University of Otago
ŌTĀKOU WHAKAIHU WAKA

Te Whatu Ora
Health New Zealand
Capital, Coast and Hutt Valley

MALAGHAN INSTITUTE OF MEDICAL RESEARCH

A workflow for longitudinal biomarker discovery studies in human disease:
a focus on major surgery

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ISAC Marylou Ingram Scholar (2024-2028)

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Surgical Immunology Research Group

Ko wai au?

Ko Remutaka te maunga e rū nei taku ngākau.

The Remutaka Range is my mountain.

Ko Te Awa Kairangi te awa e mahea nei aku māharahara.

The Hutt River is my river.

Nō Te Awa Kairangi ki Tai ahau. Kei Te Awa Kairangi ki Tai ahau e noho ana.

I live in Lower Hutt.

He Pūkenga ahau mō Ōtākou Whakaihu Waka ki Poneke.

I work at the University of Otago, Wellington campus.

Acknowledgements

Surgical Immunology Research Group

- Connor Davis
- Emma Thompson
- Thomas Hartono
- Eushin Kang

Connor Davis,
Assistant Research Fellow

Wellington Cardiovascular Research Group

- Ceridwyn Jones
- Dr Ana Holley
- AProf Peter Larsen
- Michael Roberts

Ceridwyn Jones,
PhD student

Malaghan Institute of Medical Research

- Kate Pilkington

Te Whatu Ora – Capital, Coast and Hutt Valley

- Dr Lupe Taumoepeau
- Dr Scott Harding

Project funding

HRC Emerging Researcher First Grant

Deans Research Grant

Conference funding

Predicting adverse postoperative recovery

For surgery

Admission

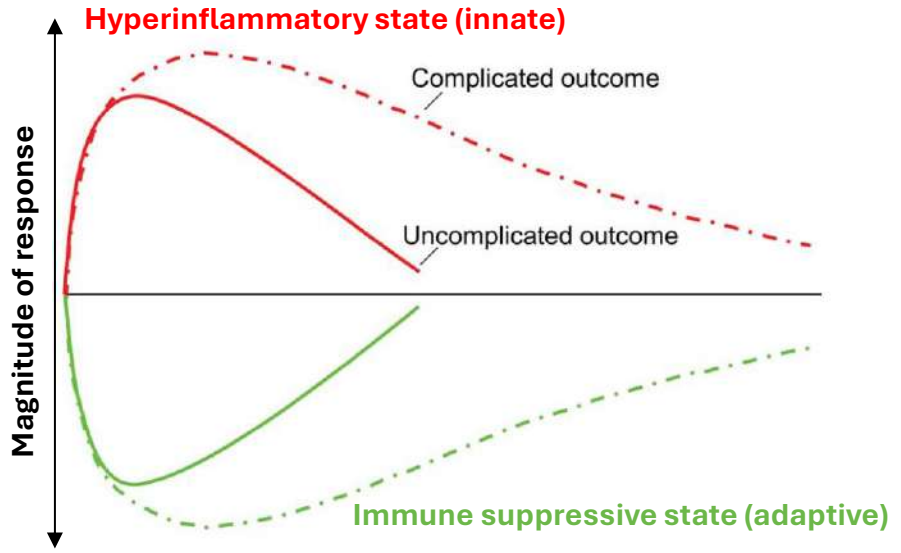
Surgery

Discharge

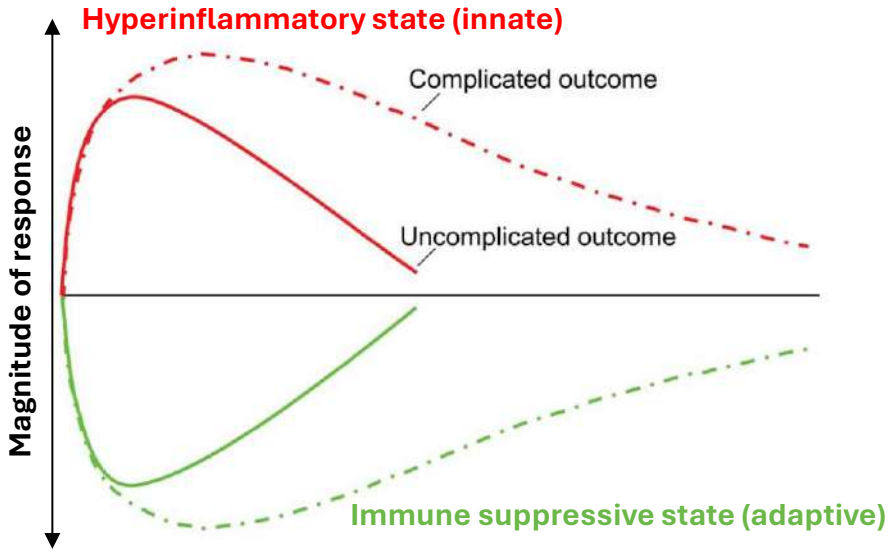
Postoperative course

Uneventful

- Acute perioperative events
- Deterioration in health
- Reoperation or readmission



Predicting adverse postoperative recovery



<u>Group aim:</u> to harness the immune response to surgery for biomarker discovery

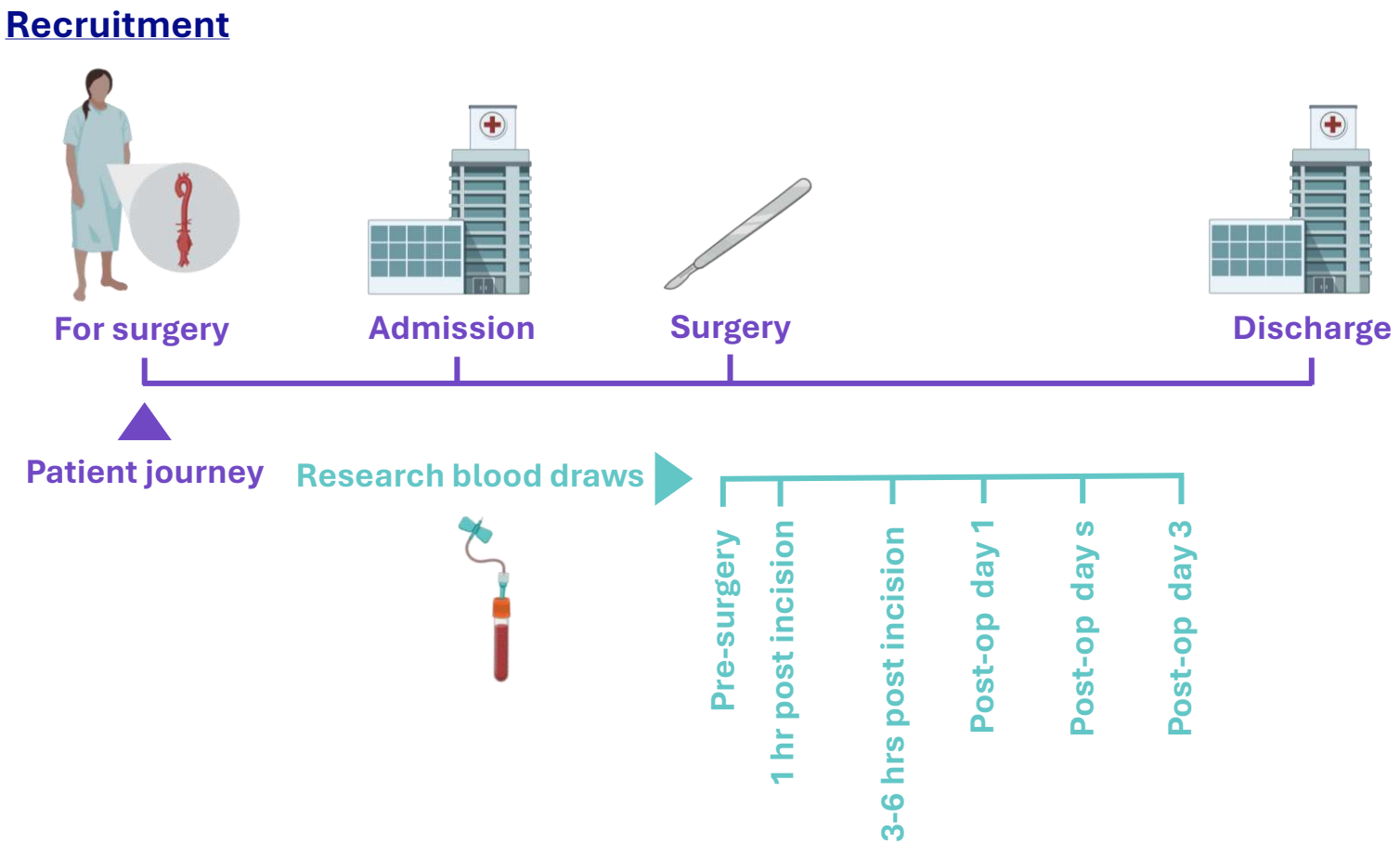
To design panels for monitoring the phenotype of circulating human immune cells.

To monitor the immune response to major surgery.

To determine what cell phenotypes are associated with a complicated postoperative course.

Predicting adverse postoperative recovery

To design panels for monitoring the phenotype of circulating human immune cells.



Predicting adverse postoperative recovery

To design panels for monitoring the phenotype of circulating human immune cells.

Panel design

Panel 1: Monocytes and DCs

Monocytes:
- Classical
- Intermediate
- Non-classical

Dendritic cells:
- pDCs
- cDC1s
- cDC2s

Panel 2: Neutrophils

- Mature
- Immature
- Immunosuppressive
- ‘Aged’

Panel 3: T, B and NK cells

T cells:
- Naïve and memory
- Tregs
- NKT cells
- γδ T cells

B cells:
- Naïve
- Memory
- Transitional
- Antibody secreting cells

NK cells:
- Early
- Mature

Adhesion | **Pattern recognition** | **Antigen presentation** | **Activation**

Predicting adverse postoperative recovery

*EDTA-anticoagulated peripheral blood

To design panels for monitoring the phenotype of circulating human immune cells.

Sample processing

Blood processing
- **Cell isolation**

Stain
- **Reagents**
- **Sequential staining**

Fix

Wash

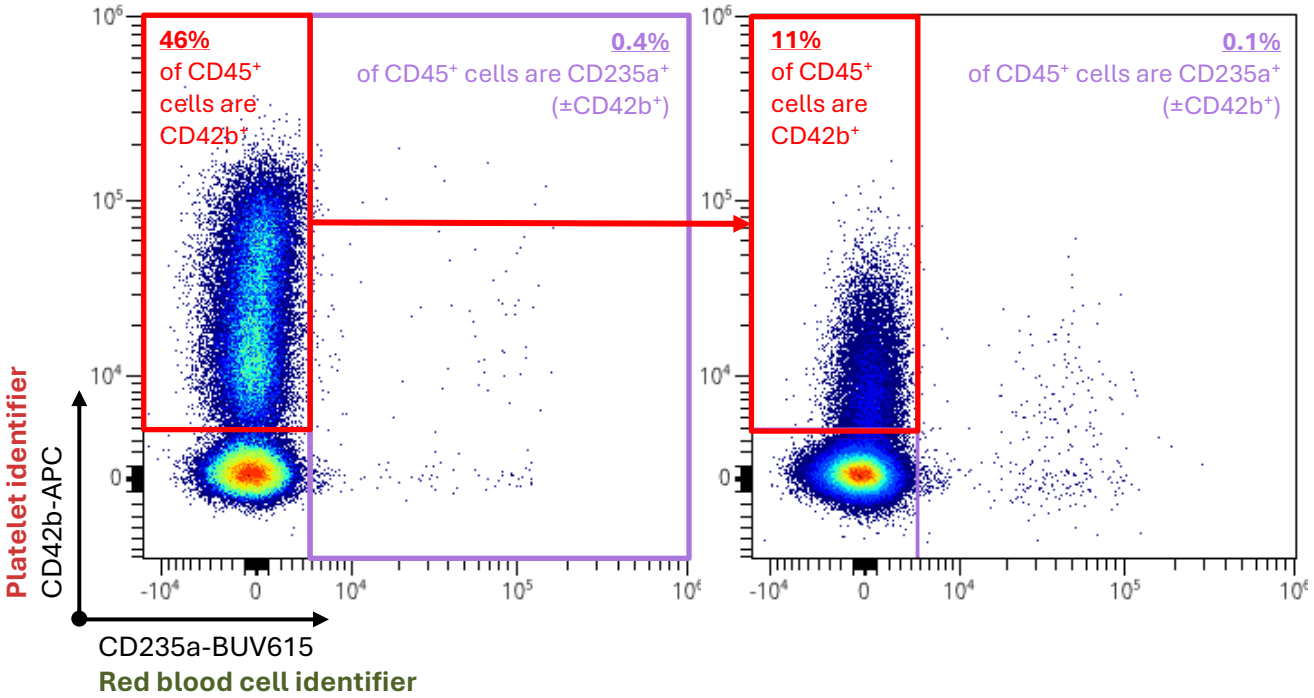
Acquire
- **Longitudinal monitoring**

Comparing platelet aggregation across isolations

Considerations for: cell isolation

*EDTA-anticoagulated peripheral blood

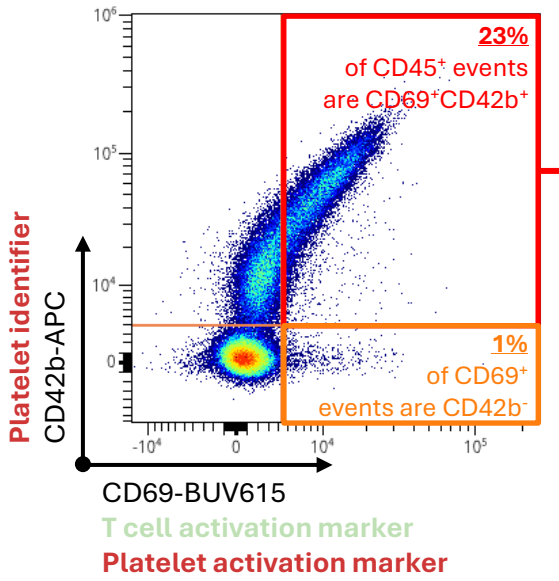
Density isolation



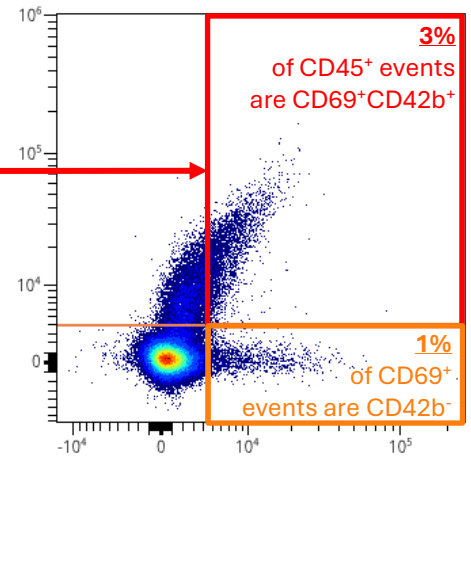
Magnetic isolation

"False" expression of markers co-expressed on platelets

Density isolation



Magnetic isolation



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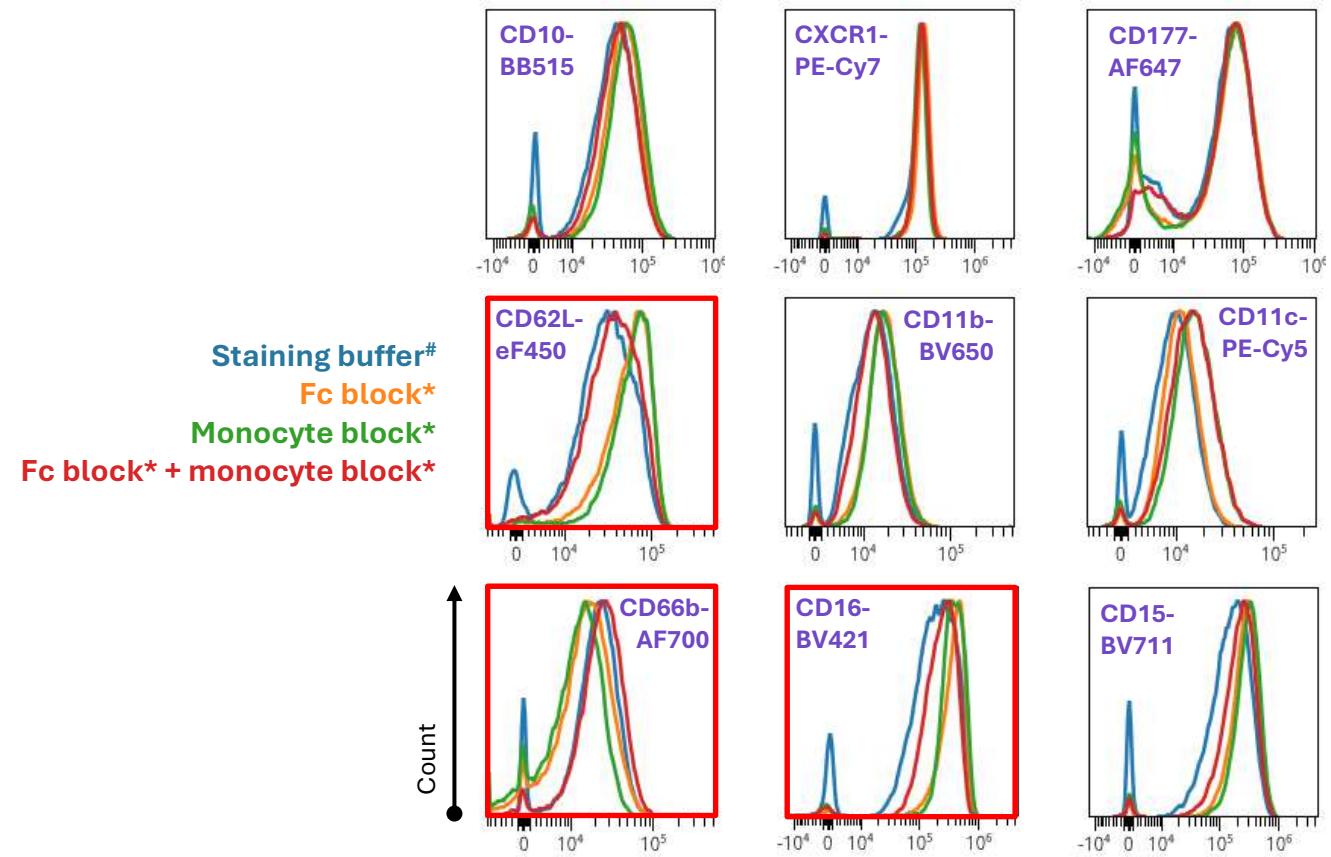
Panel 3: T, B and NK cells

Staining and blocking agents

Considerations for: cell isolation → regents



Staining buffer#
Fc block*
Monocyte block*
Fc block* + monocyte block*

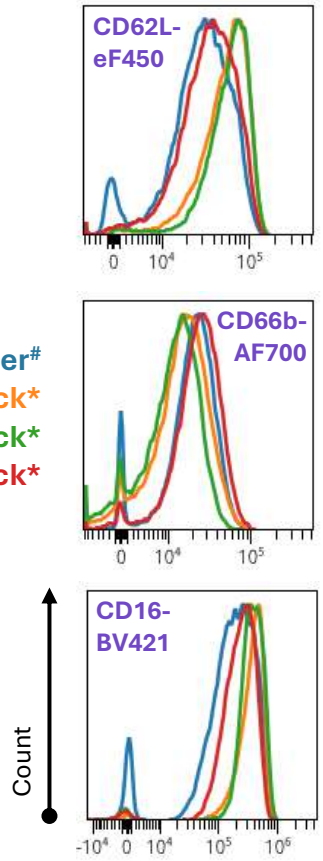
#Is Fc blocking during magnetic isolation sufficient for staining?
*BioLegend TruStain FcX blocker and True-Stain Monocyte Blocker

Panel 2: Neutrophils

Connor Davis,
Assistant Research Fellow

Staining and blocking agents

Considerations for: cell isolation → regents



Staining buffer[#]
Fc block*
Monocyte block*
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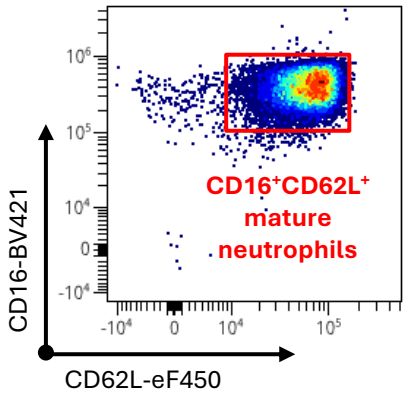
Monocyte blocker



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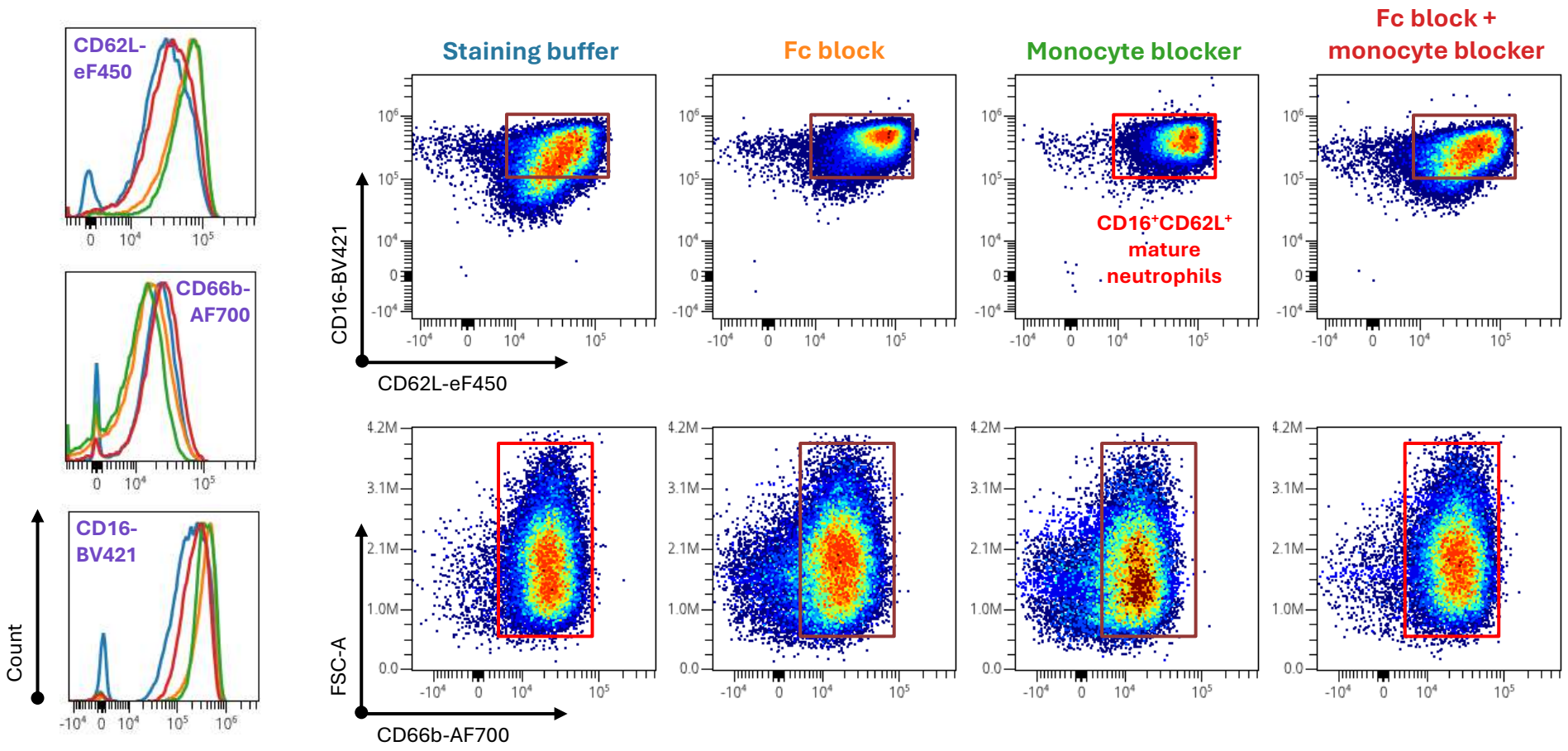

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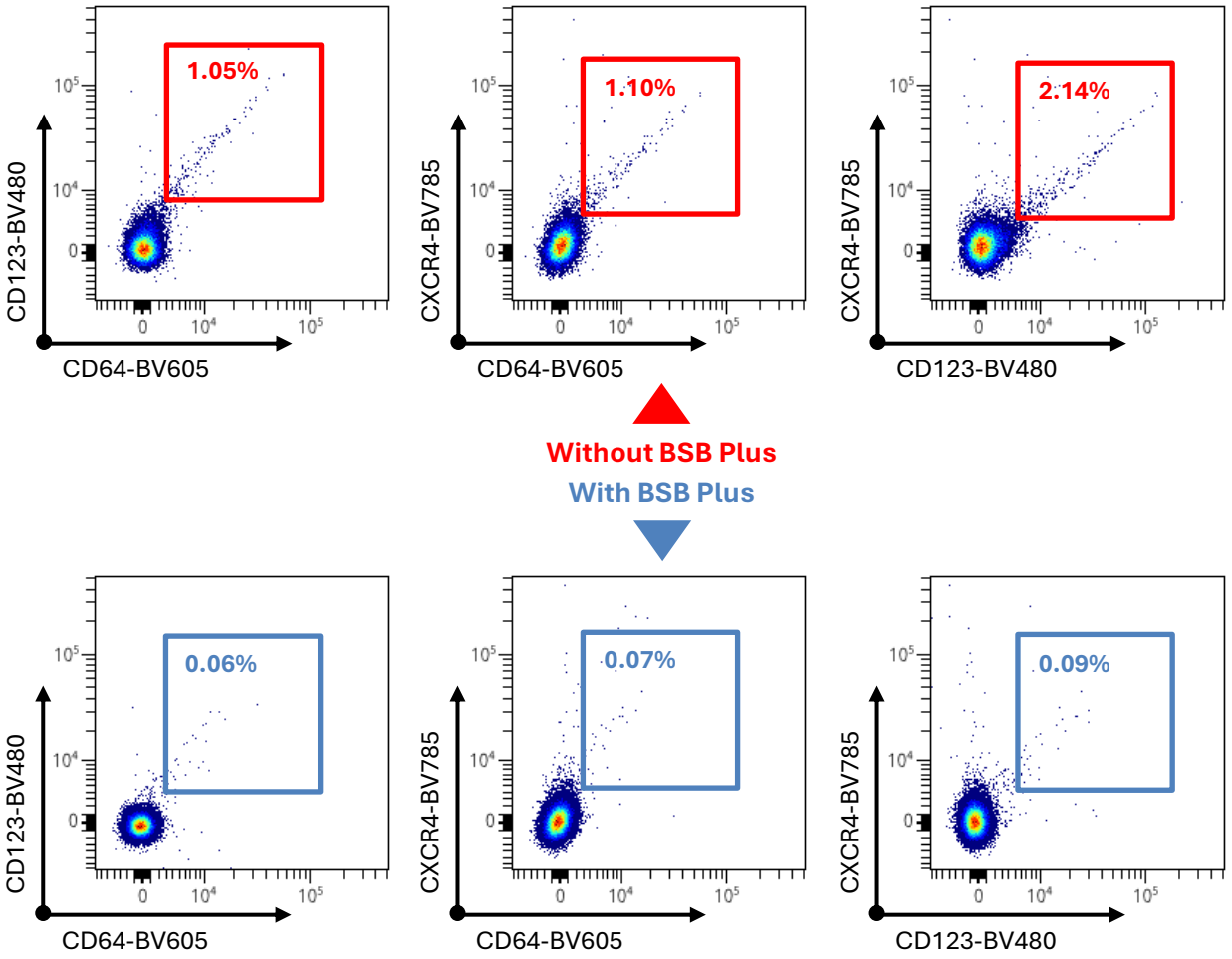
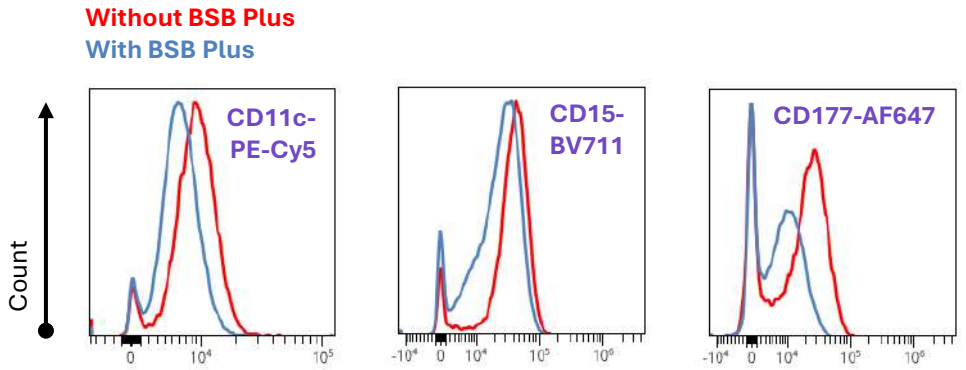




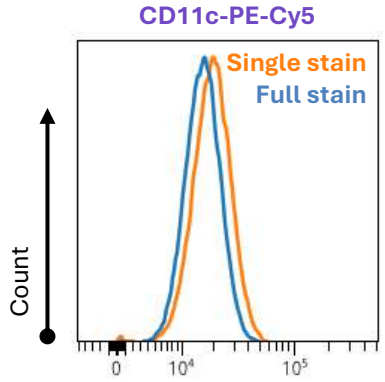
Panel 2: Neutrophils

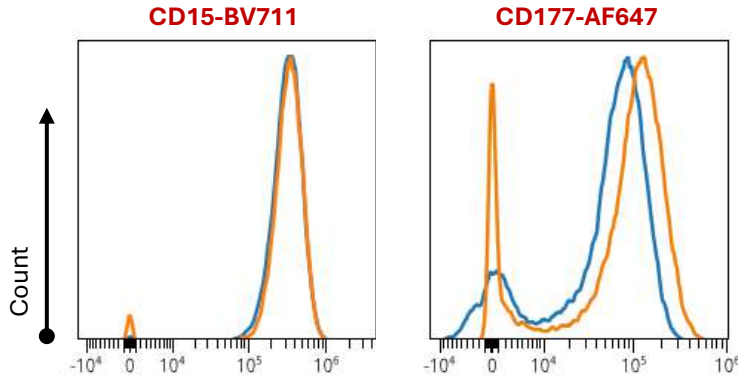
Ceridwyn Jones, PhD student

Improving marker resolution with sequential staining

Considerations for: cell isolation → regents → sequential staining

	Step 1 *30 minutes*	Step 2 *30 minutes*
1	**CD11c-PE-Cy5** CD62L-eF450 CXCR4-BV785 CXCR1-PE-Cy7	**All other antibodies**
2	**CD11c-PE-Cy5** **CD15-BV711** **CD177-AF647** CD62L-eF450 CXCR4-BV785 CXCR1-PE-Cy7	**All other antibodies**





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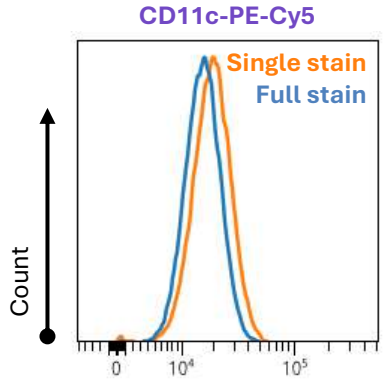
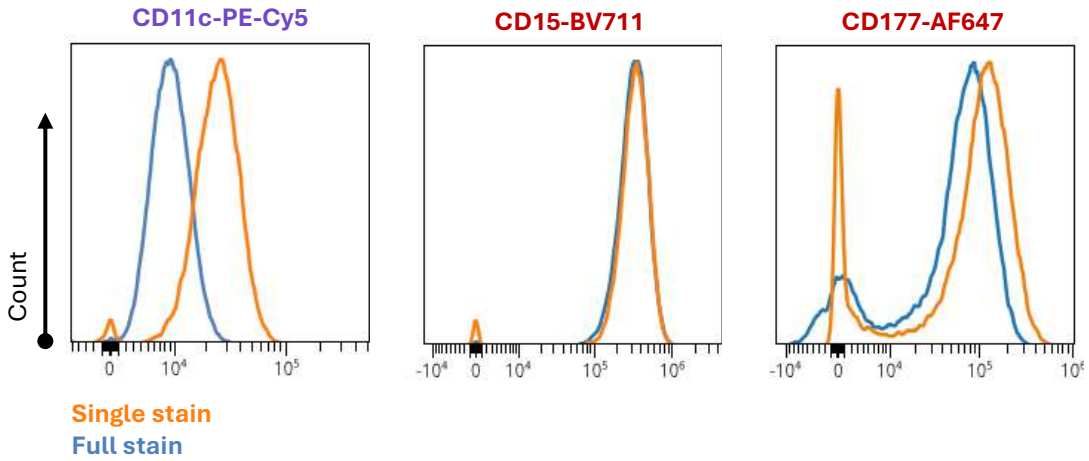




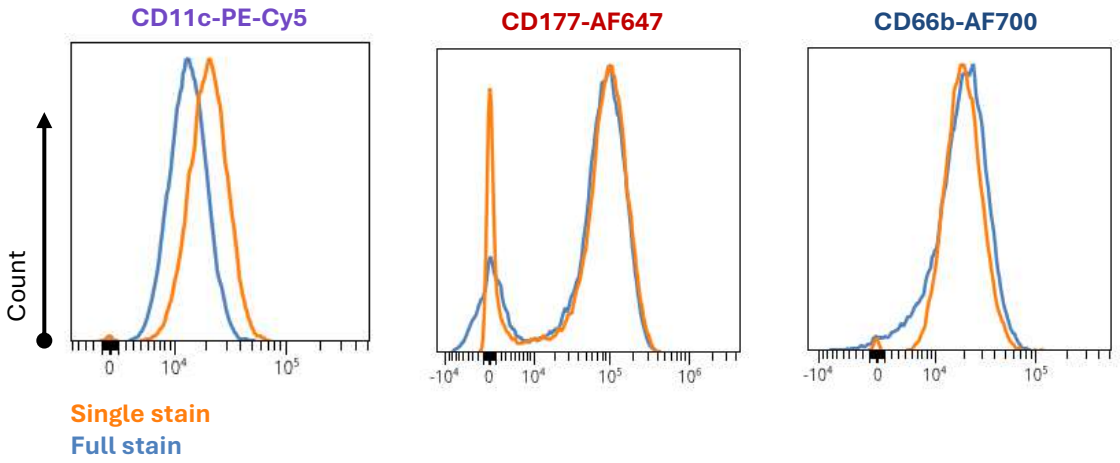
Panel 2: Neutrophils

Ceridwyn Jones,
PhD student

Improving marker resolution with sequential staining

Considerations for: cell isolation → regents → sequential staining

	Step 1 *10 minutes*	Step 2 *30 minutes*	Step 3 *30 minutes*
3	CD11c-PE-Cy5 CD177-AF647 CD66b-AF700	CD62L-eF450 CD15-BV711 CXCR4-BV785 CXCR1-PE-Cy7	All other antibodies



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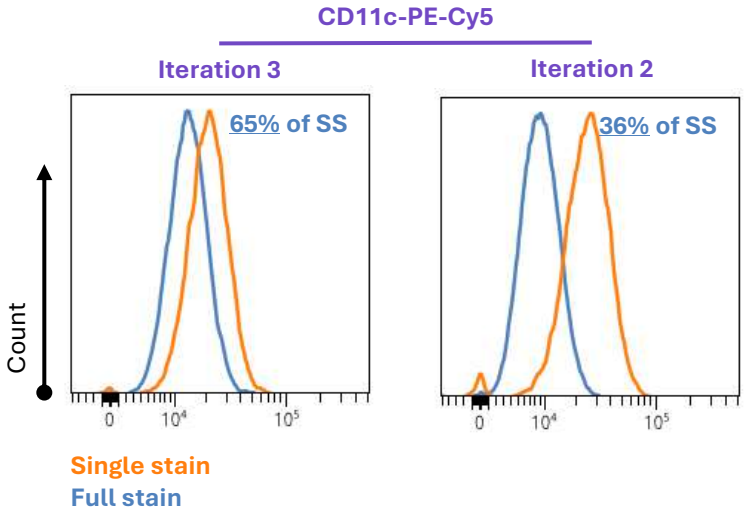


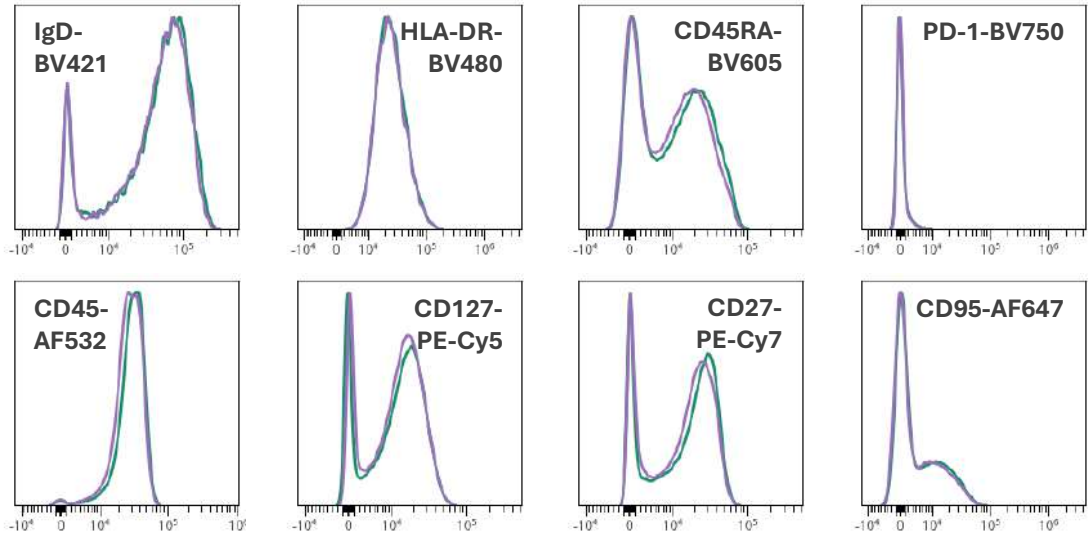
Panel 2: Neutrophils

Ceridwyn Jones,
PhD student

Validating batch preparation of the antibody cocktails

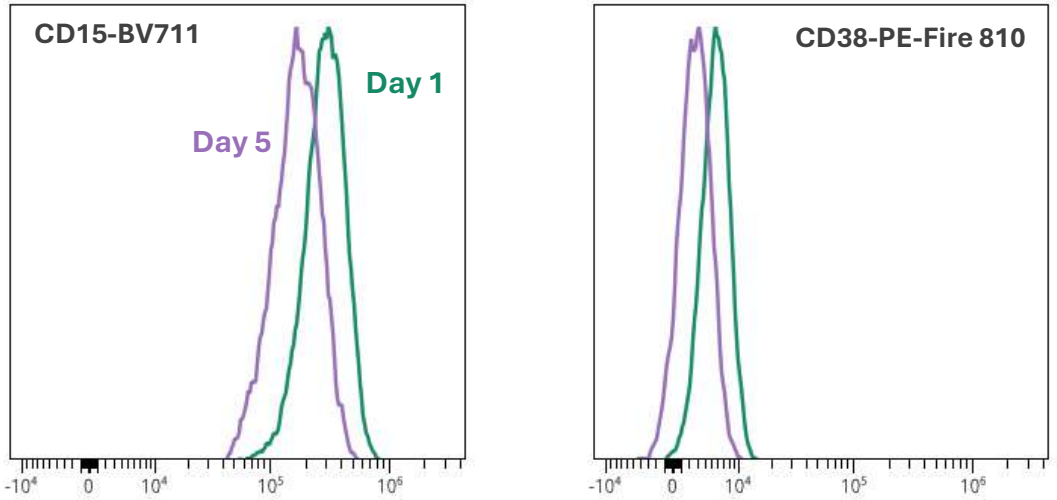
Considerations for: cell isolation → regents → sequential staining → longitudinal monitoring

Acceptable after storage



Day 1
Day 5

Added 'fresh' for each sample



Panel 3: T, B and NK cells

Panel 2: Neutrophils

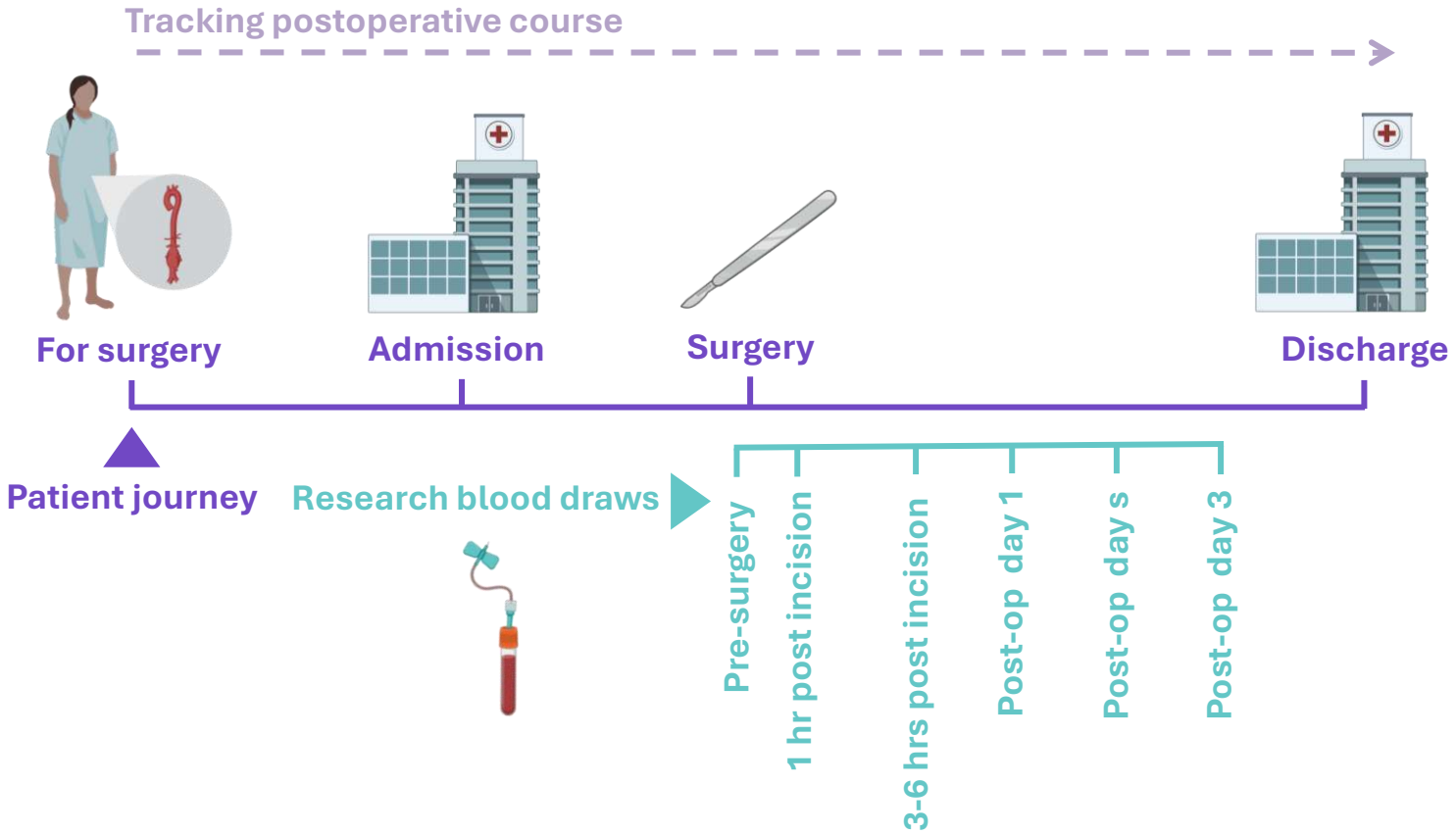
Connor Davis,
Assistant Research Fellow

To conclude

To design panels for monitoring the phenotyping circulating human immune cells.

- **Panel 1:** Monocytes and DCs
- **Panel 2:** Neutrophils
- **Panel 3:** T, B and NK cells
- **Considerations for:**
  - Cell isolation
  - Regents
  - Sequential staining
  - Longitudinal monitoring

To monitor the immune response to major surgery.



To determine what cell phenotypes are associated with a complicated course.