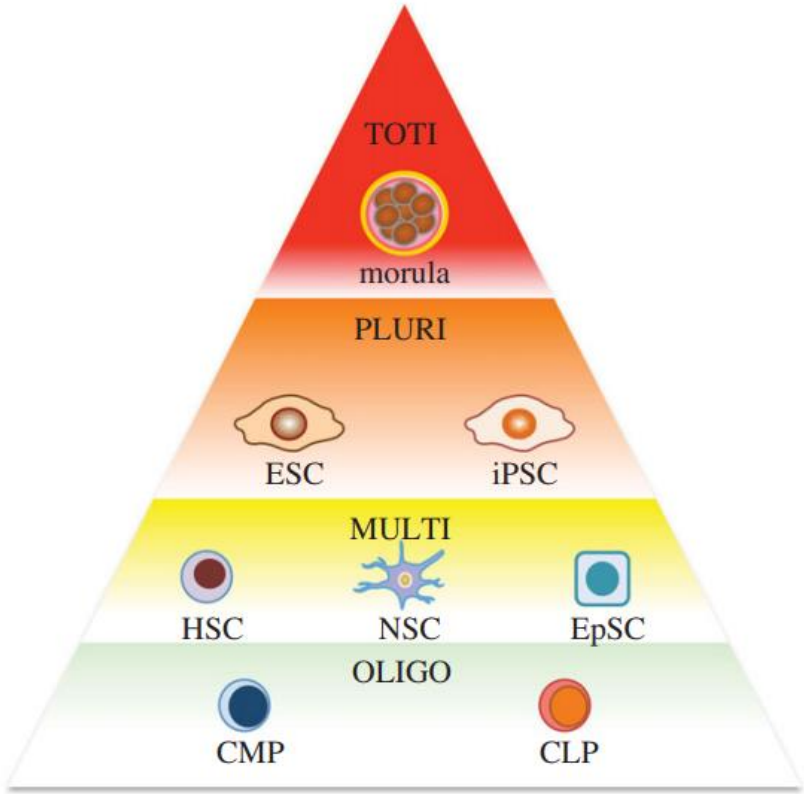
Stem cell biology

Classification of stem cells

- The stem cells classified into totipotent, pluripotent, multipotent, oligopotent and unipotent based on the range of differentiation potentials.

Table 1 Classification of stem cells derived from various sources based on their differentiation potential

Type of stem cells	Developmental potency	Examples
Totipotent	Ability to differentiate into all cell types and a functional organism	Zygote and the first few cells that result from the division of the zygote
Pluripotent	Ability to differentiate into almost all cell types but cannot form a functional organism	ESCs
Multipotent	Ability to differentiate into a closely realted family of cells	Hematopoietic (adult) stem cells, MSCs, dental pulp stem cells, etc.
Oligopotent	Ability to differentiate into a few cell types	Lymphoid (adult) or myeloid stem cells
Unipotent	Ability to only produce cells of their own type, but have the property of self-renewal required to be labeled a stem cell	Muscle (adult) stem cells



Acta Biochim Biophys Sin. 2012, 44: 463–475
Phil Trans R Soc B. 2013, 368:20110334

Stem cell biology

Advantages and disadvantages of different stem cells

Table 1. Comparison of the advantages and disadvantages of stem cells.

Type	Advantages	Disadvantages	References
Embryonic stem cells	Pluripotent, unlimited quantity, heritable gene defect correction, off-the-shelf product potential, broad biomedical applications	Allogeneic rejection, limited accessibility, complicated differentiation, teratoma risk	Zhang et al. 2002; Rideout et al. 2002; Swijnenburg et al. 2008*a*, 2008*b*; Bel et al. 2010; Lin et al. 2010; Lü et al. 2010; Song et al. 2010; Dai et al. 2011; Deuse et al. 2011; Xiong et al. 2011
Induced pluripotent stem cells	Pluripotent, autologous, pathology-specific cell production, broad biomedical applications	Susceptible to autologous pathology, low induction efficiency, awaiting standardized production, complicated differentiation, teratoma risk, methodological oncogenesis, induced histoincompatability	Maherali and Hochedlinger 2008; Nelson et al. 2009; Miura et al. 2009; Woltjen et al. 2009; Blin et al. 2010; van Laake et al. 2010; Bar-Nur et al. 2011; Mauritz et al. 2011; Pearl et al. 2011; Narsinh et al. 2011
Hematopoietic stem cells	Multipotent, paracrine effects, autologous, standardized isolation, many biomedical applications	Susceptible to autologous pathology, unable to sustain cells ex vivo, limited number, limited accessibility, limited to hematopoietic lineage	Nygren et al. 2004; Fujita et al. 2007; Templin et al. 2008; Ha et al. 2010; Sun et al. 2010; Li et al. 2010
Mesenchymal stem cells	Multipotent, paracrine effects, autologous, standardized isolation, many biomedical applications	Susceptible to autologous pathology, complicated accessibility, low transdifferentiation potential	Miyahara et al. 2006; Hare et al. 2009; Quevedo et al. 2009; Schuleri et al. 2009; Hatzistergos et al. 2010; Huang et al. 2010; Lee et al. 2010; Chong et al. 2011
Endothelial progenitor cells	Unipotent, paracrine effects, autologous, high accessibility, many biomedical applications	Susceptible to autologous pathology, low quantity, undefined or undefinable immunophenotype, awaiting standardized isolation	Werner et al. 2005; Abou-Saleh et al. 2009; Desai et al. 2009; Frederick et al. 2010; Achneck et al. 2011; Hynes et al. 2011; Richardson and Yoder 2011
Organ-specific precursors (e.g., CSCs, NSCs, SkMBs, PSCs)	Unipotent, autologous, organ-specific applications	Susceptible to autologous pathology, low quantity and (or) accessibility, undefined phenotype(s), awaiting standardized isolation	Andersen et al. 2009; Domian et al. 2009; Hansson et al. 2009; Lee et al. 2011; Le Belle et al. 2011; Leri et al. 2011; Smart et al. 2011; Smukler et al. 2011

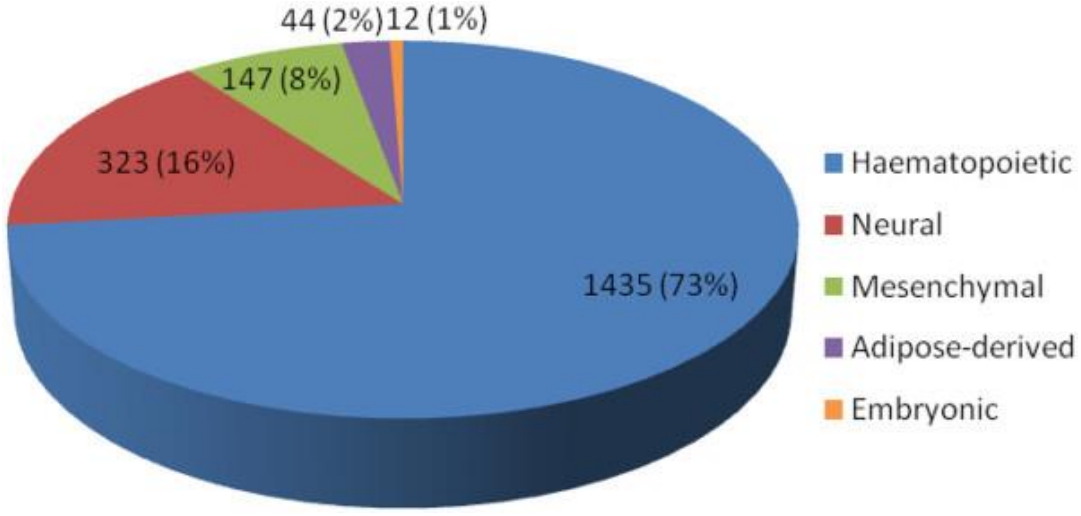
Note: CSCs, cardiac stem cells; NSCs, neural stem cells; SkMBs, skeletal myoblasts; PSCs, pancreatic stem cells.

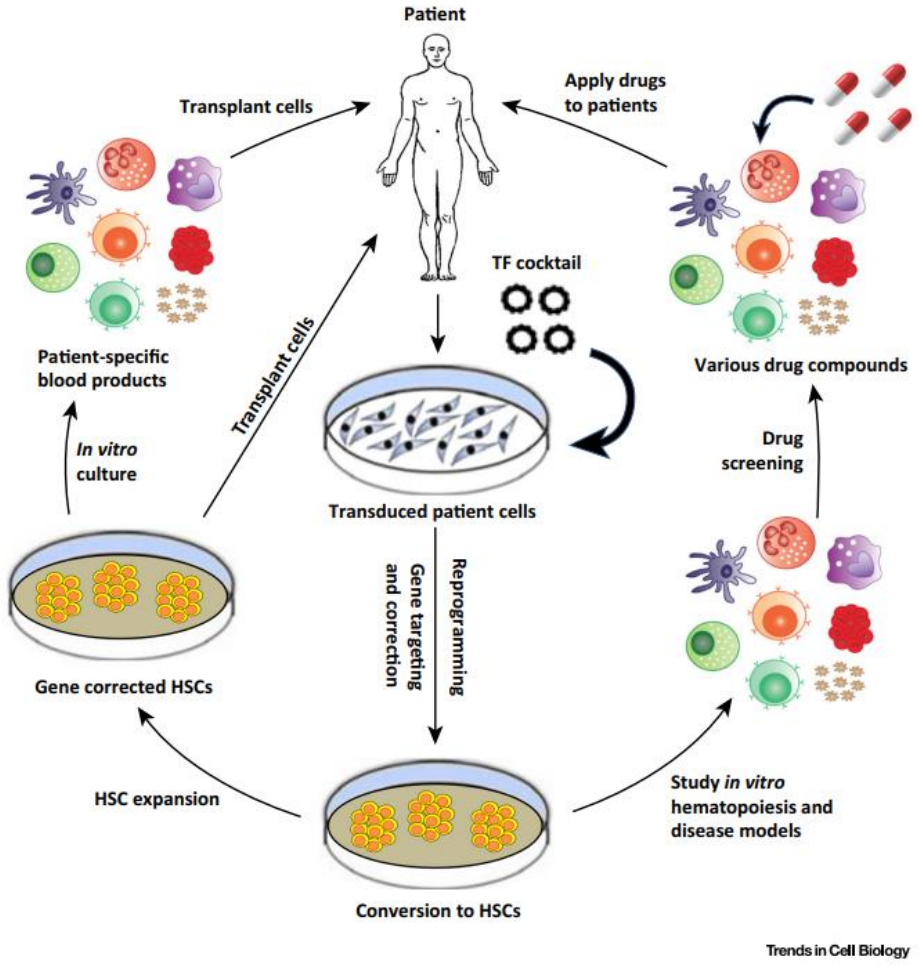
Canadian journal of physiology and pharmacology, 2012, 90(3): 327-335.

Stem cell biology

Hematopoietic stem cells

- HSCs (Hematopoietic stem cells) are the most application of stem cells in open clinical trials.
- HSCs could be used in a variety of different studies, including gene editing, transplantation, drug screening to identify novel therapeutics for a variety of diseases.

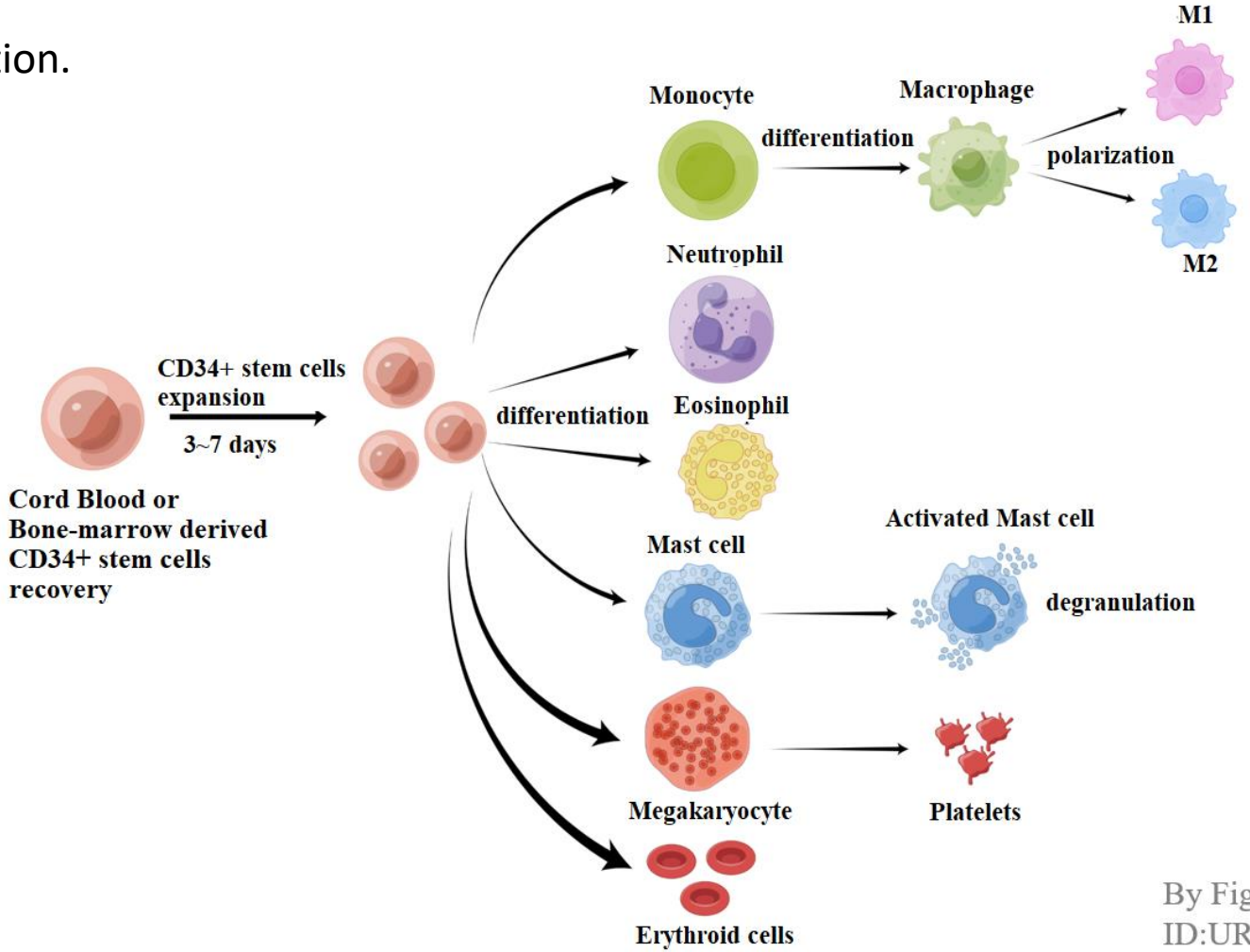




Stem Cell Research & Therapy, 2013, 4(5):54
Trends in cell biology, 2015, 26(3): 202-214.

CD34⁺ hematopoietic stem cell differentiation platform

- CD34⁺ stem cells can be obtained from **commercial umbilical cord blood** and **bone marrow**.
- CD34⁺ stem cell differentiation platform can facilitate drug testing or screening involved with immune cell differentiation.



By Figdraw
ID:URORA2ff9d

Monocyte/Macrophage differentiation and polarization

M1/M2 phenotyping and function analysis

CD34⁺ stem cell recovery and expansion

↓

CD14⁺ monocyte differentiation induced by cytokines

↓

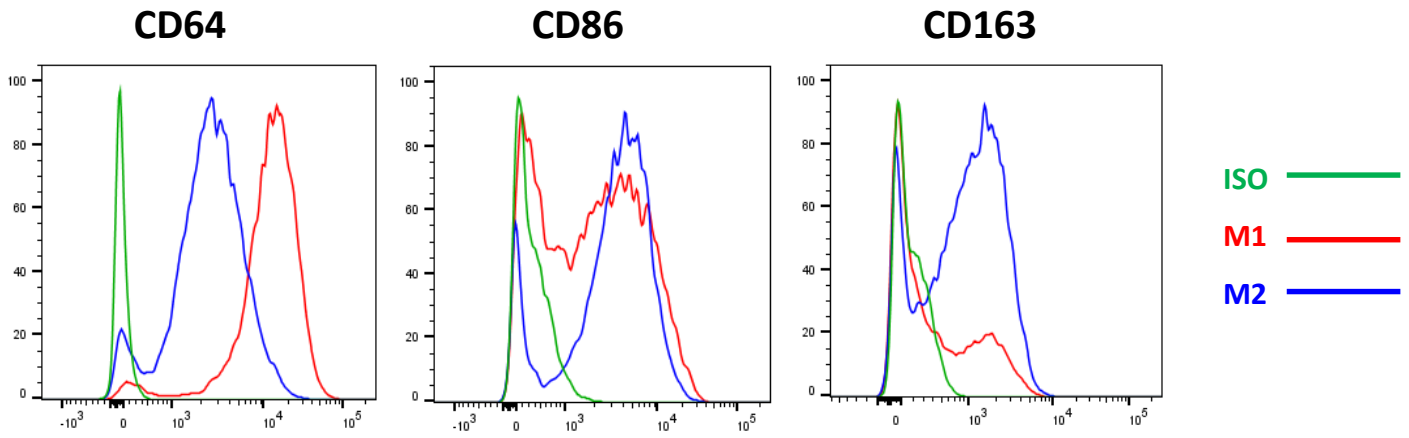
Macrophage differentiation from differentiated CD14⁺ monocytes

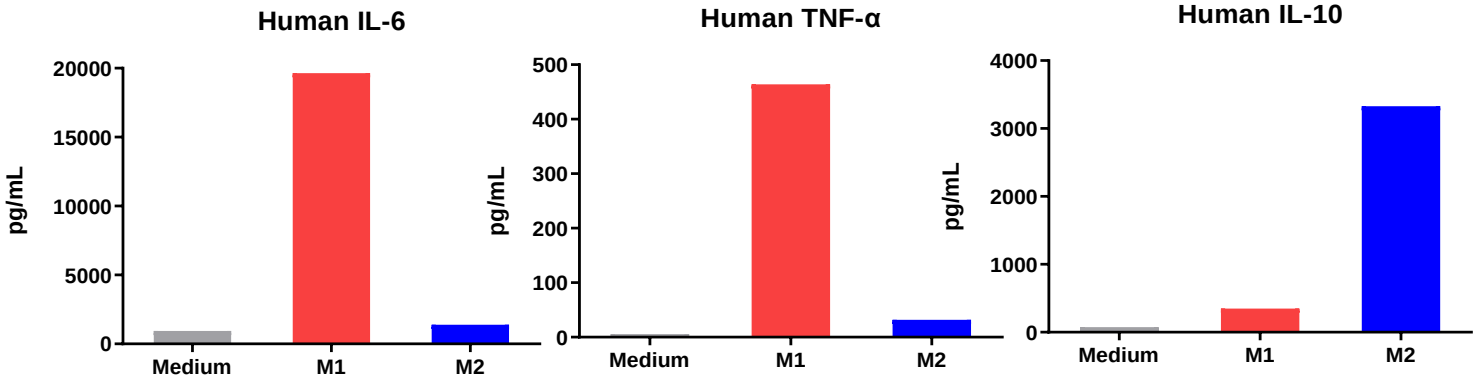
↓

Polarize macrophage to be M1 and M2

↓

Detection of M1/M2 marker by FACS (CD64/CD86/CD163) and cytokine production by ELISA





Neutrophil differentiation

FACS validation and summary of Neutrophil_CD15, CD11b and CD66b

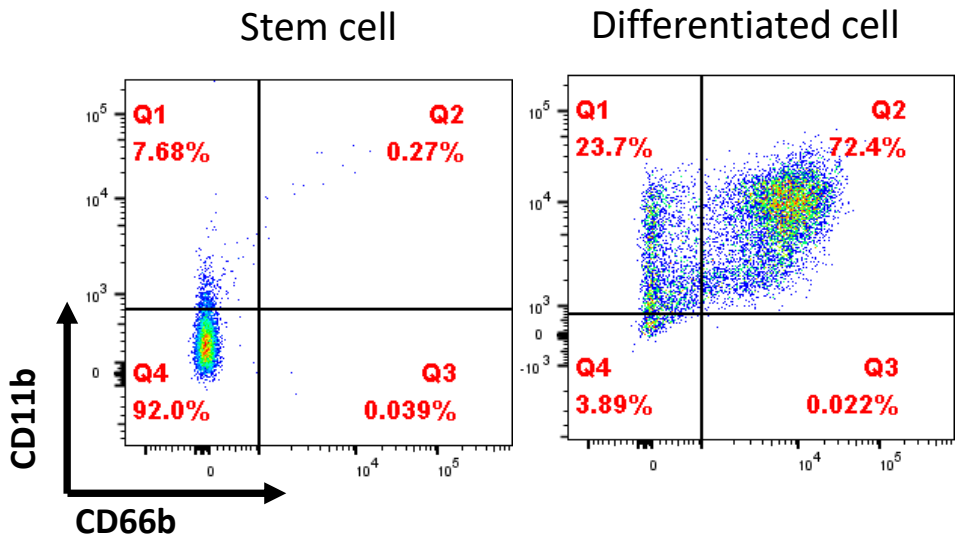
CD34⁺ stem cell recovery and expansion

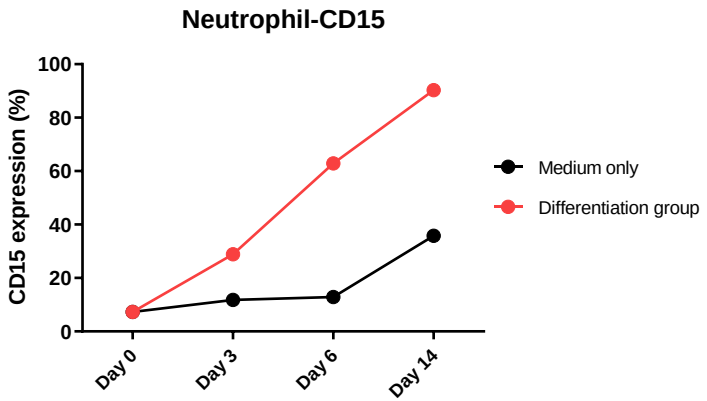
↓

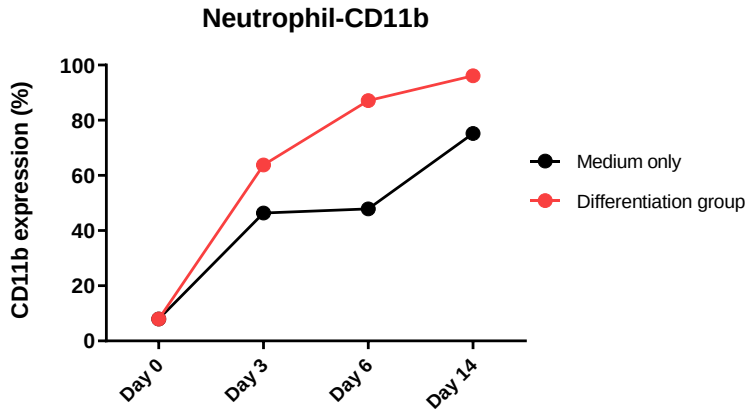
Neutrophil differentiation induced by cytokines

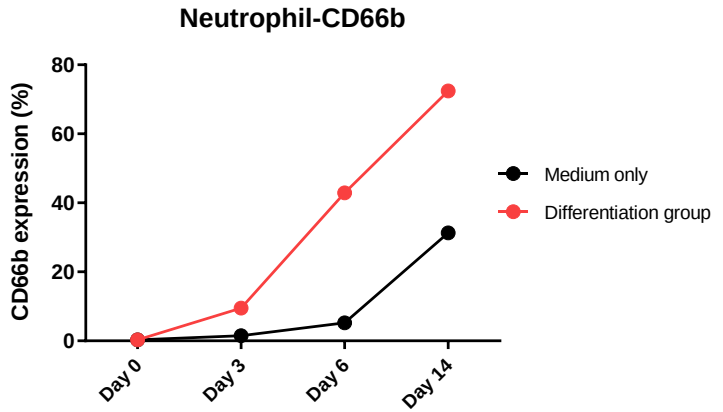
↓

FACS analysis of CD15, CD11b and CD66b on Day 0, 3, 6 and 14









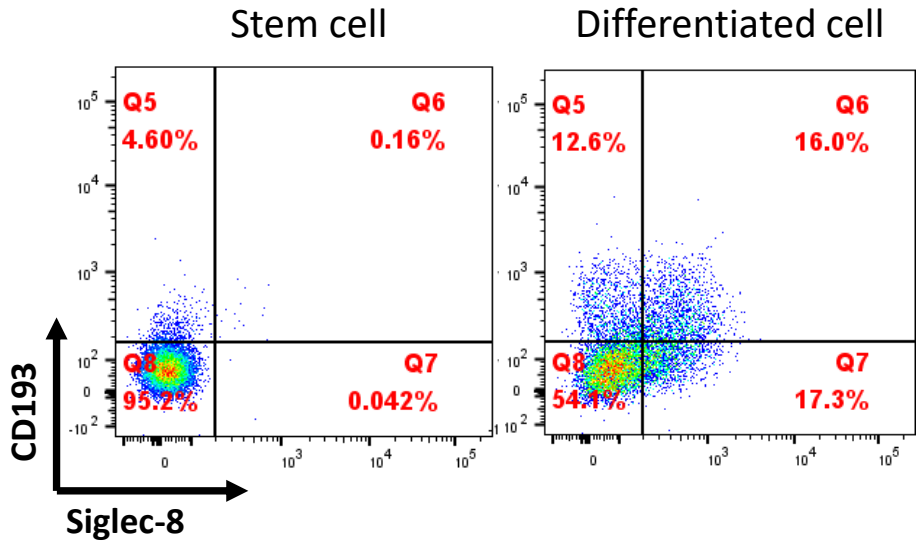
Eosinophil differentiation

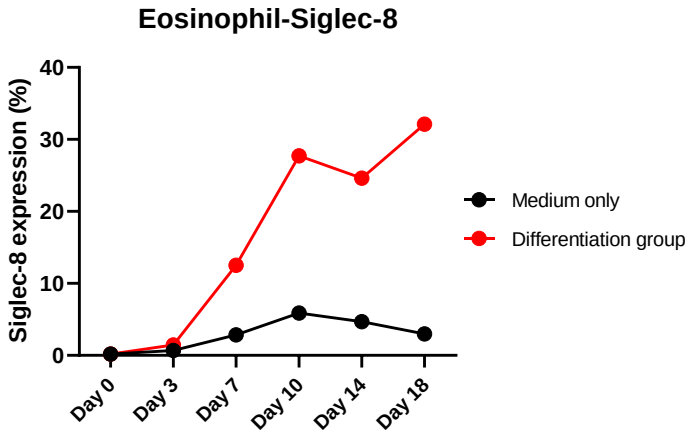
FACS validation and summary of Eosinophil_Siglec-8 and CD193

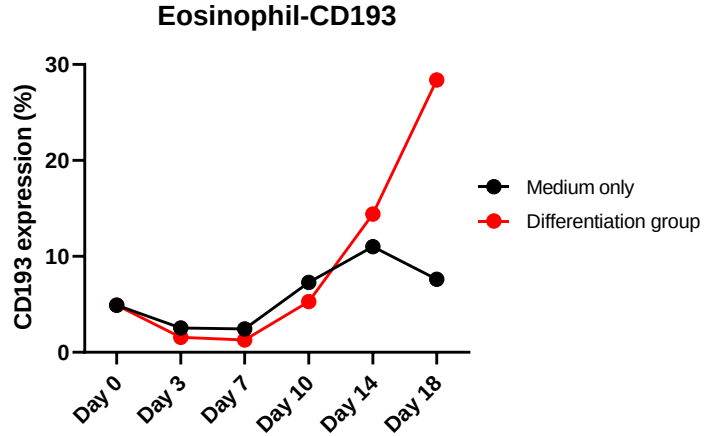
CD34⁺ stem cell recovery and expansion

Eosinophil differentiation induced by cytokines

FACS analysis of Siglec-8 and CD193
on Day 0, 3, 7, 10, 14 and 18







Mast cell differentiation

FACS validation and summary of Mast cell_C-kit and FcεRI

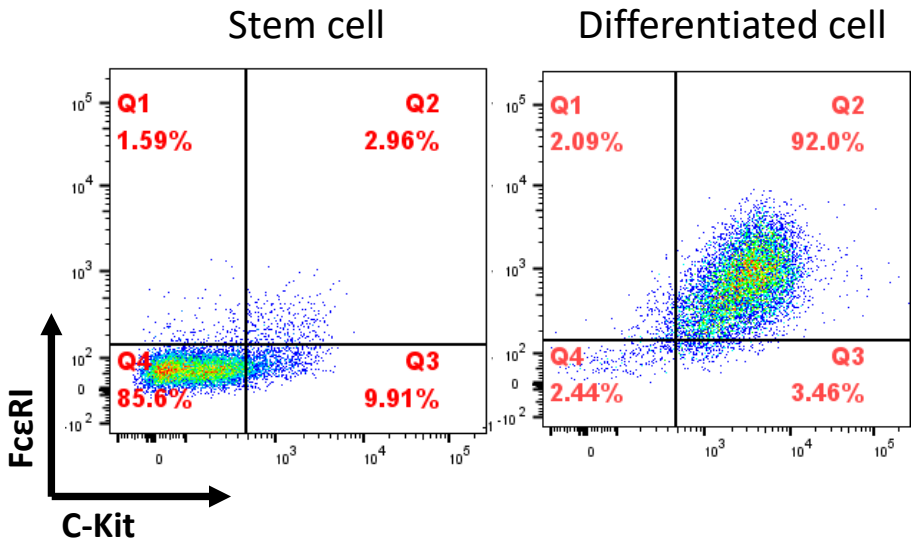
CD34⁺ stem cell recovery and expansion

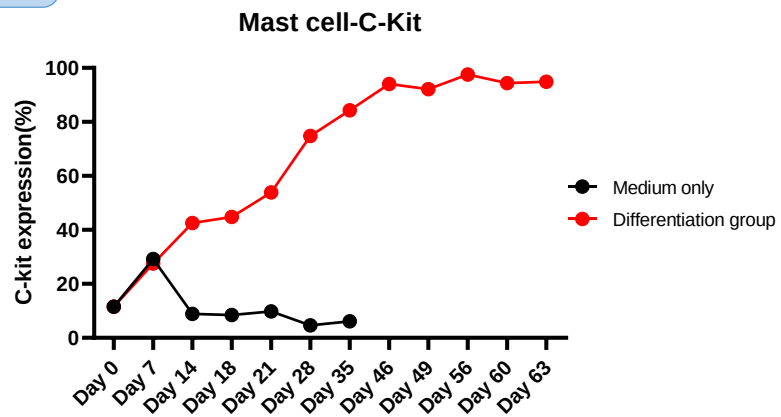
↓

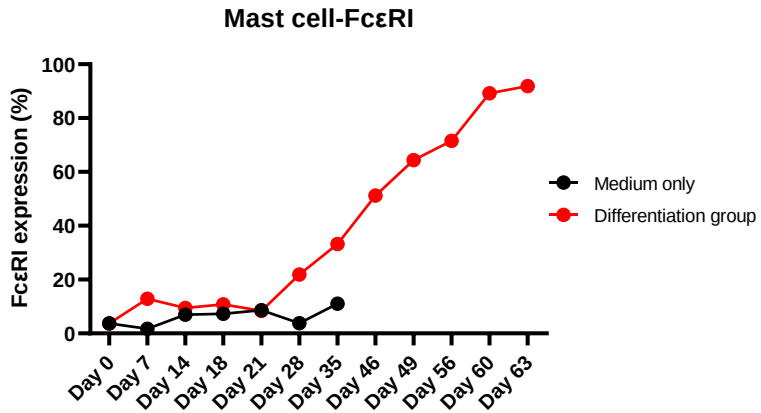
Mast cell differentiation induced by cytokines

↓

FACS analysis of C-kit and FcεRI on Day 0, 7, 14, 18, 21, 28, 35, 46, 49, 56, 60 and 63







Mast cell differentiation

Mast cell degranulation assay by β-hexosaminidase release

Plate differentiated mast cell in 96-well plate

↓

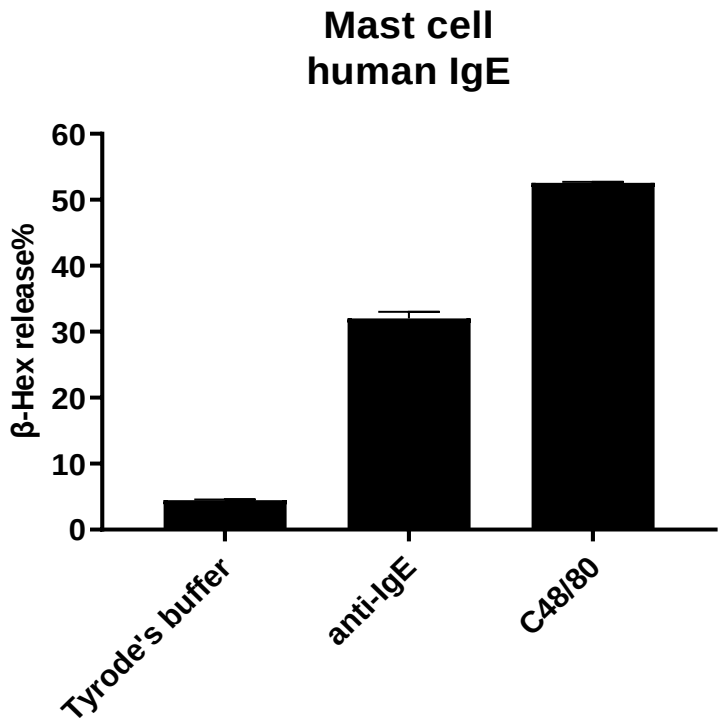
Stimulate with human IgE for 24h

↓

Add anti-human IgE for 1h

↓

Collect the supernatant for β-hexosaminidase



Megakaryocyte and Platelet differentiation

FACS validation and summary of Megakaryocyte_CD41, CD42b and CD61

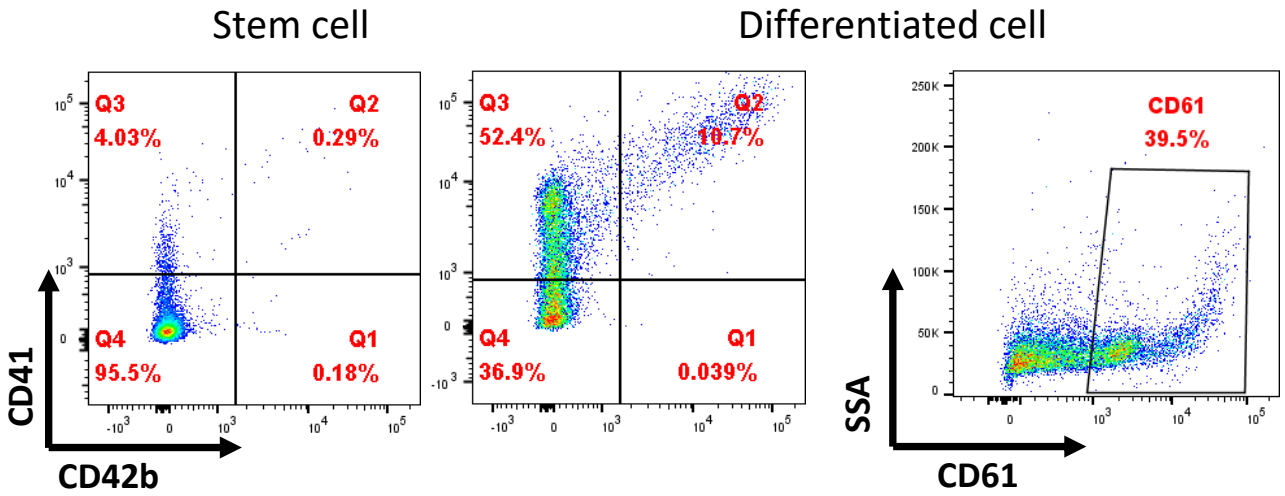
CD34⁺ stem cell recovery and expansion

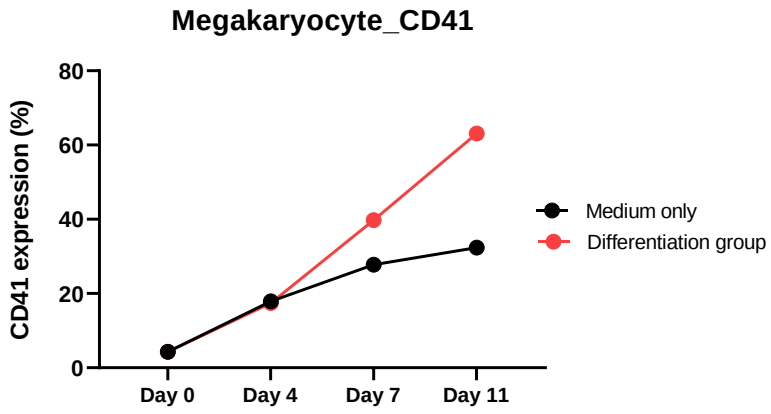
↓

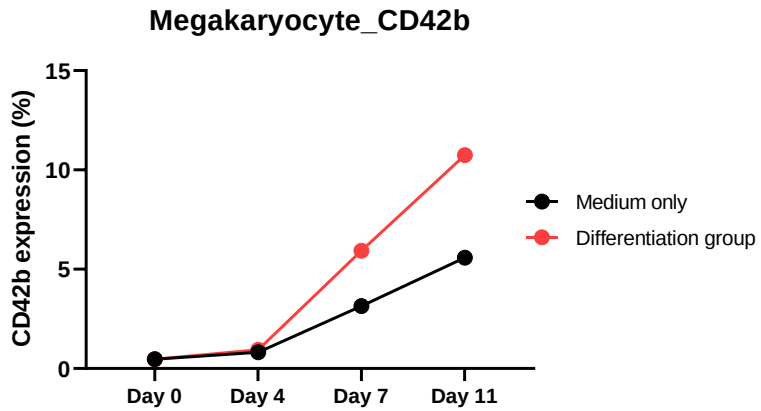
Megakaryocyte differentiation and maturation induced by cytokines

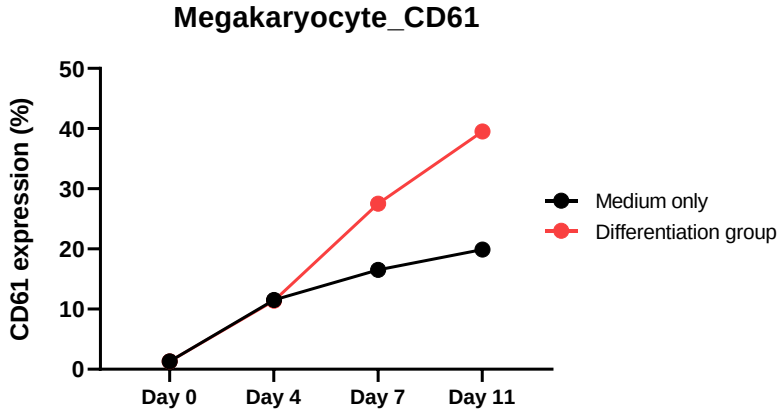
↓

Centrifuge at low speed and collect cell pellets for FACS analysis of CD41, CD42b and CD61 on Day 0, 4, 7 and 11









Megakaryocyte and Platelet differentiation

FACS validation and summary of Platelet_CD41, CD42b and CD61

CD34⁺ stem cell recovery and expansion

↓

Megakaryocyte differentiation and maturation induced by cytokines

↓

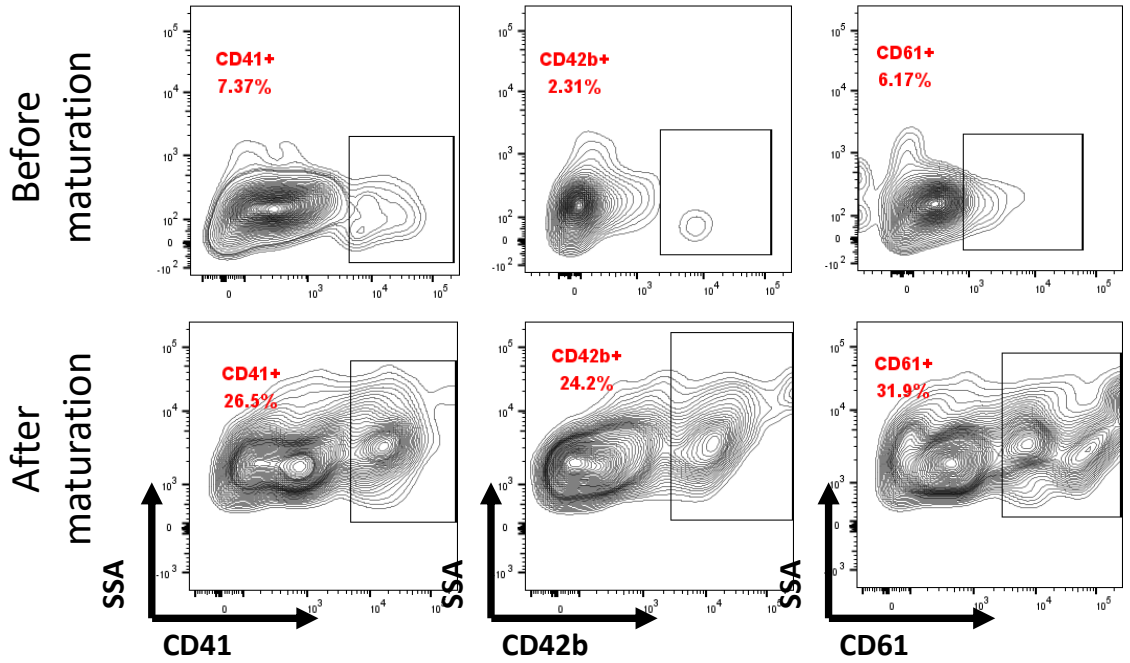
Centrifuge at low speed and collect cell supernatant on Day 11 and 14

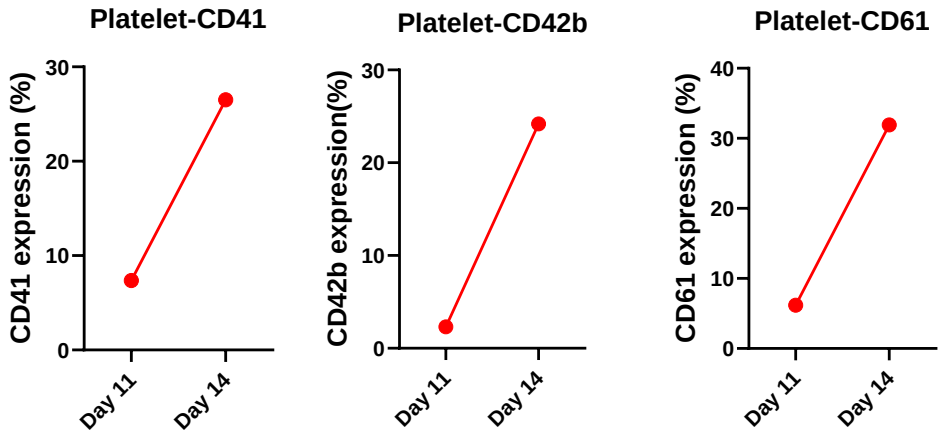
↓

Centrifuge the supernatant at high speed to collect Platelets

↓

FACS analysis of CD41, CD42b and CD61 on Day 11 and 14





Erythroid differentiation

FACS validation and summary of Erythroid_CD71 and CD235a

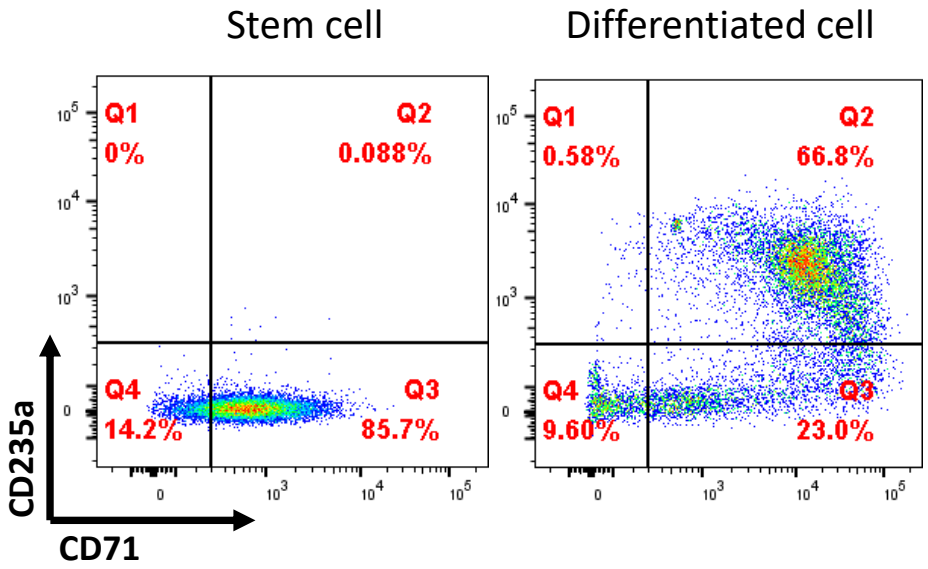
CD34⁺ stem cell recovery and expansion

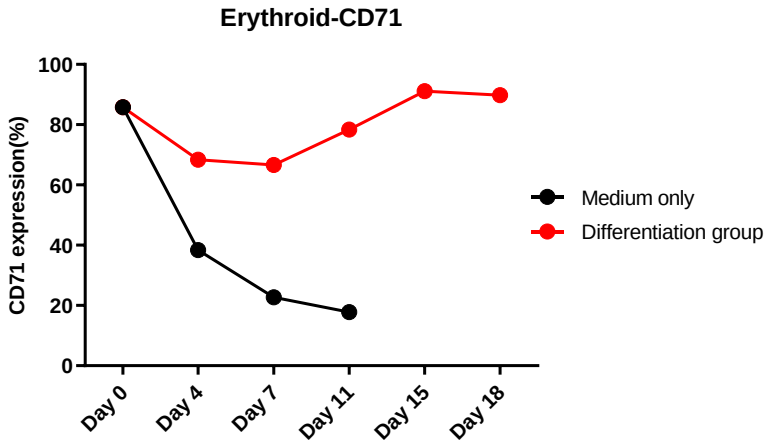
↓

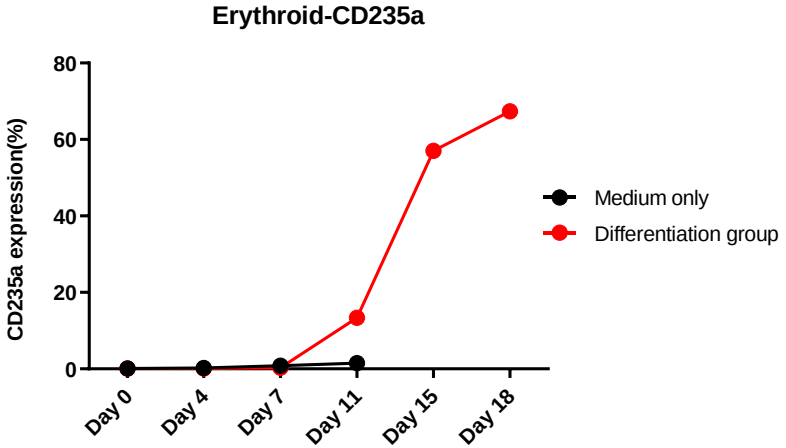
Erythroid differentiation induced by cytokines

↓

FACS analysis of CD71 and CD235a
on Day 0, 4, 7, 11, 15 and 18







OUR COMMITMENT

Improving Health. Making a Difference.

For questions and requests, please email to OIU-BD-Translation@wuxiapptec.com

https://onco.wuxiapptec.com