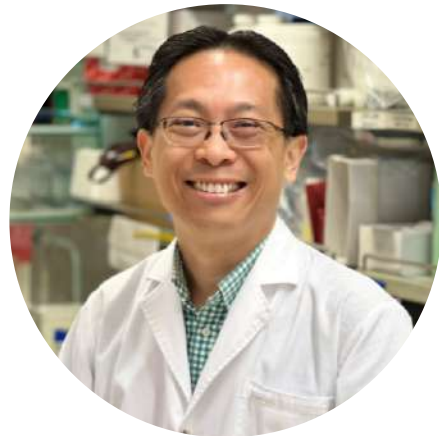


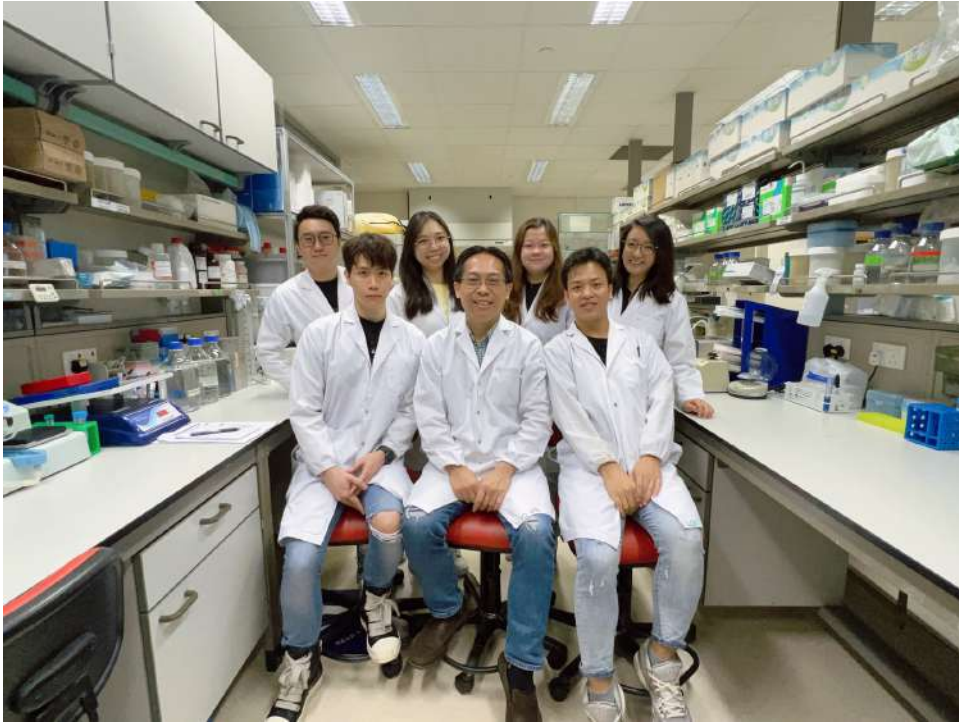
# COMPLEX CELLULAR PHENOTYPE ANALYSIS



**Loo Lit Hsin**

Senior Principal Investigator  
Bioinformatics Institute, A\*STAR  
loolh@bii.a-star.edu.sg

# COMPLEX CELLULAR PHENOTYPE ANALYSIS GROUP



Cuithbert, Carmen, Claresta, Joey  
Oscar, Lit-Hsin, Guorui



## Spatial profiling of cells and tissues

- Spatial multi-omics profiling of human tissues
- High-throughput image-based phenotypic profiling



## Bioimage databases and portals

- Visualization and sharing of cellular and tissue images
- HistoPath Analytics (HPA) Platform and ImmunoAtlas



## Digital medicine for cancers

- Biomarkers for patient stratification and targeted intervention
- Pathogenicity of mutations








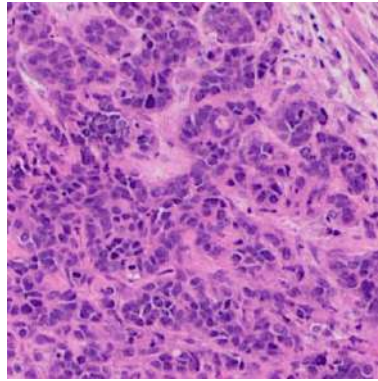
## Predictive models for therapeutic development

- Tumor-associated antigen prediction
- Xenobiotic-induced toxicity prediction



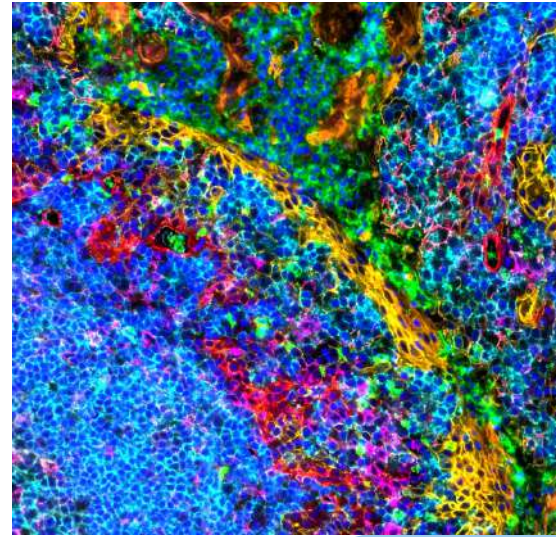
# The Quest of **Spatial** Biology

The goal is to map and visualize biology across biological scales, from  DNAs to  RNAs to  proteins to  metabolites to  phenotypes



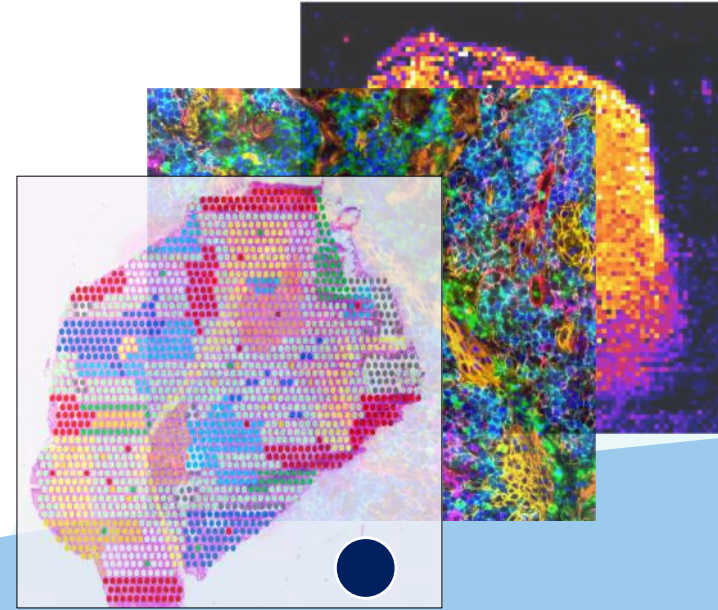
## Bright-field imaging

- **Histology**
- Tech: H&E, IHC and MxP imaging
- 1 or 2 markers



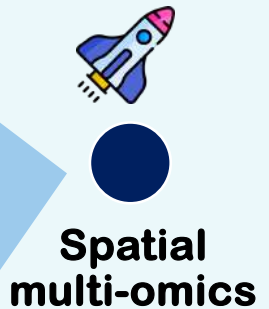
## Multiplex imaging

- **DNAs, RNAs, or Proteins**
- Tech: Vectra, CODEX, COMET, MERFISH, MIBI, IMC
- 5 to 100 markers



## Spatial omics

- **Genomics, Transcriptomics, Proteomics or metabolomics**
- Tech: Visium, Xenium, GeoMX, CosMX, StereoSeq, MALDI-MSI, DESI-MSI
- 1,000 to 20,000 markers

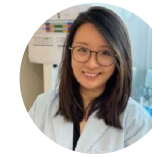


**Spatial multi-omics**



# Spatial Proteomics

Joey Lee

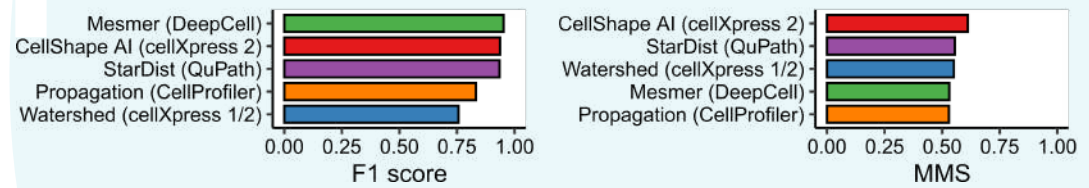
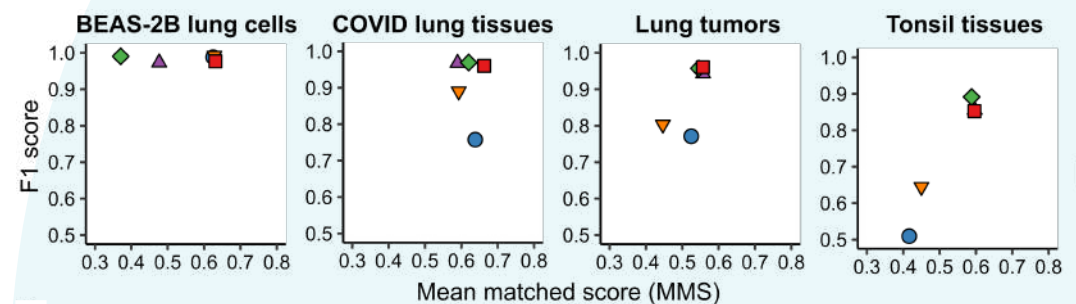
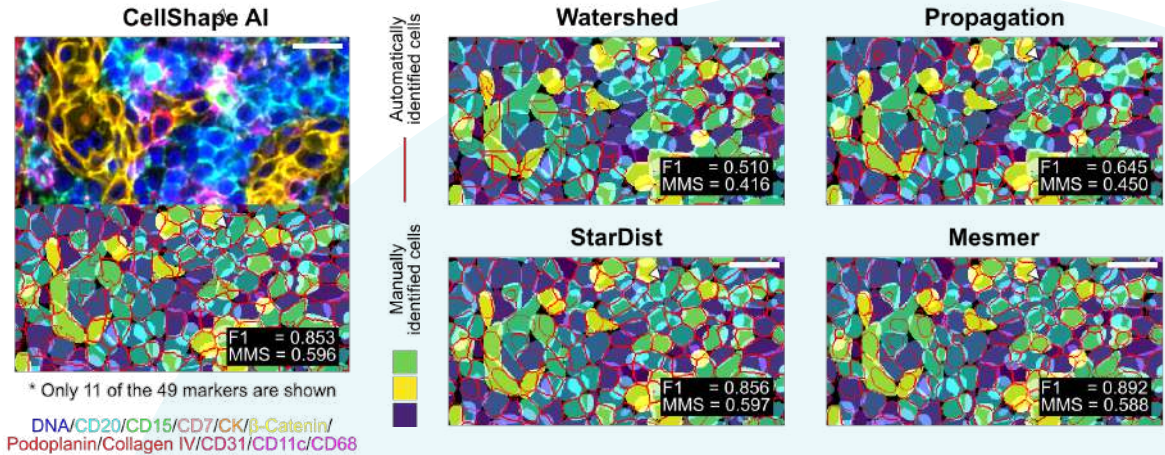
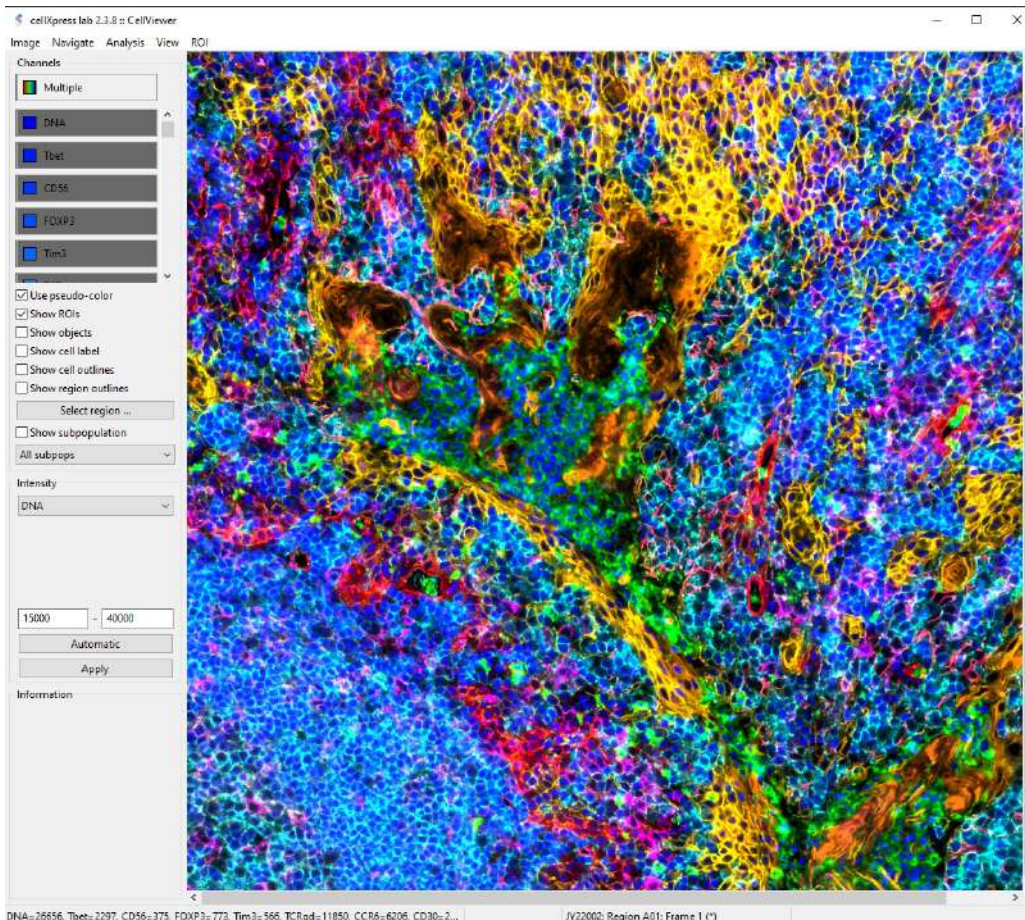


Sizon Jiang



cellXpress 2 <https://cellXpress.org>

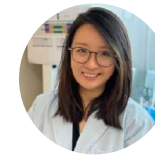
- We have updated cX2 to handle large hyperplexed tissue images
- An example of a tonsil tissue stained with 49 protein markers using the CODEX technology
- We have developed CellShapeAI to segment diverse and overlapping cell types at tissues





# Spatial Metabolomics

Joey Lee

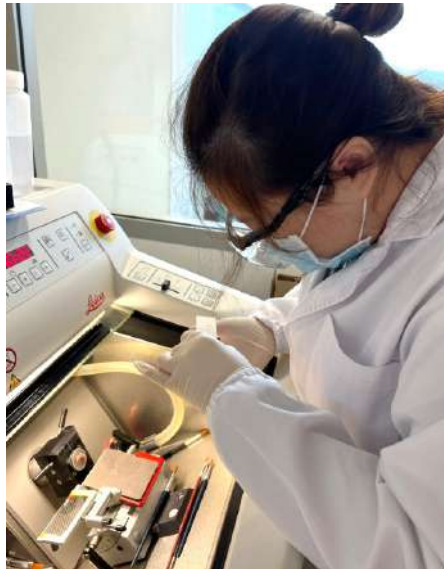


Wang Yulan

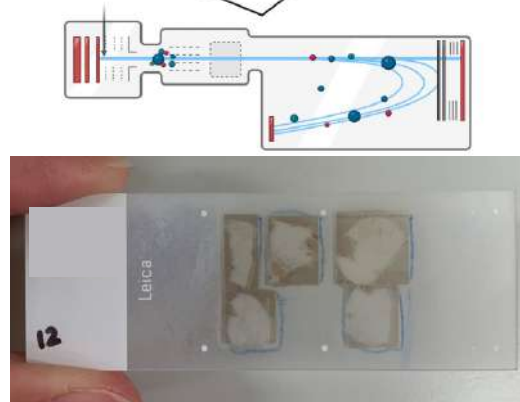
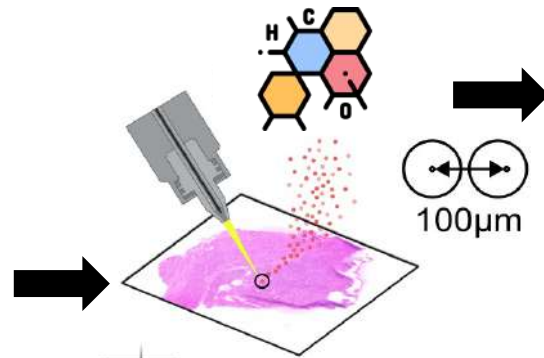


**Mass spectrometry imaging (MSI)** can measure the spatial expressions of metabolites, such as **amino acids, lipids, steroids**, and other small molecules in human tissues

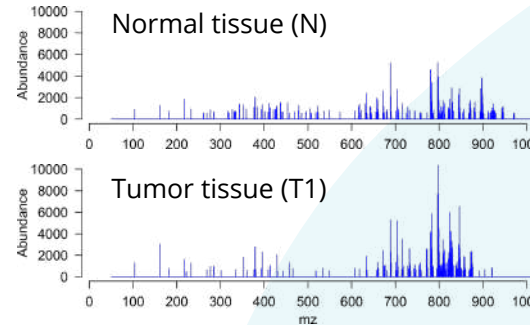
We have developed computational pipelines to process, QC, analyze, and visualize MSI data



Tissue sectioning  
(Our PhD student, Claresta)

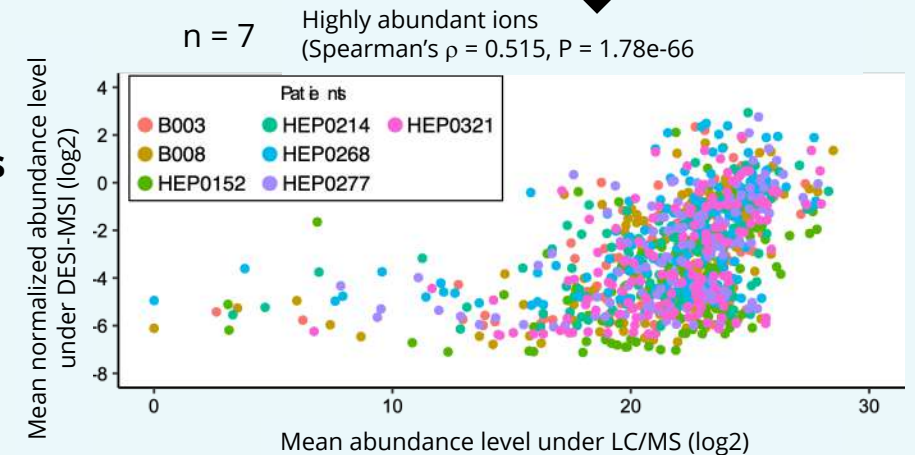
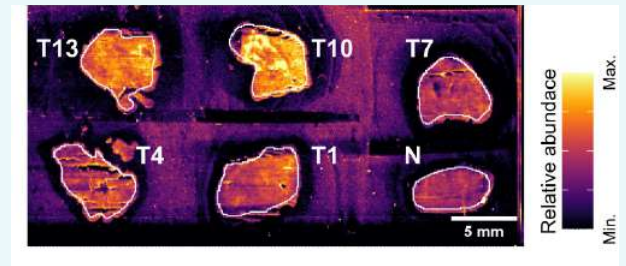


Multiple sections from one patient



**Comparing spatial (MSI) and bulk metabolomics (LC/MS)**

Total ion current from MSI



n = 7 Highly abundant ions (Spearman's  $\rho = 0.515$ ,  $P = 1.78e-66$ )

- | Patients  |           |           |
|-----------|-----------|-----------|
| ● B003    | ● HEP0214 | ● HEP0321 |
| ● B008    | ● HEP0268 |           |
| ● HEP0152 | ● HEP0277 |           |

# COMPLEX CELLULAR PHENOTYPE ANALYSIS GROUP



Cuithbert, Carmen, Claresta, Joey  
Oscar, Lit-Hsin, Guorui



## Spatial profiling of cells and tissues

- Spatial multi-omics profiling of human tissues
- High-throughput image-based phenotypic profiling



## Bioimage databases and portals

- Visualization and sharing of cellular and tissue images
- HistoPath Analytics (HPA) Platform and ImmunoAtlas



## Digital medicine for cancers

- Biomarkers for patient stratification and targeted intervention
- Pathogenicity of mutations



## Predictive models for therapeutic development

- Tumor-associated antigen prediction
- Xenobiotic-induced toxicity prediction



# Online public portal for **immuno-oncology** images and markers

Joey Lee

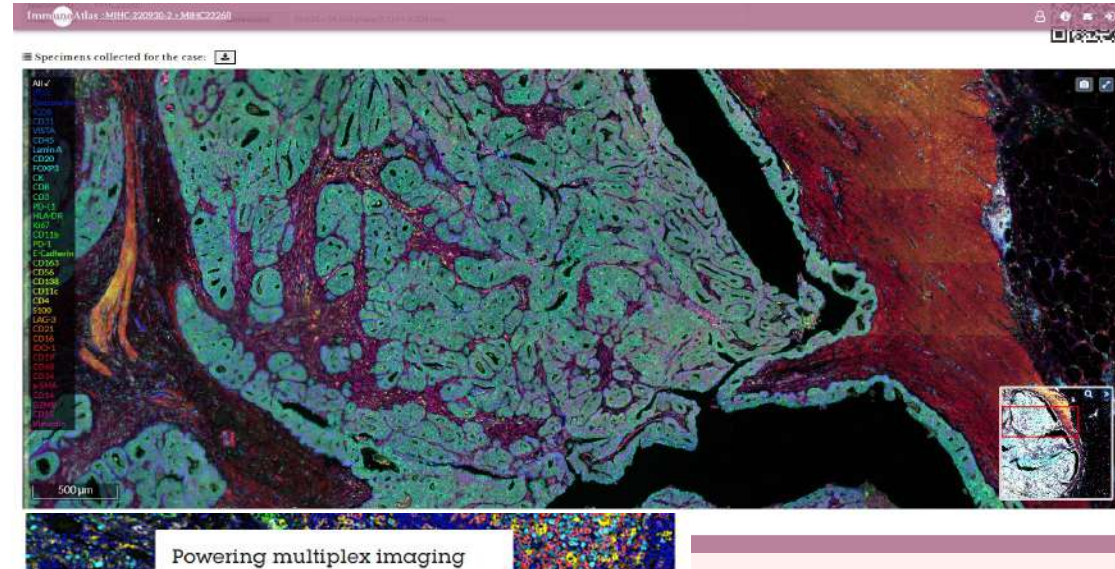


Joe Yeong



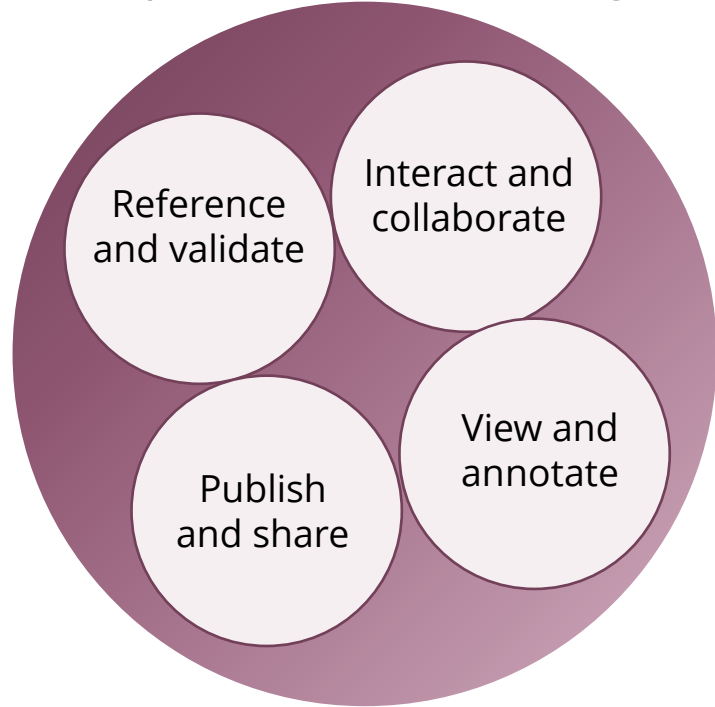
## ImmunoAtlas

<https://ImmunoAtlas.org>

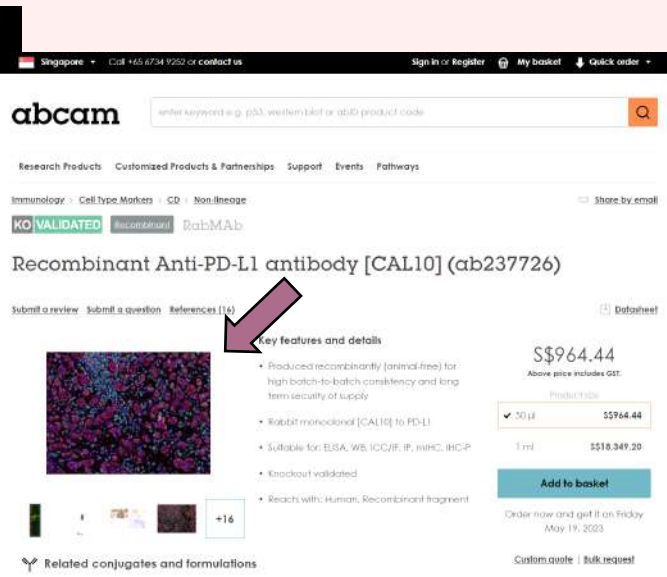


Support whole-slide hyperplexed images  
Over 30 markers and 180GB!!

Find out more!

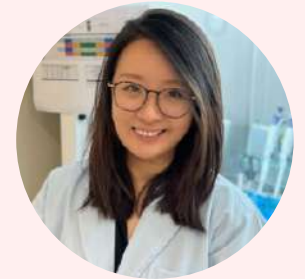
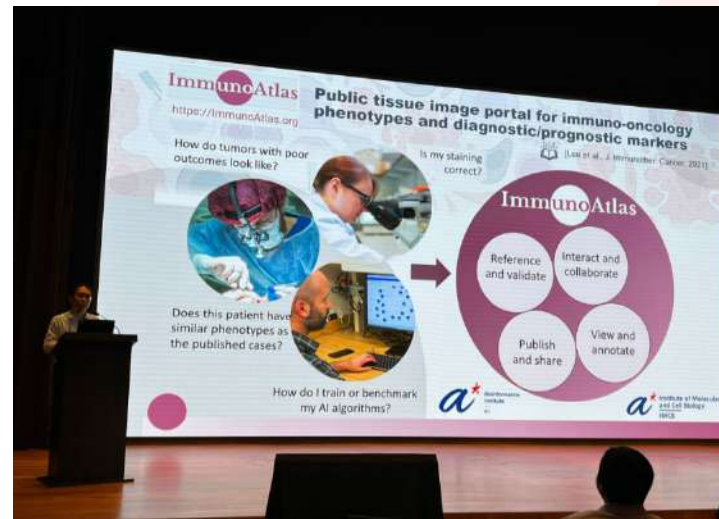
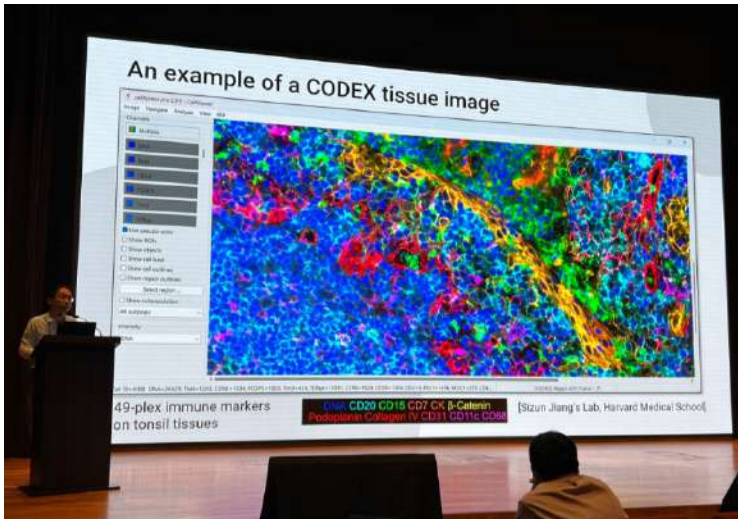
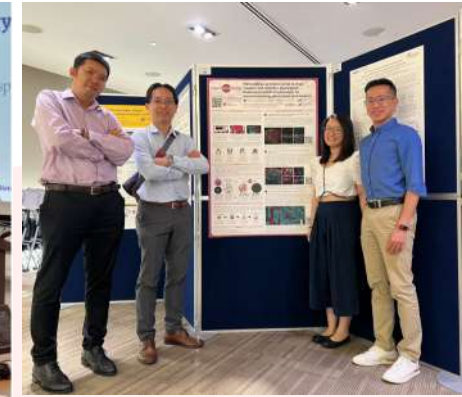


Marketing campaign with **abcam** (April 2022)!





# SingHealth Immunopathology Workshop (8<sup>th</sup> – 9<sup>th</sup> May 2023)



Joey Lee



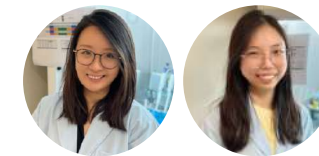
Poster award  
(Second Prize)





# HistoPath Analytics (HPA) Platform

Joey Lee



View and share anywhere, anytime

- Cloud-based digital platform for organizing, sharing, visualizing, and analyzing large histological images
- Offering as a service via A\*STAR RSC since 2019

HPA currently supports **spatial proteomics** images

Original Article | [Open Access](#) | [Published: 04 June 2022](#)

### Choice of PD-L1 immunohistochemistry assay influences clinical eligibility for gastric cancer immunotherapy

[Joe Yeong](#), [Huey Yew Jeffrey Lum](#), [Chong Boon Teo](#), [Benjamin Kye Jyn Tan](#), [Yiong Huak Chan](#), [Ryan Yong Kiat Tay](#), [Joan Rou-Fn Choo](#), [Anand D. Jayasekharan](#), [Qing Hao Miow](#), [Lit-Hsin Loo](#), [Wei Peng Yong](#) & [Raghav Sundar](#)

[Gastric Cancer](#) **25**, 741–750 (2022) | [Cite this article](#)

4212 Accesses | 19 Citations | 6 Altmetric | [Metrics](#)

ASCO Daily News  
Clinical News From the American Society of Clinical Oncology

NEWS COMMENTARIES MEETINGS TOPICS PODCASTS ABOUT [Edit your words / photos / DOI / EURL / alerts](#)

2022 ASCO ANNUAL MEETING

### Significant Discrepancies Found Among PD-L1 Assays Used in Gastric Cancer

May 26, 2022



We will support **spatial transcriptomics** and **metabolomics** soon!

Home > Tech Platforms > Bioinformatics > High Throughput Image-based Phenotypic Profiling

**Technology Platforms**

- National Shared Platform
- Mass Spectrometry Cluster (A\*STAR)
- Fluor Cytometry Cluster (A\*STAR)
- Animal Research and Testing
- Bioinformatics
- AlloSentry Platform
- Computational Immunology
- High Throughput Image-based Phenotypic Profiling
- Multiscale Simulation: Modeling and design
- Bioimaging
- Bioprocess Engineering

**High Throughput Image-based Phenotypic Profiling (HIPP) Platform by Bioinformatics Institute (BII)**

High Throughput Image-based Phenotypic Profiling Platform provides biological image analysis, management, and visualization services for core imaging facilities.

HPAScore - NCIR-210524-1 > NCIR21317 > [39861,11944] Joey Jia Ying About us

All

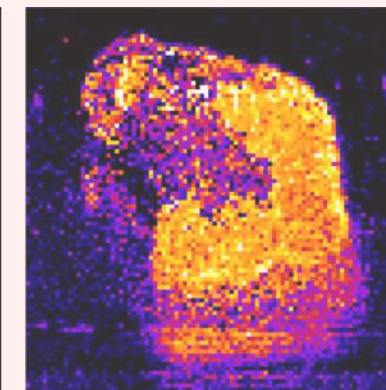
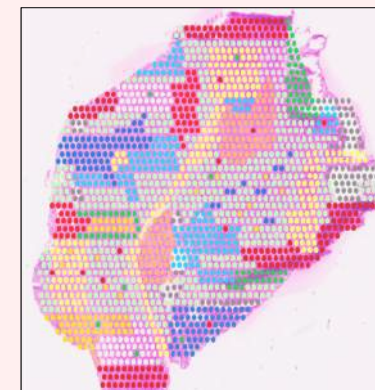
- PD-L1 (2203) ✓
- PD-L1 (SP142) ✓
- PD-L1 (28-8) ✓
- CK ✓

Virtual subpopulation staining:

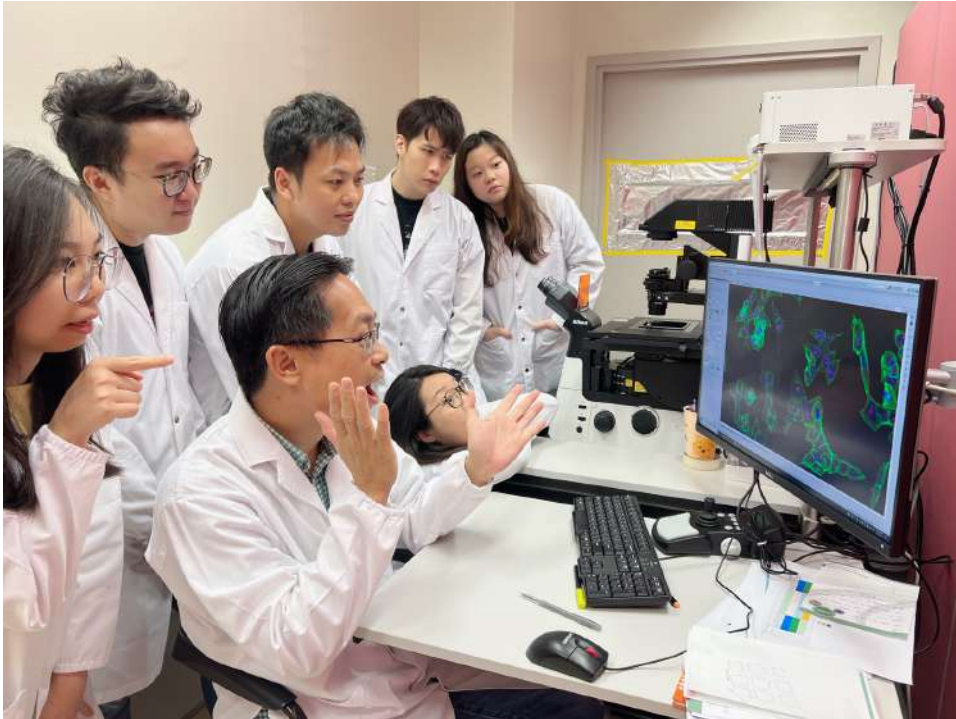
Subpopulation scores

Case 16/41 : Sample 1/1

Powered by HPA and cellXpress 2. © 2019-2021, A\*STAR.



# COMPLEX CELLULAR PHENOTYPE ANALYSIS GROUP



Cuithbert, Carmen, Claresta, Joey  
Oscar, Lit-Hsin, Guorui



## Spatial profiling of cells and tissues

- Spatial multi-omics profiling of human tissues
- High-throughput image-based phenotypic profiling



## Bioimage databases and portals

- Visualization and sharing of cellular and tissue images
- HistoPath Analytics (HPA) Platform and ImmunoAtlas



## Digital medicine for cancers

- Biomarkers for patient stratification and targeted intervention
- Pathogenicity of mutations



## Predictive models for therapeutic development

- Tumor-associated antigen prediction
- Xenobiotic-induced toxicity prediction



# Biomarkers for Liver Cancer

- Liver cancer is the third most common cause of cancer death in Singapore
- There is currently NO predictive biomarker for hepatocellular carcinoma (HCC) immunotherapy

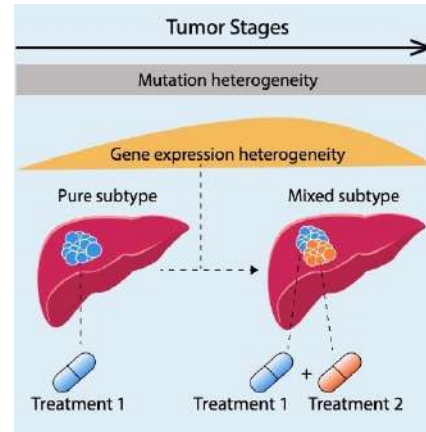
Pierce Chow



## Precision Medicine in Liver Cancer across an Asia Pacific Network (PLANet)

### PLANet 1 (2016-2021)

Characterized the dynamic evolutionary and heterogeneity in HCC



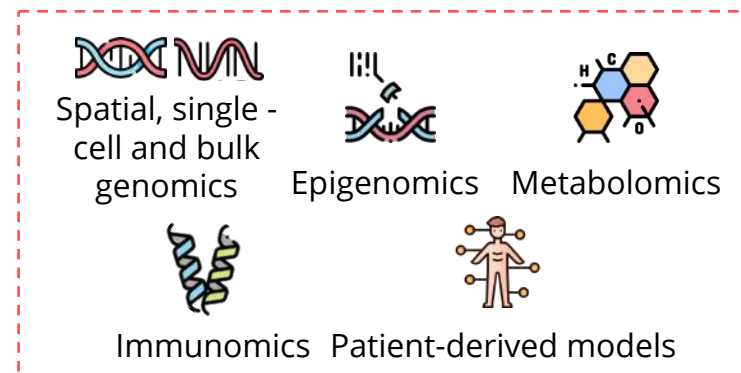
### PLANet 2 (2022-2027)

To identify predictive biomarkers for HCC

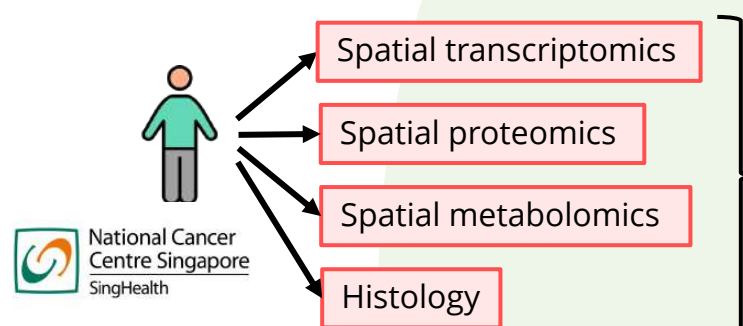
### Current Trials

PLANet 2.0 – AHCC12 and AHCC13	
	Precision Medicine in Liver Cancer across an Asia Pacific Network 2.0 (PLANet 2.0)
	AHCC12:
	Deep, Multi-omics Phenotyping to Predict Response, Resistance and Recurrence to Adjuvant Atezolizumab plus Bevacizumab in Resected Hepatocellular Carcinoma (HCC)(EMPHASIS)(NCT05516628)
AHCC12 & AHCC13	AHCC13:
	Liquid Biopsy based Identification of Predictive Biomarkers of Response in Intermediate/Locally Advanced HCC receiving Y90 +/- Atezolizumab plus Bevacizumab (NCT05377034)

### Curated data for >1000 patient samples



### Spatial multi-omics data before and after treatments



BII will use the HPA Platform to **integrate and analyze** all the generated spatial multi-omics data

Biomarker for patient stratification



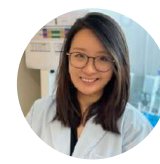
View and share anywhere, anytime





# Spatial metabolomics landscape of Liver Cancer

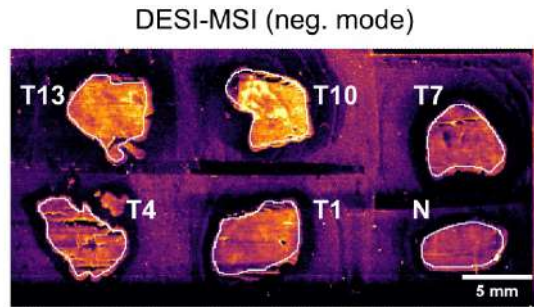
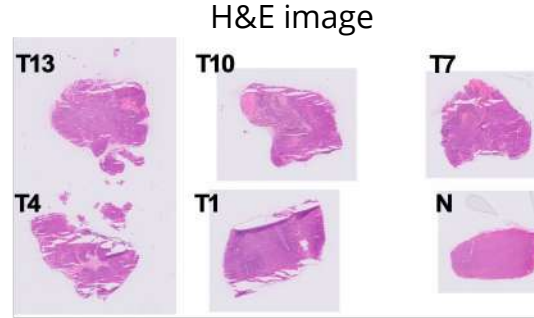
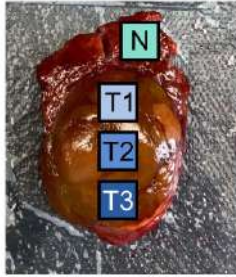
Joey Lee



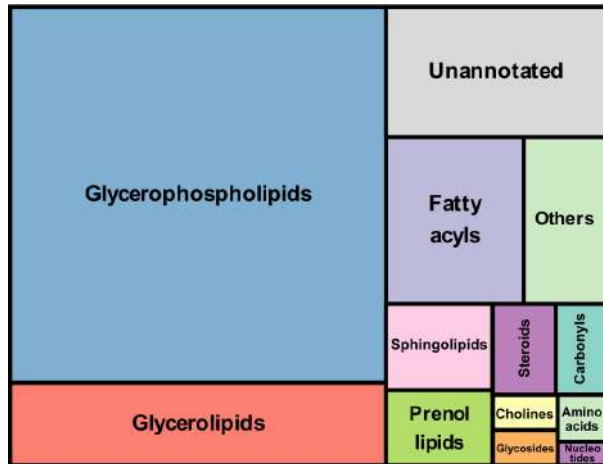
Wang Yulan



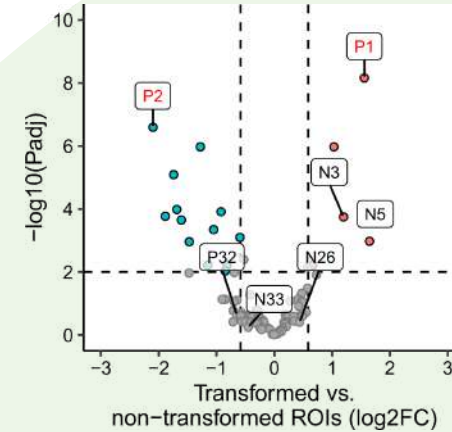
24 HCC patients



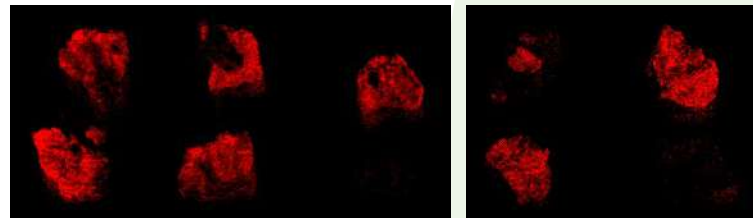
Our analysis found highly-abundant metabolites in both MSI and LC/MS samples



We used machine learning to integrate **histopathological and spatial metabolomics** information and identify potential markers for the normal and tumor tissues



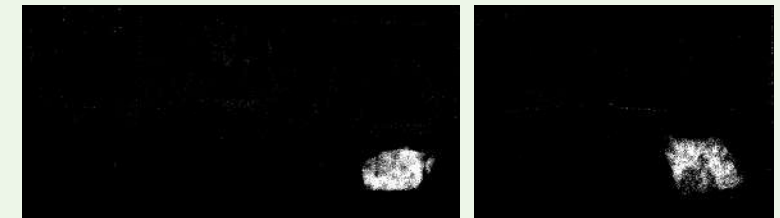
P1 (m/z = 810.6001)



HEP0152

B0003

P2 (m/z = 437.3528)



HEP0152

B0003



# The 5<sup>th</sup> Singapore Liver Cancer Consortium Scientific Symposium

## (1 June 2023)

PROF PIERCE CHOW  
PROTOCOL CHAIR  
ASIA-PACIFIC HEPATOCELLULAR CARCINOMA (AHCC) TRIALS GROUP  
National Cancer Centre Singapore  
Singapore General Hospital



1 | THE SPATIAL METABOLOMICS LANDSCAPE OF HEPATOCELLULAR CARCINOMA

Dr Loo Lit Hsin  
Senior Principal Investigator,  
Bioinformatics Institute, A\*STAR



2 | SPATIAL MAP OF PRIMARY AND RECURRENT HEPATOCELLULAR CARCINOMA

Prof Vinay Tergaonkar  
Research Director, Institute of Molecular and Cell Biology, A\*STAR  
Professor, Yong Loo Lin School of Medicine



3 | A GENETIC EVOLUTIONARY PORTRAIT OF HEPATOCELLULAR CARCINOMA THROUGH DISEASE PROGRESSION

Dr Tam Wai Leong  
Group Leader and Associate Director, Genome Institute of Singapore, A\*STAR;  
Principal Investigator, Cancer Science Institute of Singapore, NUS



4 | WORKING TOWARDS METABOLIC AND MICROBIOME UNDERSTANDING OF HCC DEVELOPMENT

Dr Sunny Wong  
Associate Professor, Lee Kong Chian School of Medicine, Nanyang Technological University Singapore



Prof Wang Yulan  
Director of Singapore Phenome Centre  
Professor of Metabolomics



5 | ONCO-FETAL REPROGRAMMING AS A PRIMARY MECHANISM FOR IMMUNE ESCAPE IN HEPATOCELLULAR CARCINOMA

Dr Ankur Sharma  
Laboratory Head, Harry Perkins Institute of Medical Research



6 | ADJUVANT THERAPY FOR LIVER CANCER: IS IT READY FOR PRIME TIME?

Prof Toh Han Chong  
Senior Consultant & Deputy Chief Executive Officer (Strategic Partnerships), National Cancer Centre Singapore



7 | SHOULD SYSTEMIC THERAPY BE FIRST-LINE FOR INTERMEDIATE STAGE HEPATOCELLULAR CARCINOMA RATHER THAN LOCO-REGIONAL THERAPY?

Prof Masatoshi Kudo  
Professor and Chairman, Department of Gastroenterology and Hepatology, Kindai University Faculty of Medicine



8 | MONO-THERAPY WITH CHECK-POINT INHIBITORS IN HCC: WHY DID THE RANDOMIZED CONTROLLED TRIALS FAIL?

Prof Thomas Yau  
Consultant Medical Oncologist  
Department of Medicine, Queen Mary Hospital



9 | IMBRAVE050: TOWARD A NEW PAGE OF MULTI-MODALITY MANAGEMENT OF HEPATOCELLULAR CARCINOMA

Prof Chiun Hsu  
Graduate Institute of Oncology, National Taiwan University College of Medicine  
Department of Medical Oncology, National Taiwan University Cancer Center



MORNING TEA AND LUNCH WILL BE SERVED  
FREE ADMISSION AND CME POINTS WILL BE AWARDED

Date: 1 June 2023

Time: 0830 - 1200

Venue: NCCS Lecture Theatre Level 1

30 Hospital Boulevard Singapore 168583

Direct Link to Outram MRT (Exit 7)

Scan here to register:



Asia-Pacific  
Hepatocellular Carcinoma  
Trials Group

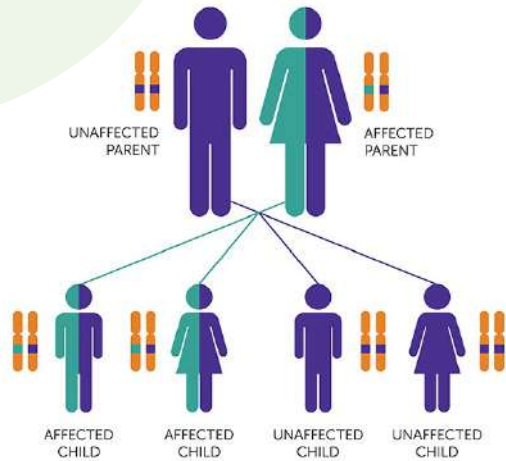
National Cancer  
Centre Singapore  
SingHealth

Or click this link to register: <https://form.gov.sg/64112a144d531c001124558b>



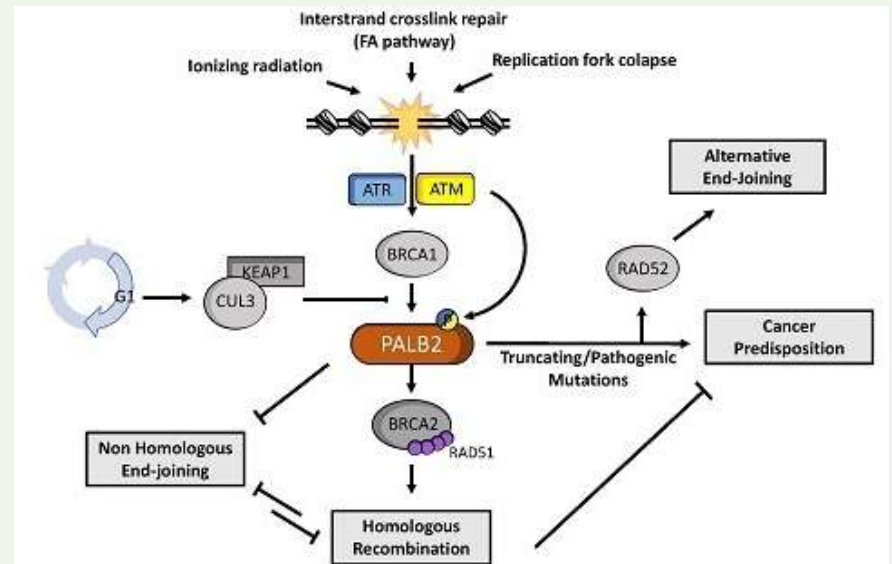
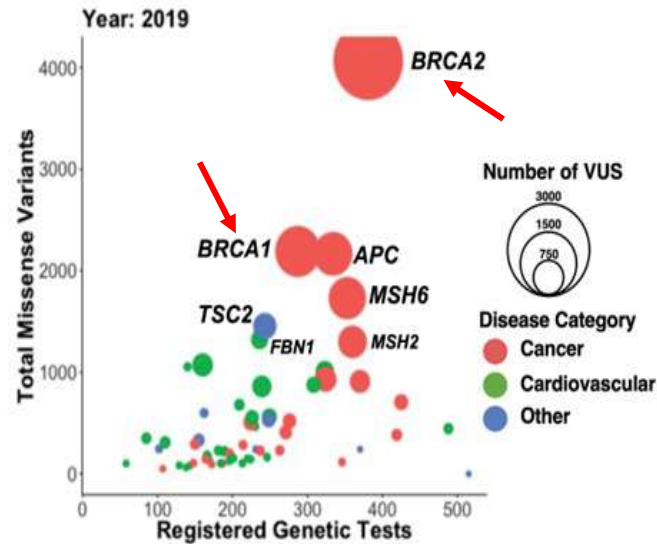
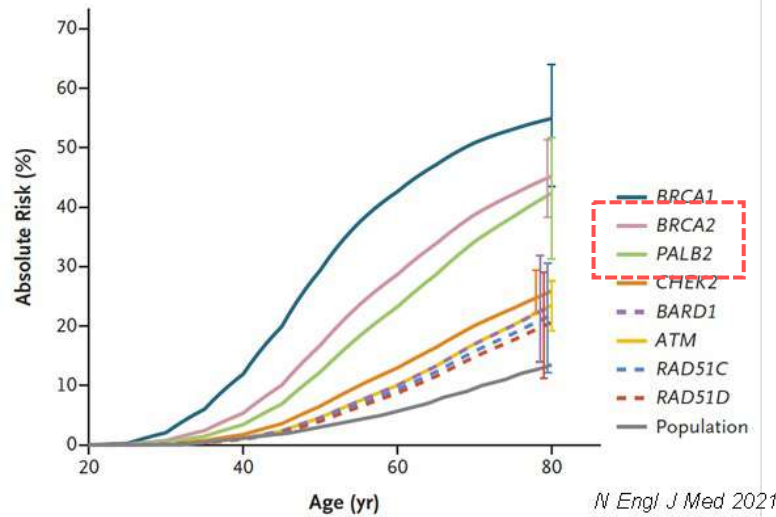
# Hereditary mutations associated with cancers

Joanne Ngeow



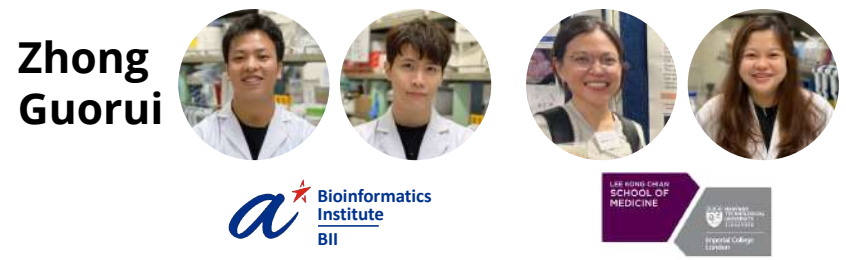
## Variants of Uncertain Significance (VUS)

When analysis of a patient's genome identifies a variant, but it is unclear if the variant is actually connected to a health condition

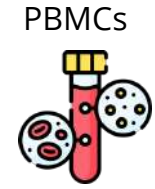
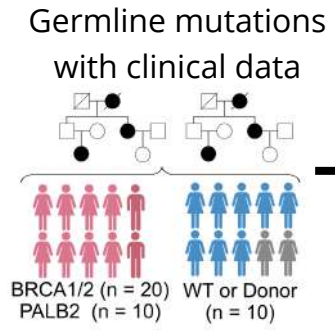




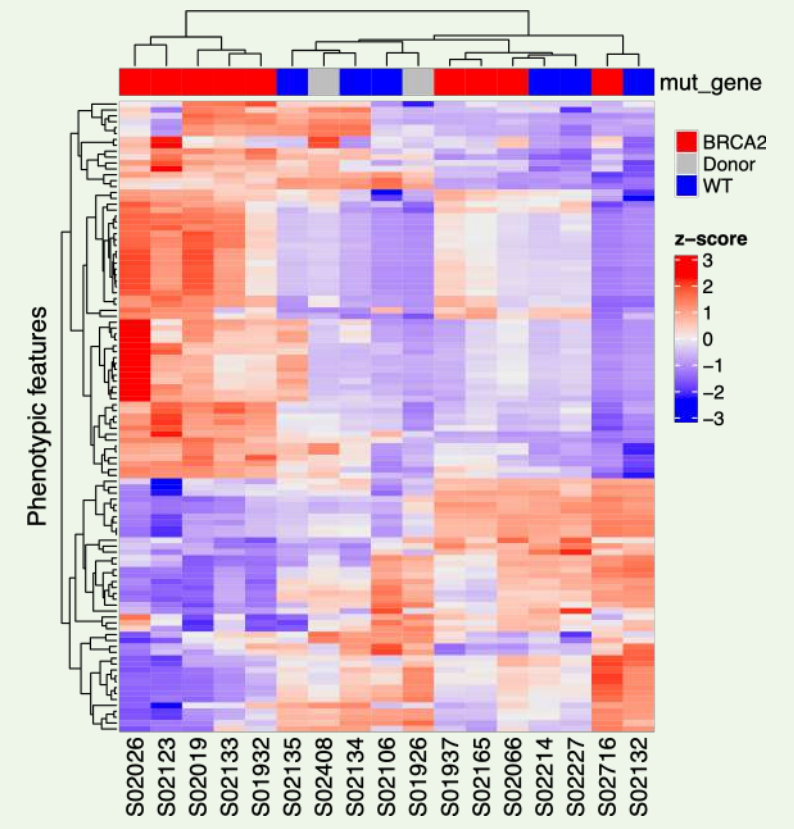
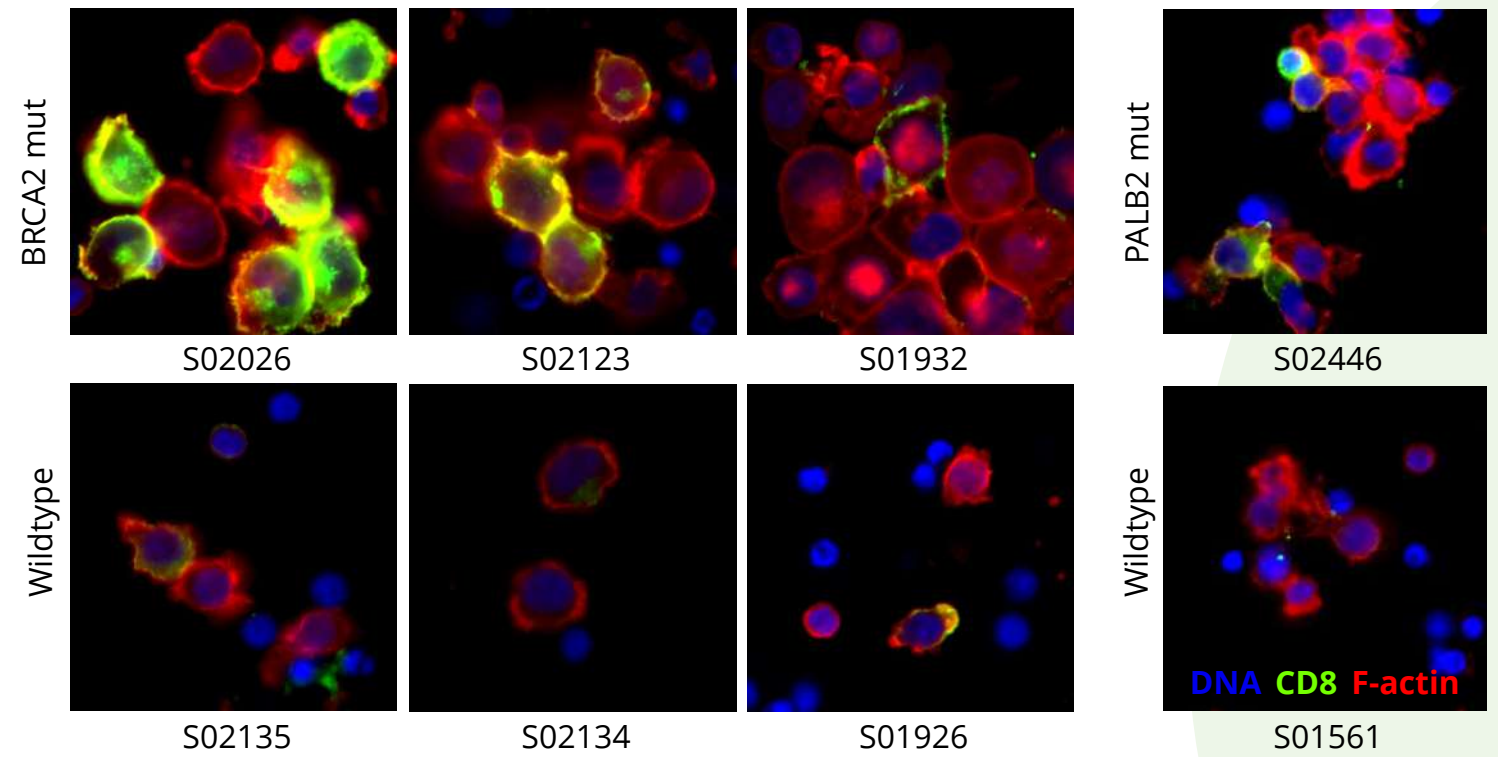
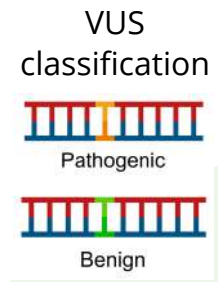
# Imaging-based phenotypic profiling to predict pathogenic mutations



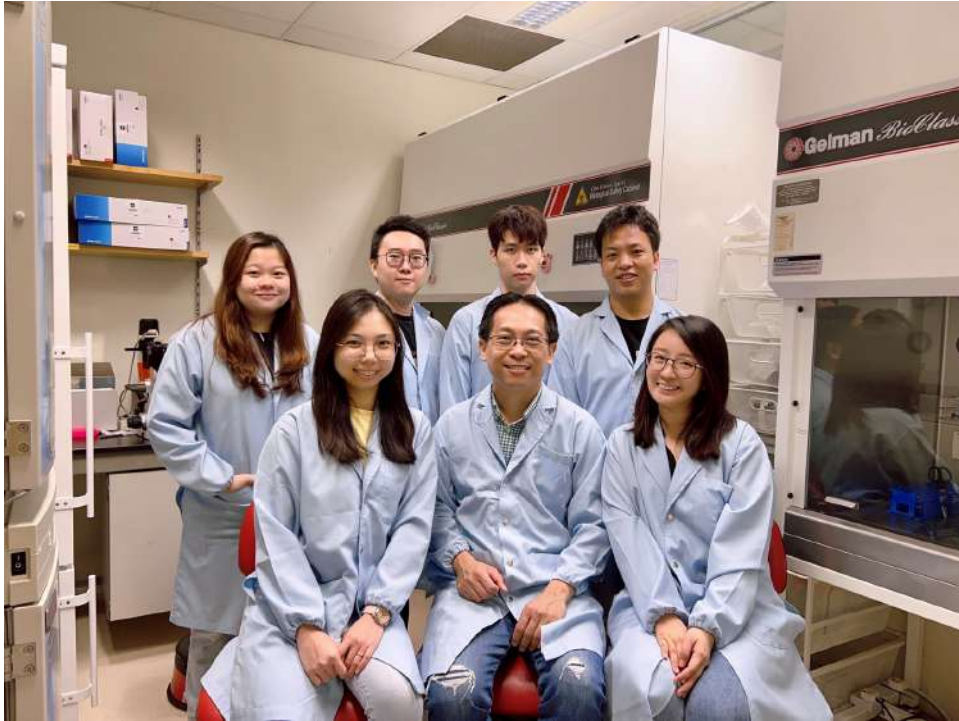
National Cancer Centre Singapore SingHealth  
Cancer Genetic Service



Phenotypic profiling



# COMPLEX CELLULAR PHENOTYPE ANALYSIS GROUP



Claresta, Cuithbert, Oscar, Guorui  
Carmen, Lit-Hsin, Joey



## Spatial profiling of cells and tissues

- Spatial multi-omics profiling of human tissues
- High-throughput image-based phenotypic profiling



## Bioimage databases and portals

- Visualization and sharing of cellular and tissue images
- HistoPath Analytics (HPA) Platform and ImmunoAtlas



## Digital medicine for cancers

- Biomarkers for patient stratification and targeted intervention
- Pathogenicity of mutations



## Predictive models for therapeutic development

- Tumor-associated antigen prediction
- Xenobiotic-induced toxicity prediction



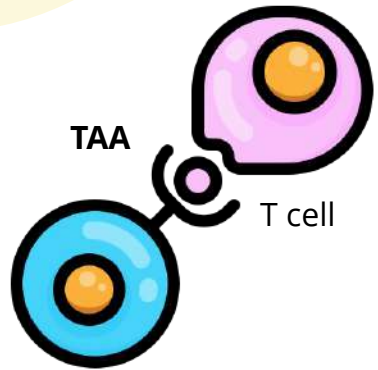


# High-throughput assays and AI models for **tumor-associated antigen classification**

Joe Yeong

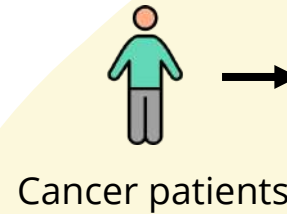
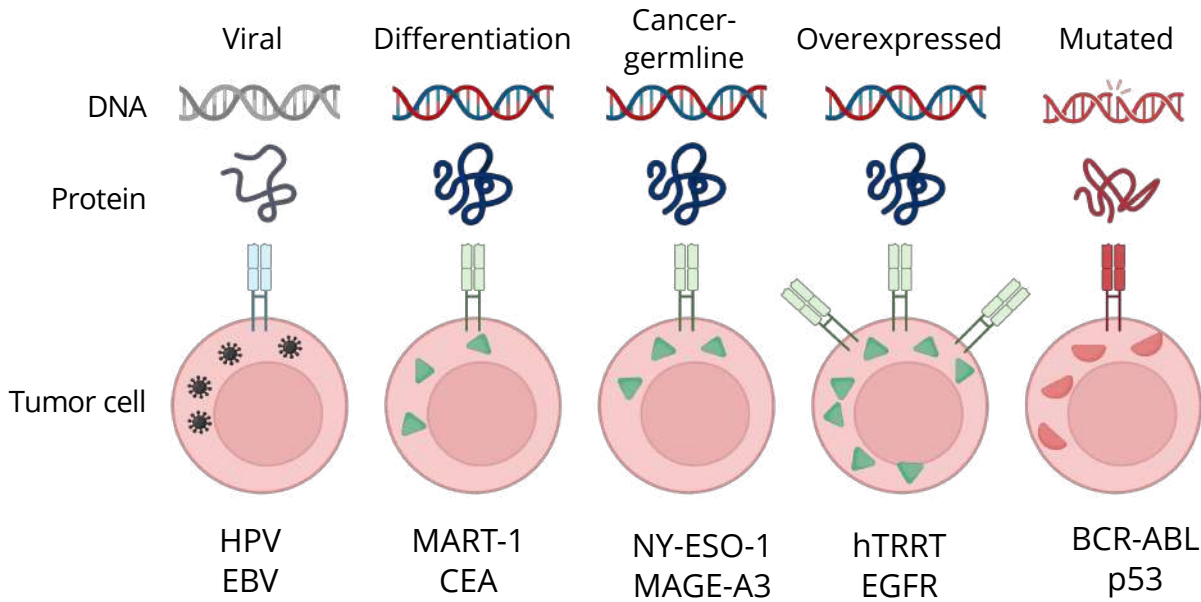


**IAF-ICP** funded: \$3,897,195  
(1 Sept 2022 to 31 Aug 2025)

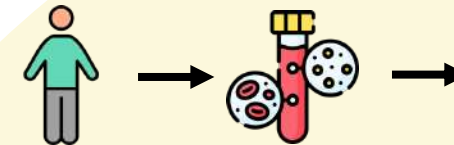


Tumor cell

## Tumor-associated antigens (TAAs)

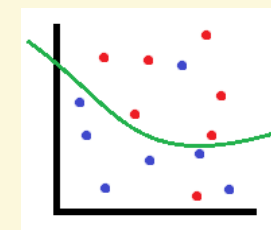
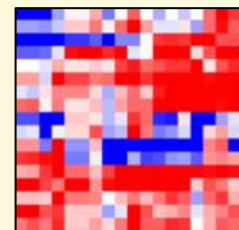
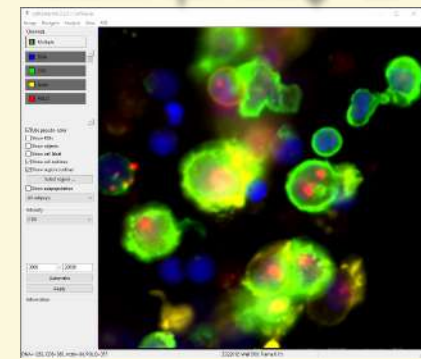


Cancer patients



Machine learning

cellXpress 2



**Classification of TAAs**



Personalized treatment



# Imaging-based phenotypic profiling to predict tumor-associated antigens

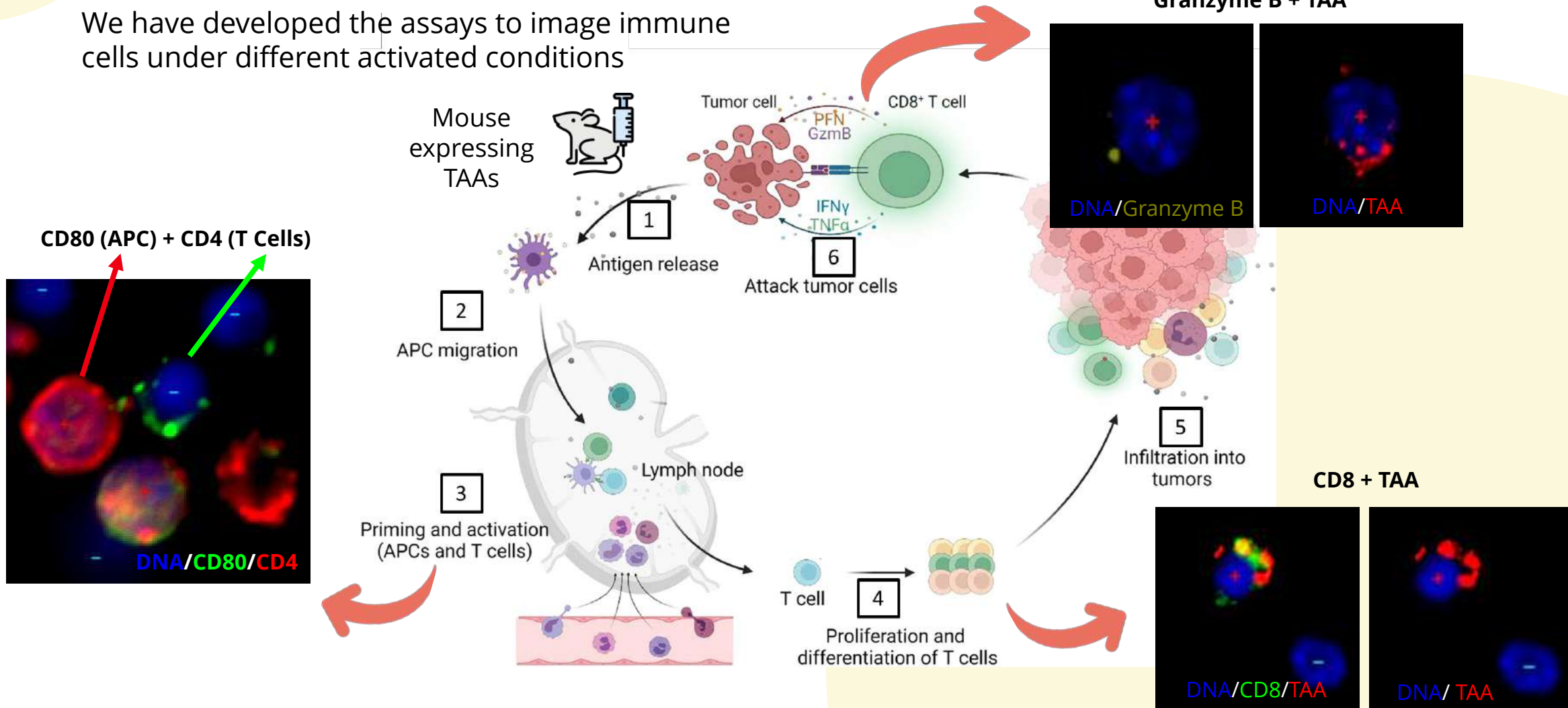
Oscar Fu



Bioinformatics Institute BII

Institute of Molecular and Cell Biology IMCB

We have developed the assays to image immune cells under different activated conditions



# COMPLEX CELLULAR PHENOTYPE ANALYSIS GROUP



Claresta, Cuithbert, Oscar, Guorui  
Carmen, Lit-Hsin, Joey

# Thank you



## IMCB, A\*STAR

- Dr Joe Yeong
- Jeffrey Lim
- Dr Meng Jia
- Prof Vinay Tergaonkar
- Dr Abdul Aziz Aiderus



## National Cancer Center

- Prof Pierce Chow
- Dr Chew Sin Chi



## Singapore General Hospital

- Dr Leow Weiqiang
- Dr Shihleone Loong



## LKC School of Medicine

- Prof Joanne Ngeow
- Chong Siao Ting
- Prof Wang Yulan



## Harvard BIDMC

- Dr Jiang Sizun
- Yeo Yao Yu

