Immune Contexture of Transforming Naevi

Conceptual Developments in Cancer Immunology

[1]Cancer immunoediting

[2]Immune Reaction

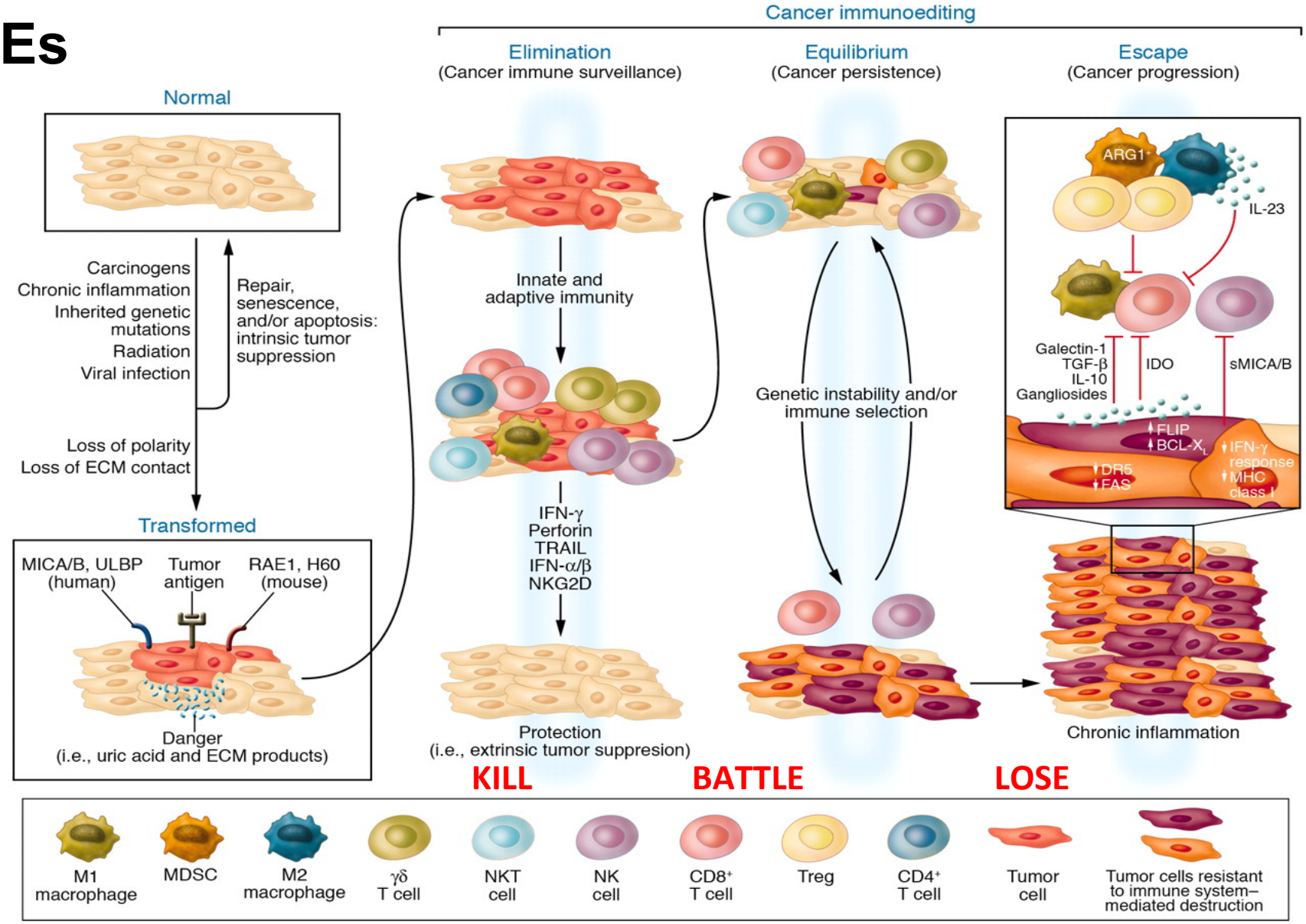
[3]Tumor Neoantigen

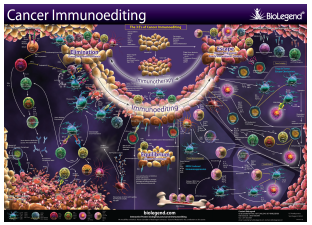
[4]Combination mAb-based therapy

[5]Tumor induced Immune suppression

QIMR Berghofer
Medical Research Institute

3Es





Smyth et al. JEM 2000, Shankaran et al. Nature 2001
Swann et al. J. Clin. Invest. 2007, Koebel et al. Nature 2007
Teng et al., JLB 2008; Schreiber..Smyth. Science 2011
Teng et al., Cancer Res 2012, Teng et al., JCI 2015

QIMR Berghofer
Medical Research Institute

Immune contexture dictates clinical outcome

Immune contexture associated with good prognosis in CRC

Chemokine
↑CXCL9 ↑CX3CL1
↑ CXCL10 ↑ CCL2
↑ CCL5 ↑ CXCL13

Adhesion
↑MADCAM1
↑ ICAM1
↑ VCAM1

Cytotoxic
↑ Granzymes
↑ Perforin
↑ Granulysin

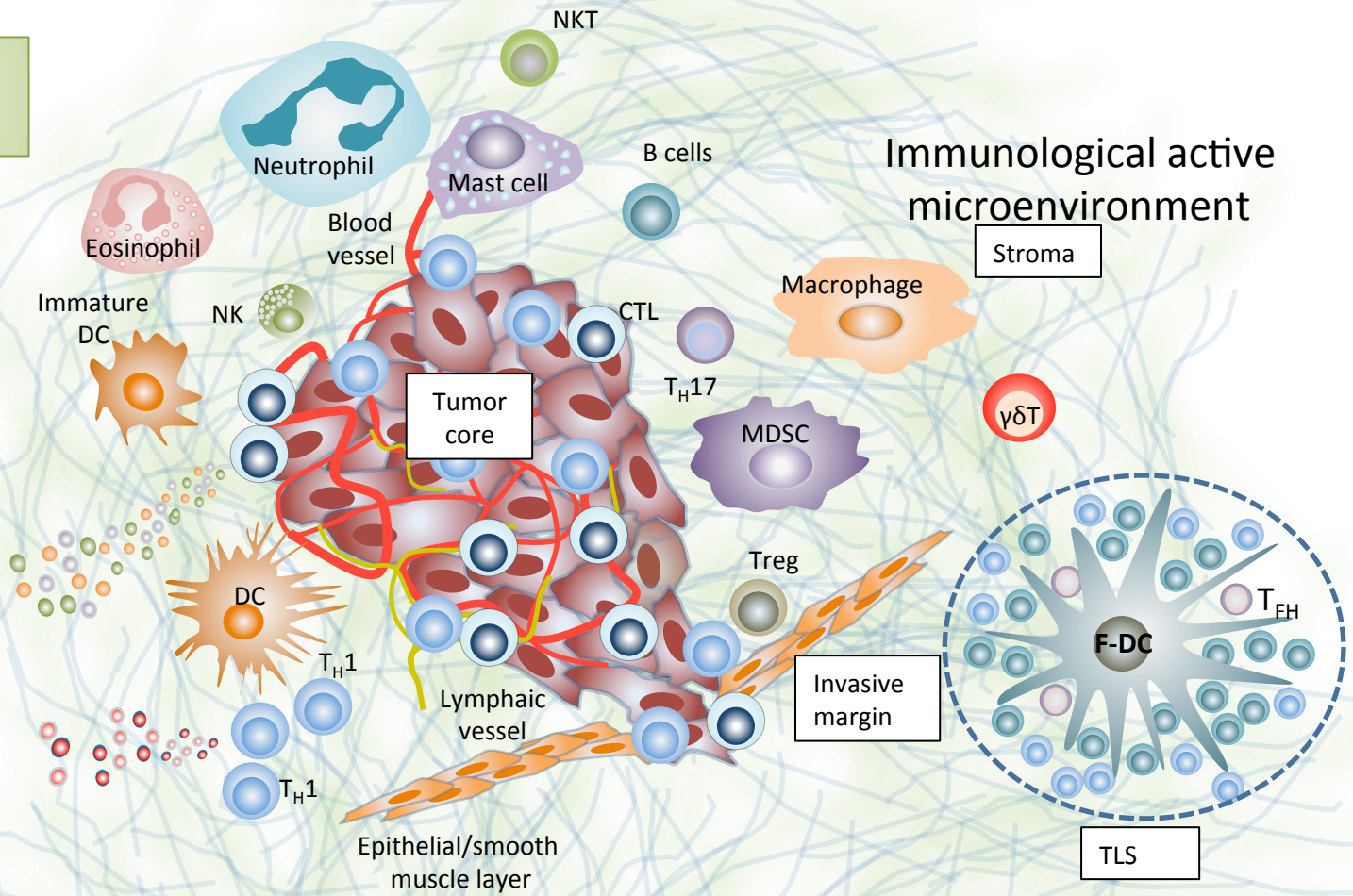
T_H1
↑T-bet
↑ IRF1
↑ STAT1

T_H1
↑ IL-12
↑ IFN-γ
↑IL-15

T_{FH}
↑ IL21

B cells

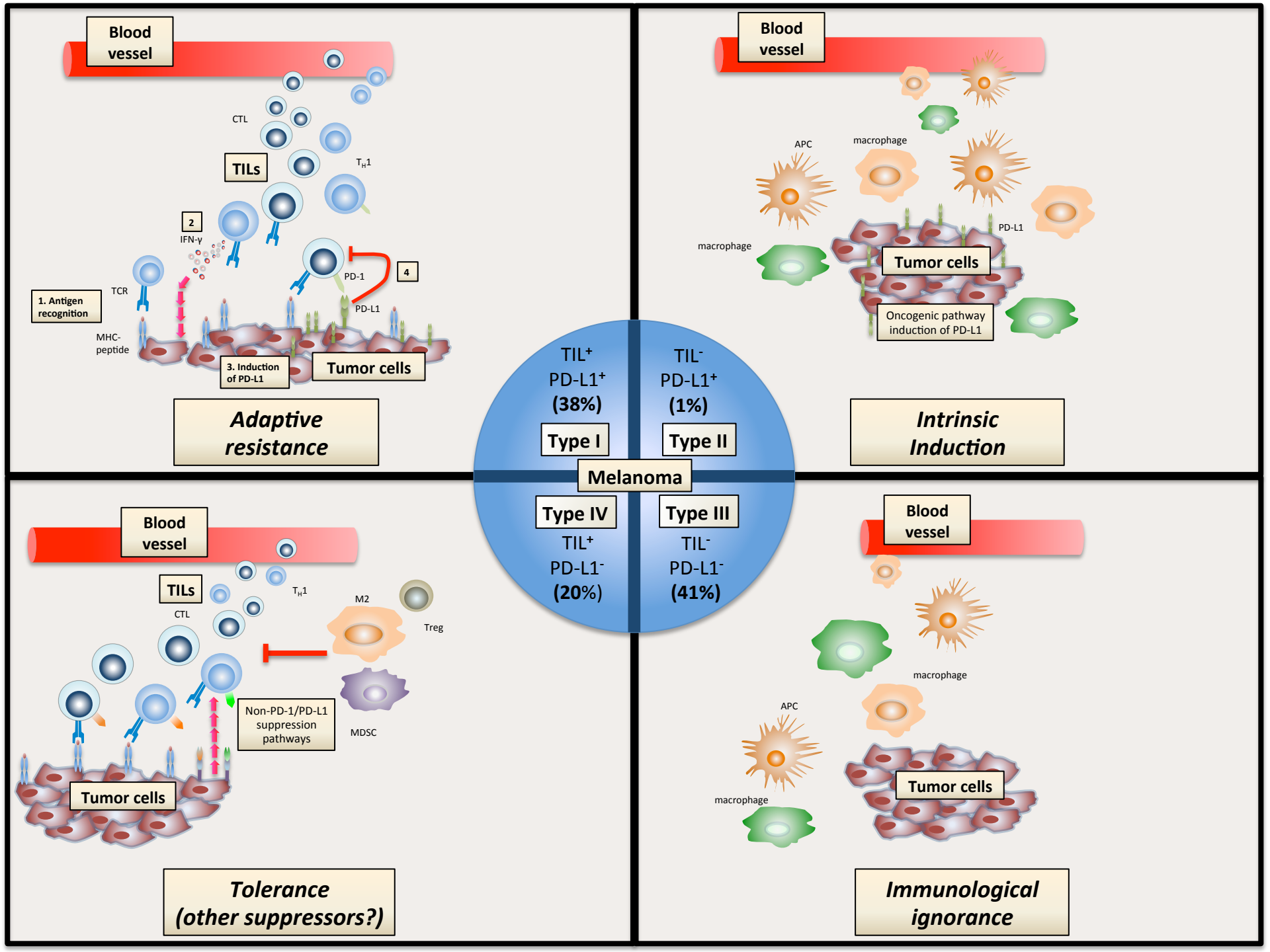
Tumor margin
↑ CD3+, CD8+, CD45RO+ T cells



Immunological active microenvironment

Galon et al., 2006 Science
Pages et al., 2005 NEJM
Bindea el al., 2013 Immunity

QIMR Berghofer
Medical Research Institute


Blood vessel
CTL
TILs
T_H1
2
IFN-γ
1. Antigen recognition
TCR
MHC-peptide
PD-1
4
PD-L1
3. Induction of PD-L1
Tumor cells
Adaptive resistance
Blood vessel
APC
macrophage
macrophage
PD-L1
Tumor cells
Oncogenic pathway induction of PD-L1
Intrinsic Induction
TIL+ PD-L1+ (38%)
Type I
TIL- PD-L1+ (1%)
Type II
Melanoma
Type IV
TIL+ PD-L1- (20%)
Type III
TIL- PD-L1- (41%)
Blood vessel
TILs
CTL
T_H1
M2
Treg
Non-PD-1/PD-L1 suppression pathways
MDSC
Tumor cells
Tolerance (other suppressors?)
Blood vessel
macrophage
APC
macrophage
Tumor cells
Immunological ignorance

Background

The clinical problem is to identify naevi with high transformation potential for more informed preventative measures.

The complicated interplay between cancer and the host immune system has been studied for decades, but only now new insights into the mechanisms by which tumours evade immune control have led to innovative therapeutic strategies that are considered amongst the medical breakthroughs of the last few years.

Multiplex immunohistochemistry (IHC) to detect proteins and define cell types in a spatio-temporal manner is revolutionizing our understanding of the natural immune reaction to cancer and immune response under therapeutic intervention.

Application thus far has been largely limited to assessing pre-treatment and on-treatment advanced cancers, in particular, malignant melanoma. By contrast, almost nothing is known about the earliest immune reactions to melanocytes in naevi that first transform.

Aims

The hypotheses of this enabling grant proposal are that:
1) an immune signature to early transformation of melanocytes can be characterized by multiplex IHC;
2) this immune signature can be further explored and substantiated by genetic analysis of the same lesions;
3) that naevi cultures can be used to correlate intrinsic genetic alterations with surface immune profile and immune contexture of the lesions from which the naevus cells originate.

To multiplex IHC stain several series of benign and dysplastic naevi, and thin melanomas.

To immunophenotype existing and new naevi cultures.

Part 1

Brian Gabrielli identified a set of eight markers that distinguish naevi from melanoma from three separate cohorts totaling 279 lesions. Naevi with a strong potential for transformation had lost β-catenin, gained nuclear N-cadherin, but retained nuclear p16

We have this series including 27 benign, 25 dysplastic, 55 thin melanoma, 54 thick melanoma, 54 lymph node and 55 distant metastasis samples.

We also have 17 benign naevi and 14 dysplastic naevi from the Brisbane Naevus Morphology Study (BNMS) study with pairs of lesions from 10 patients. We have germline and somatic tissue whole exome sequence for these samples (82% are BRAF mutation positive, 18% have NRAS mutations).

FFPE sections of the above samples will be assessed by multispectral fluorescent IHC with a panel including CD45RO, CD3, CD4, CD8, FoxP3, γδTCR (all T cell subsets), CD20 (B cells), CD68 (myeloid cells), NKp46, CD56, CD57 (NK cells), S100A8, and Ki67 will be used as a marker of immune cell proliferation. One of the pigmentation antigens, TYRP1, will be used to detect melanocytes (clone B8G is a monoclonal to TYRP1) routinely used. Nuclear counterstain DAPI and PanCK are also included.

Part 2

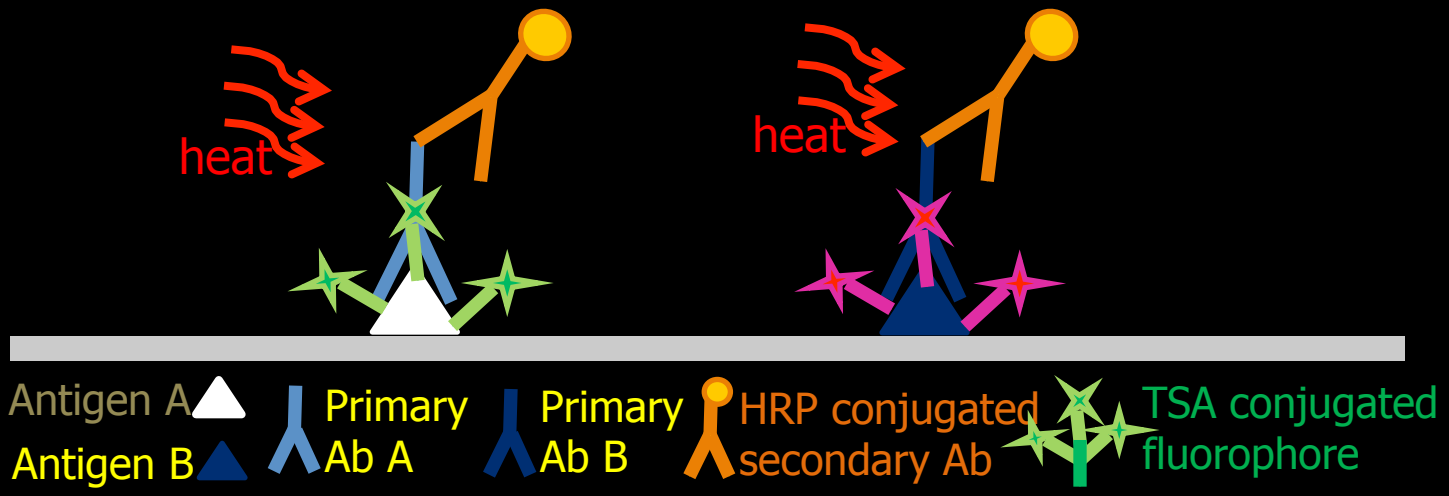
From the BNMS we have so far processed 10 naevi and established cells from 4 of these. More will be generated at a rate of one per month in 2017.

Each of these individuals has germline genotype data available allowing somatic mutations to be identified by whole exome sequencing of naevus cultures and tissue samples.

We will now include a flow cytometry profile of immune markers on the surface of these cells by QIMRB team, examining; MHC class I, MICA, MICB, ULBP-1, HLA-E, HLA-G, CD47, CD54 (ICAM-1), and CD155. This will present an immune profile of these naevocytes.

Immune profiling of the tumor microenvironment

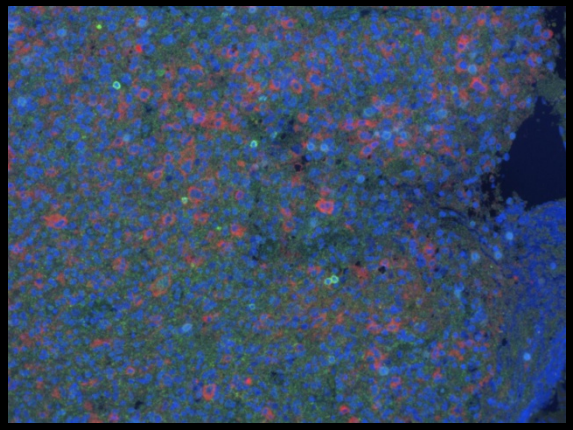
Multiplex IHC using Tyramide Signal Amplification



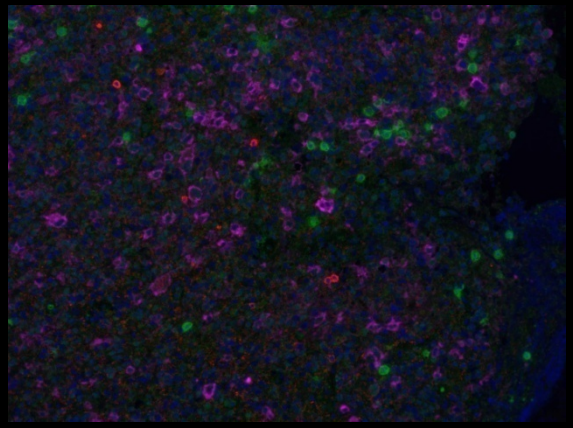
Immune profiling of the tumor microenvironment using mIHC

- Enumeration of immune cells (immunoscore)
- Location and spatial relationships in different tumor regions
- correlation to clinical outcome pre and post immunotherapy

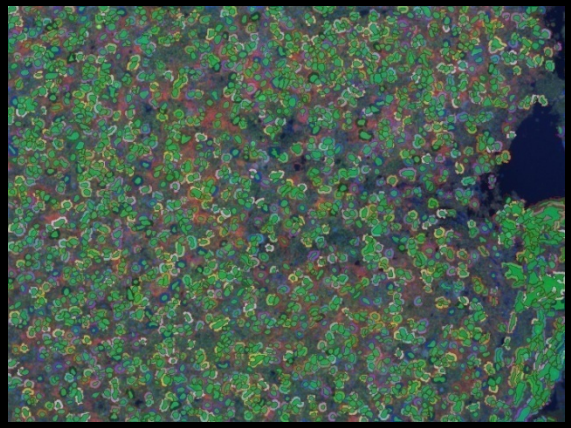
mIHC Digital Image Analysis



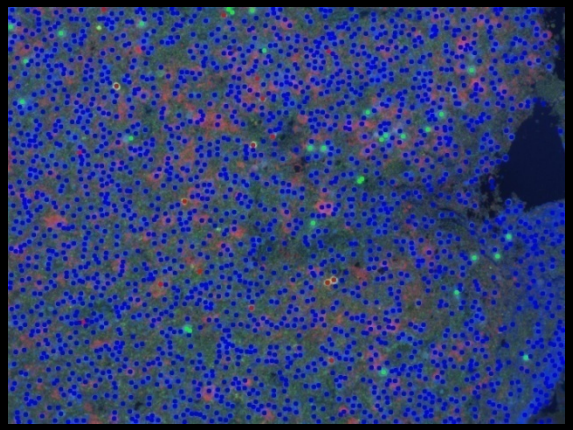
Raw →



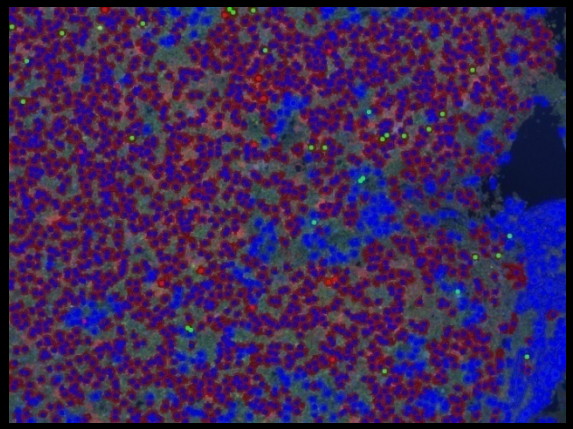
spectral unmixing →



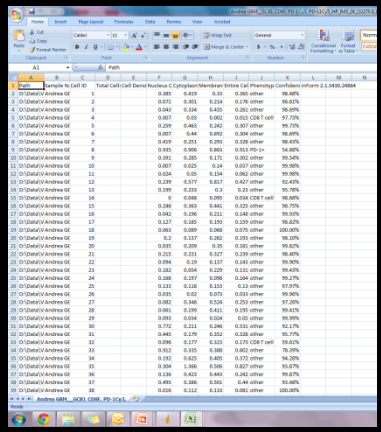
cell segmentation →



phenotyping →



scoring →



data analysis

Acknowledgements

- **QIMR Berghofer Medical Research Institute**
  - **Immunology in Cancer & Infection**
  - Mark Smyth
  - **Cancer Immunoregulation and Immunotherapy**
  - Michele Teng
  - **Tumor Immunology**
  - Rajiv Khanna
  - Reshma Shakya
- **University of Queensland**
  - Richard Sturm
  - Mitchell Stark
  - Brian Gabrielli
  - Helmut Schaider
- **MIA**
  - Richard Scolyer
  - Georgina Long

ASSC

QIMR Berghofer Medical Research Institute

80 YEARS of MEDICINE
The University of Queensland AUSTRALIA
Preparing Global Leaders in Medicine

Australian Government
National Health and Medical Research Council
NHMRC