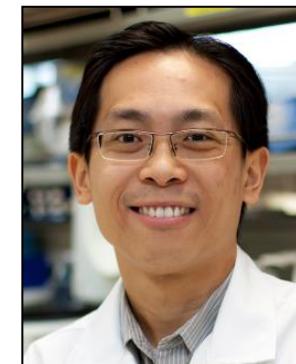
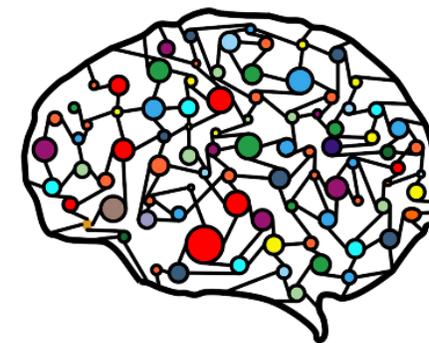
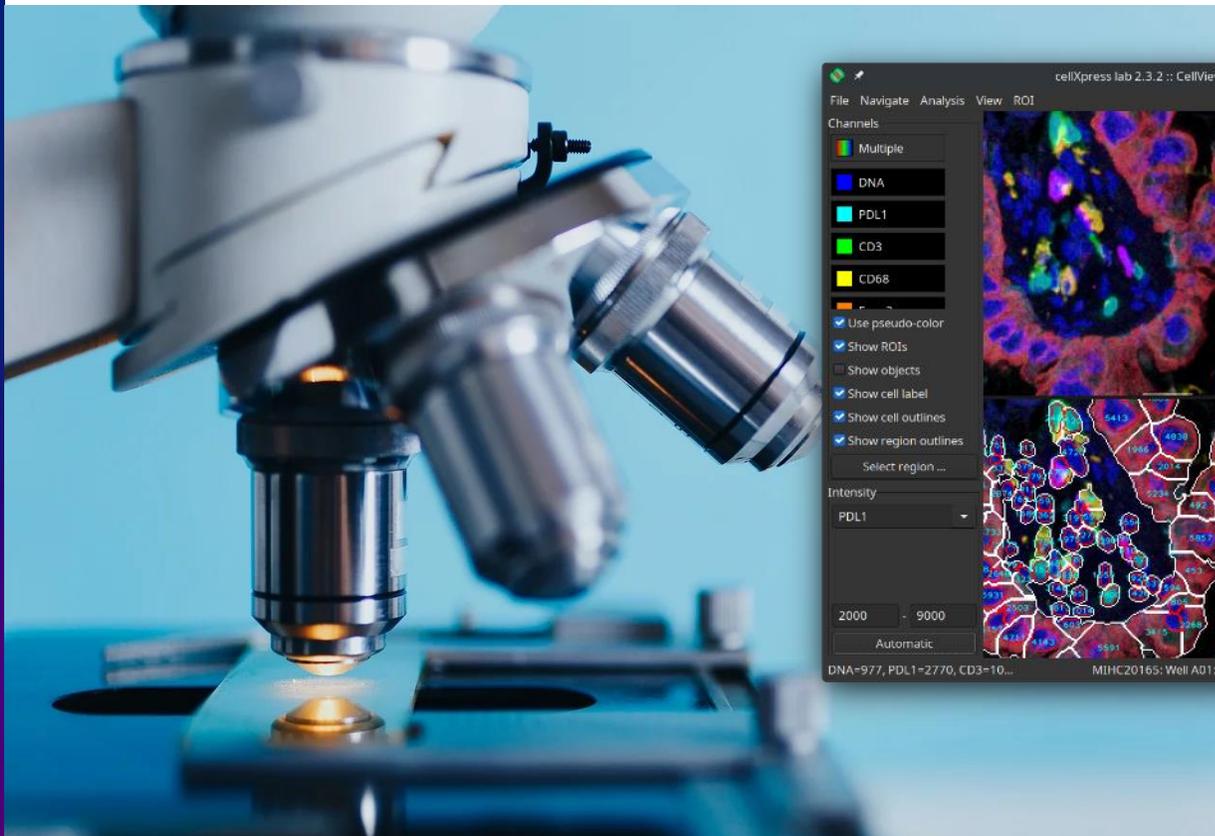




CREATING GROWTH, ENHANCING LIVES



# Complex Cellular Phenotype Analysis

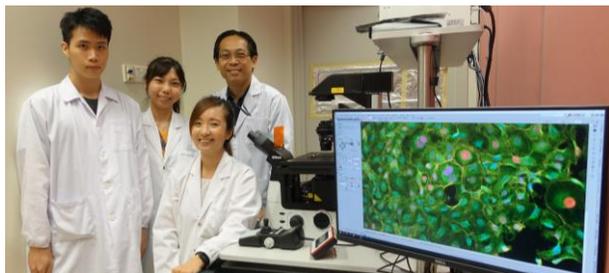


**Loo Lit Hsin**  
Senior Principal Investigator  
BII, A\*STAR  
l00lh@bii.a-star.edu.sg





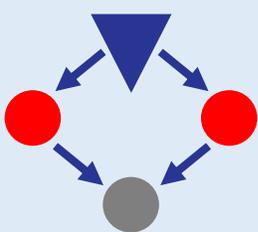
# Complex Cellular Phenotype Analysis Group, BII



## Computational Pharmacology/Toxicology



- Chemical/drug safety or efficacy assessment and prediction
- High-throughput Imaging-based Phenotypic Profiling (HIPP)
- Machine learning, data analysis, and assay automation



### ToxMAD

Toxicity Mode-of-Action  
Discovery Platform

Assess chemical safety based  
on mechanistic reasoning



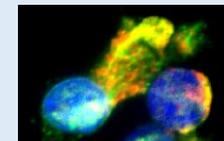
### Bioimage Databases

HPAShare  
HPAScore **ImmunoAtlas**

View and share anywhere, anytime

Bioimage management, visualization,  
standardization, and analytics

### Digital Medicine for Cancer



Data-driven cancer  
treatment and care



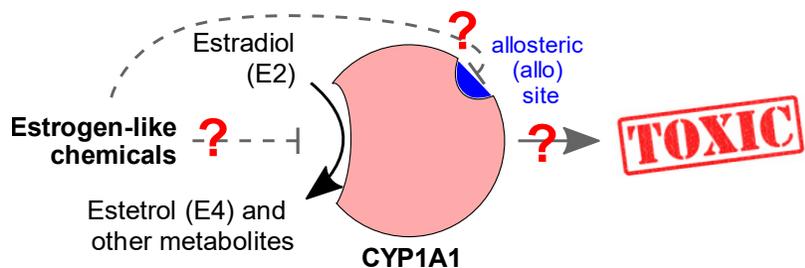
## Six Publications in 2021/2022

\* IF>5.0 (5 papers)

- **\*Choice of PD-L1 immunohistochemistry assay influences clinical eligibility for gastric cancer immunotherapy.** J Yeong, HYJ Lum, CB Teo, BKJ Tan, YH Chan, RYK Tay, JRE Choo, AD Jeyasekharan, QH Miow, **LH Loo**, WP Yong, R Sundar. *Gastric Cancer*. (in press).
- **\*ImmunoAtlas: an online public portal for sharing, visualizing, and referencing multiplex immunohistochemistry/immunofluorescence (mIHC/IF) images and results for immuno-oncology.** **JYJ Lee**, LWJN Lee, **J Dong**, J Yeong, **LH Loo**. *Journal for ImmunoTherapy of Cancer*. (2021) 9(Suppl 2):A657.
- **Group VIII metal carbonyl cluster-boronic acid conjugates: cytotoxicity and mode of action studies.** **JW Kong**, Z Lam, KH Chan, R Ganguly, **JYJ Lee**, **LH Loo**, RD Webster, ZX Wong, WK Leong. *ACS Omega*. (2021) 6:29045-29053
- **\*Leveraging advances in immunopathology and artificial intelligence to analyze in vitro tumor models in composition and space.** TKM Leong, WS Lo, WEZ Lee, B Tan, XZ Lee, LWJN Lee, **JYJ Lee**, N Suresh, **LH Loo**, E Szu, J Yeong. *Advanced Drug Delivery Reviews*. (2021) 177:113959.
- **\*Structure-based virtual screening of CYP1A1 inhibitors: towards rapid tier-one assessment of potential developmental toxicants.** **JJN Goh**, J Behn, CS Chong, **G Zhong**, S Maurer-Stroh, H Fan, **LH Loo**. *Archives of Toxicology*. (2021) 95:3031-3048.
- **\*Virtual screening of potentially endocrine-disrupting chemicals against nuclear receptors and its application to identify PPAR $\gamma$ -bound fatty acids.** CK Jaladanki, Y He, LN Zhao, S Maurer-Stroh, **LH Loo**, H Song, and H Fan. *Archives of Toxicology*. (2021) 95:355-374



# A truly Inter-division Collaboration from BII!



Archives of Toxicology (2021) 95:3031–3048  
<https://doi.org/10.1007/s00204-021-03111-2>

IF: 5.153

BIOINFORMATICS AND STATISTICS

## Structure-based virtual screening of CYP1A1 inhibitors: towards rapid tier-one assessment of potential developmental toxicants

Janice Jia Ni Goh<sup>1</sup> · Julian Behn<sup>1</sup> · Cheng-Shoong Chong<sup>1,2</sup> · Guorui Zhong<sup>1</sup> · Sebastian Maurer-Stroh<sup>1,2,3</sup> · Hao Fan<sup>1,4,5</sup> · Lit-Hsin Loo<sup>1,6</sup>

Received: 25 May 2021 / Accepted: 17 June 2021 / Published online: 28 June 2021  
 © The Author(s) 2021



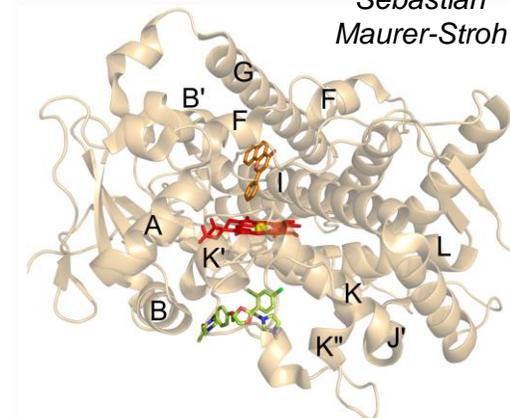
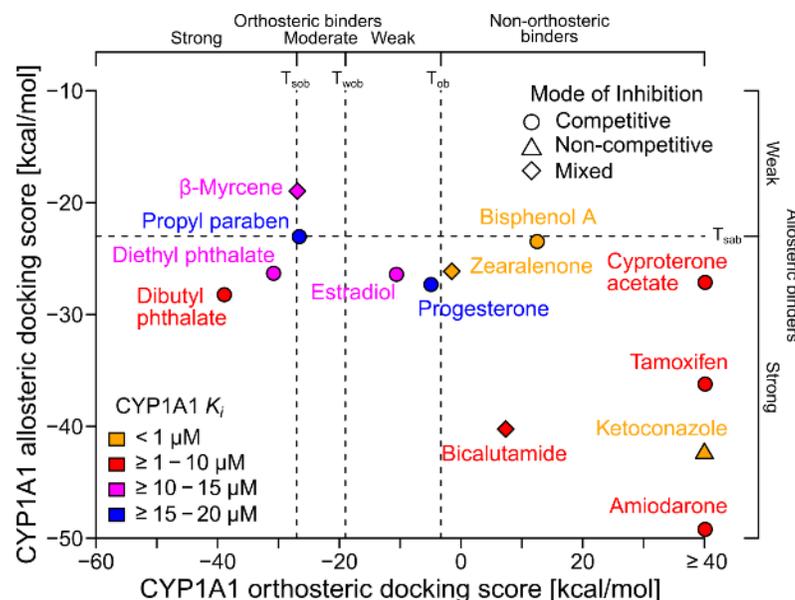
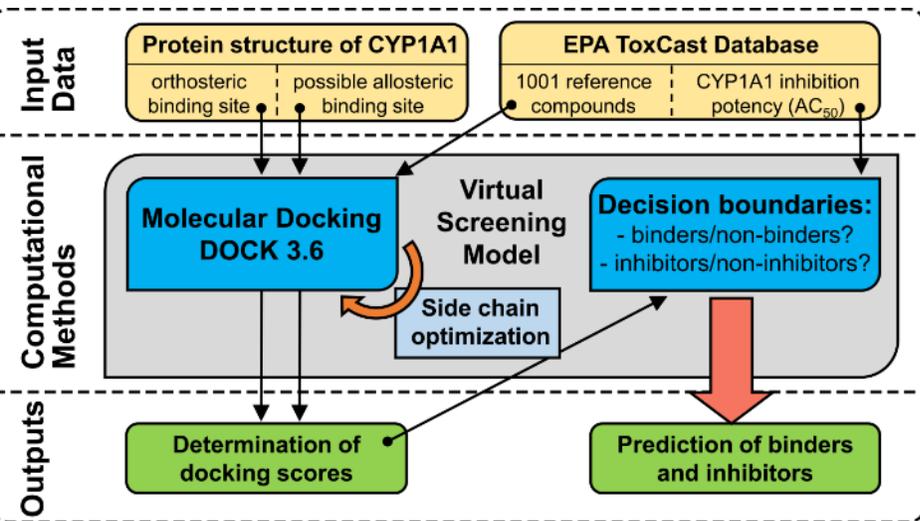
Lit-Hsin Loo



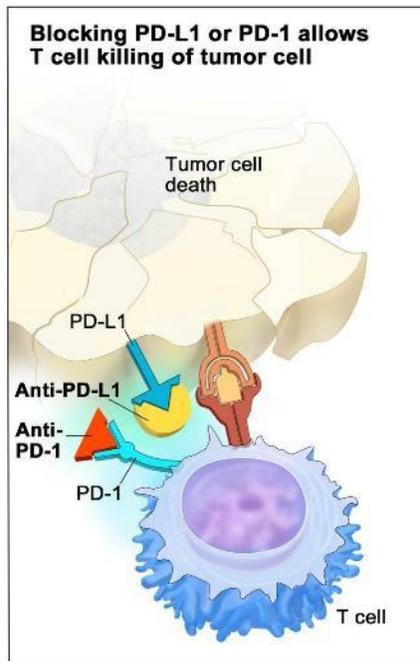
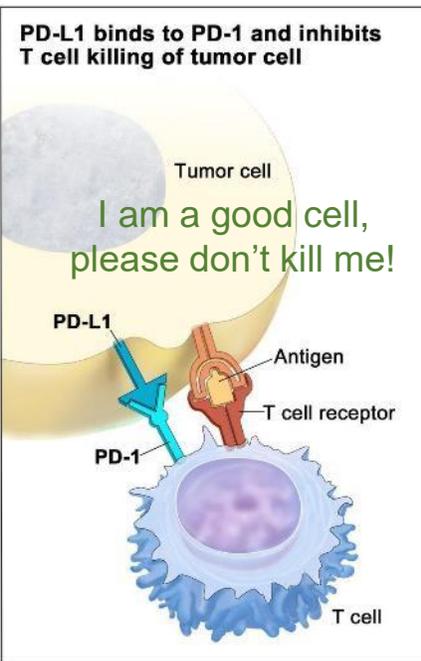
Hao Fan



Sebastian Maurer-Stroh



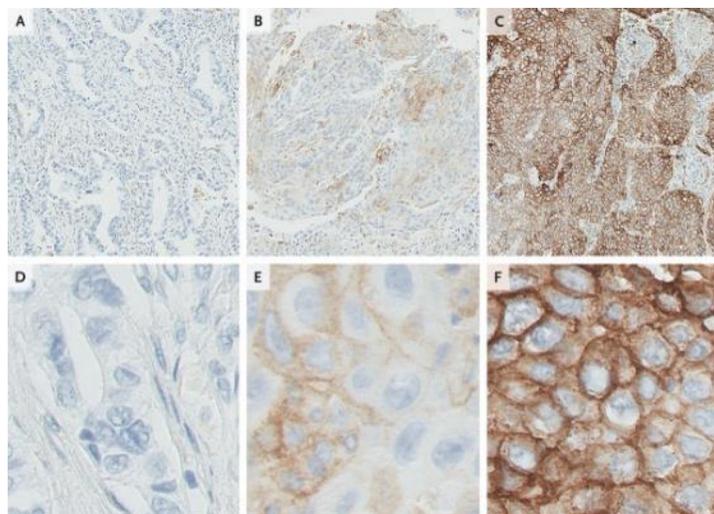
We confirmed that most predicted inhibitors, including **drugs contraindicated during pregnancy** (amiodarone, bicalutamide, cyproterone acetate, ketoconazole, and tamoxifen) and **chemicals suspected to be endocrine disruptors** (bisphenol A, diethyl and dibutyl phthalates, and zearalenone), are indeed potent inhibitors of CYP1A1.



© 2015 Terese Winslow LLC. U.S. Govt. has certain rights

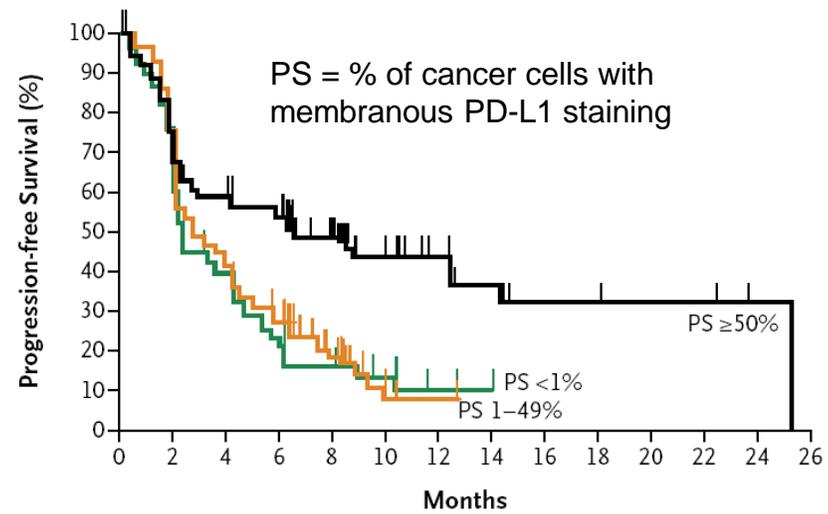
# PD-L1 scoring is critical for immunotherapy patient selection

- PD-1 is an immune-checkpoint receptor expressed on the surface of many immune cells. Its ligands (PD-L1 and -L2) inhibits T cell activations.
- Thus, abnormally-high PD-L1 expression in tumor and/or other antigen-presenting cells prevents the immune system from attacking the tumor cells.
- PD-1 or PD-L1 inhibitors (e.g., nivo or pembro) may reactivate the immune responses, but not all patients may respond to the drugs



PS < 1%    PS 1-49%    PS > 50%

Manual scoring by pathologists



No. at Risk	0	2	4	6	8	10	12	14	16	18	20	22	24	26
PS ≥ 50%	119	86	66	60	38	20	13	8	4	3	3	3	1	0
PS 1-49%	161	122	70	45	21	4	1	0	0	0	0	0	0	0
PS < 1%	76	52	29	17	11	6	2	0	0	0	0	0	0	0

[Garon et al., NEJM, 2015] KEYNOTE-001



# Several PD-L1 antibodies have been approved as companion diagnostic (CDx) assays

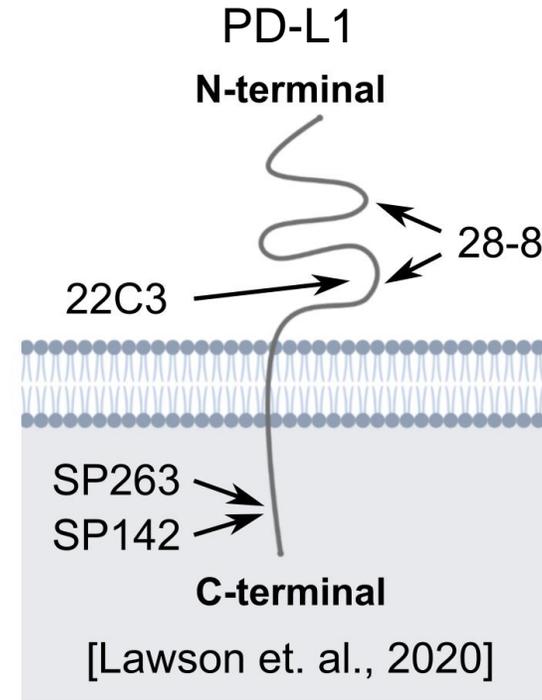
Clones	Approved	Cancer types	Drugs	Selection cutoff points
22C3 (Dako)	FDA (CDx)	TNBC	Pembrolizumab	CPS ≥ 10
	FDA (CDx)/ CE-IVD	NSCLC	Pembrolizumab	TPS ≥ 1% (Stage III and treated metastatic); TPS ≥ 50% (untreated metastatic)
	FDA (CDx)	NSCLC	Cemiplimab-rwlc	TPS ≥ 50%
	FDA (CDx)	GC	Pembrolizumab	CPS ≥ 1
	FDA (CDx)/ CE-IVD	UC	Pembrolizumab	CPS ≥ 10
	FDA (CDx)/ CE-IVD	HNSCC	Pembrolizumab	CPS ≥ 1
	FDA (CDx)	Cervical cancer	Pembrolizumab	CPS ≥ 1
	FDA (CDx)	ESCC	Pembrolizumab	CPS ≥ 10
28-8 (Dako)	FDA (CDx)/CE-IVD	NSCLC	Nivolumab with Ipilimumab	TPS ≥ 1%
	FDA (CoDx)/CE-IVD	UC	Nivolumab	TPS ≥ 1%
	FDA (CoDx)/CE-IVD	HNSCC	Nivolumab	TPS ≥ 1%
	FDA (CoDx)/CE-IVD	nsNSCLC	Nivolumab	TPS ≥ 1% or ≥ 5% or ≥ 10%
SP142 (Ventana)	FDA (CDx)/CE-IVD	TNBC	Atezolizumab	IC ≥ 1%
	FDA (CDx)/CE-IVD	NSCLC	Atezolizumab	TPS ≥ 50% or IC ≥ 10%
	FDA (CDx)/CE-IVD	UC	Atezolizumab	IC ≥ 5%
SP263 (Ventana)	CE-IVD	NSCLC	Durvalumab	TPS ≥ 1%
	FDA (CoDx)/CE-IVD	UC	Durvalumab	TPS ≥ 25%; or ICP > 1% and IC ≥ 25%; or ICP = 1% and IC = 100%
	CE-IVD	NSCLC	Pembrolizumab	TPS ≥ 50% (1st line); TPS ≥ 1% (2nd line)
	CE-IVD	NSCLC	Nivolumab	TPS ≥ 1%, ≥ 5% and ≥ 10%

### Note:

$$\text{Tumor Proportion Score (TPS)} = \frac{\text{\# of PD-L1+ tumor cells}}{\text{\# of viable tumor cells}} \times 100\%$$

$$\text{Combined Positive Score (CPS)} = \frac{\text{\# of PD-L1+ cells (include tumor and immune cells)}}{\text{\# of viable tumor cells}}$$

$$\text{Tumor-infiltrating Immune Cell (IC)} = \frac{\text{Tumor area covered by PD-L1+ immune cells}}{\text{Total tumor area}} \times 100\%$$

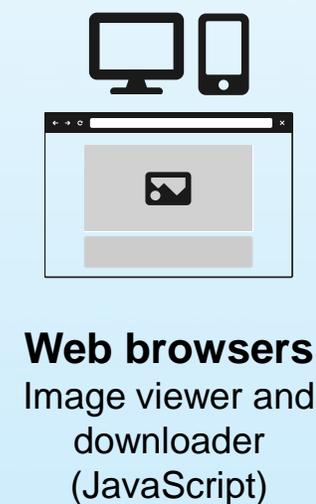
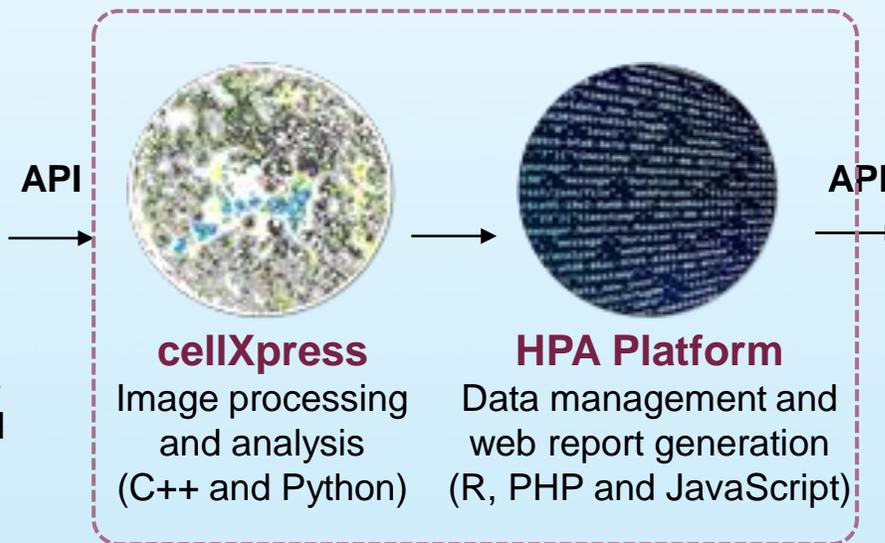
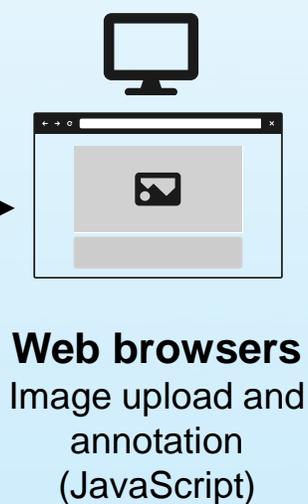
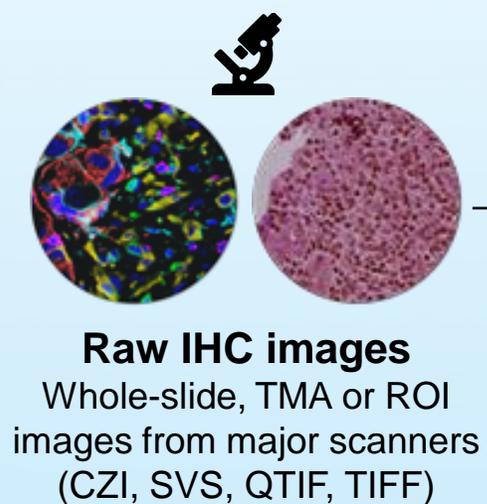


Different PD-L1 antibodies may have different staining patterns, intensities, thresholds, or even clinical decisions!

# HPAScore

View and share anywhere, anytime

- Our cloud-based and digital solution **simplifies** and **accelerates** the management and scoring of immunofluorescence and histopathological images of cells, organoids, or tissues.
- Provided as a service via RSC



Joey Lee  
(Platform manager)



Carmen Kong



Jiahui Dong



CREATING GROWTH, ENHANCING LIVES



View and share anywhere, anytime

# An example of HPA applications: PD-L1 scoring for gastric cancers



PD-L1 IHC 28-8 pharmDx is CE-IVD Marked to Identify Gastric Adenocarcinoma, Gastroesophageal Junction Adenocarcinoma, and Esophageal Adenocarcinoma Patients for Treatment with OPDIVO®

More personalized cancer results. One test makes it possible.



Agilent Dako

PD-L1 IHC 28-8 pharmDx is the only clinically validated test which aids in identifying appropriate advanced or metastatic gastric, GEJ, and esophageal adenocarcinoma with HER2-negative patients whose tumors express PD-L1 with CPS  $\geq 5$  for the first-line treatment with OPDIVO (nivolumab) in combination with fluoropyrimidine and platinum-based chemotherapy.



EUROPEAN MEDICINES AGENCY  
SCIENCE MEDICINES HEALTH

[<https://www.agilent.com/cs/library/brochures/29457-d68866-pd-l1-28-8-gastric-brochure-en-eu.pdf>]



[<https://news.bms.com/news/details/2021/U.S.-Food-and-Drug-Administration-Approves-Opdivo-nivolumab-in-Combination-with-Chemotherapy-for-Patients-with-Advanced-or-Metastatic-Gastric-Cancer-Gastroesophageal-Junction-Cancer-and-Esophageal-Adenocarcinoma/default.aspx>]



U.S. Food and Drug Administration Approves Opdivo® (nivolumab) in Combination with Chemotherapy for Patients with Advanced or Metastatic Gastric Cancer, Gastroesophageal Junction Cancer, and Esophageal Adenocarcinoma, **Regardless of PD-L1 Expression Status**

04/16/2021

CATEGORY: Corporate/Financial News

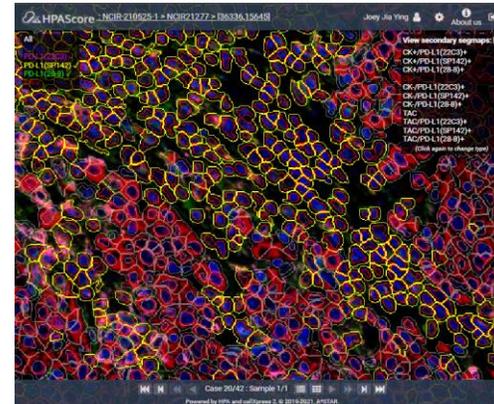
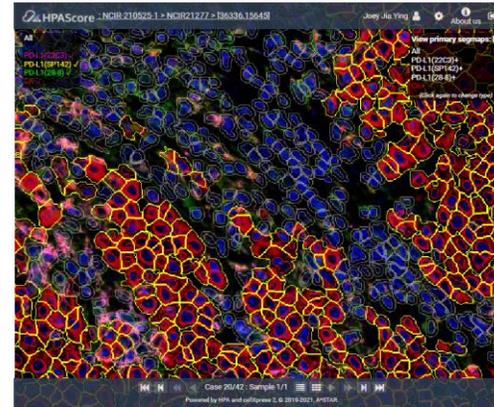
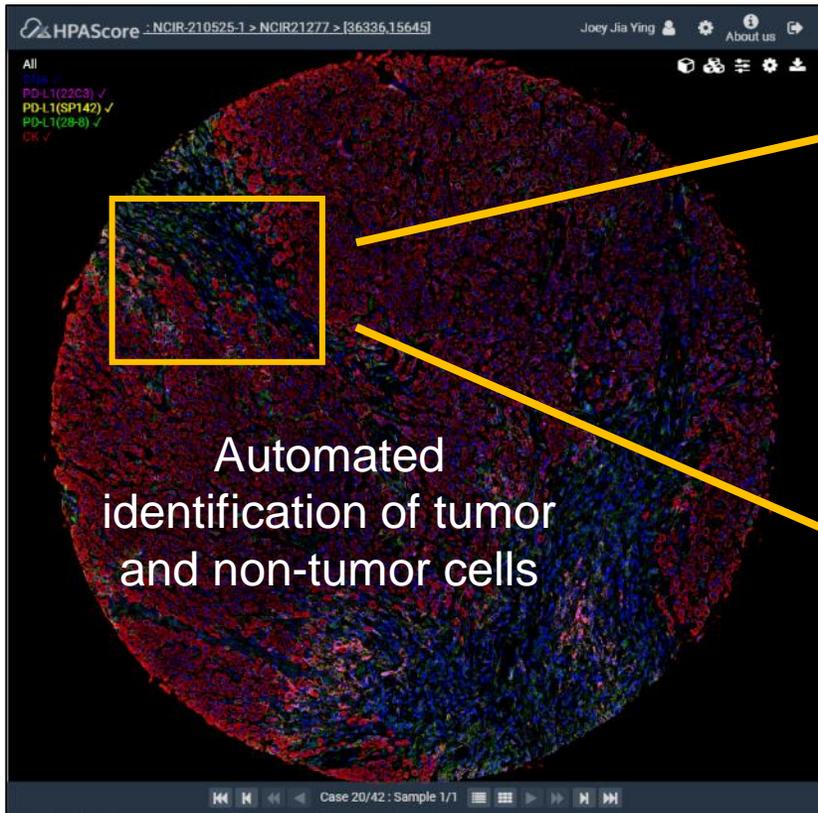
*Opdivo is the first and only immunotherapy in combination with chemotherapy to deliver superior overall survival versus chemotherapy alone in a trial of this patient population<sup>1</sup>*

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

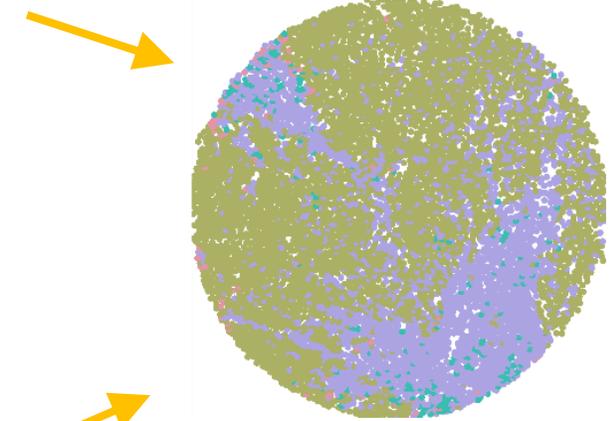
J Yeong, HYJ Lum, CB Teo, BKJ Tan, YH Chan, RYK Tay, JRE Choo, AD Jeyasekharan, QH Miow, **LH Loo**, WP Yong, R Sundar. *Gastric Cancer*, (in press). IF:7.37

- To investigate the interchangeability among three clinically-used PD-L1 CDx assays (22C3, 28-8, SP142) for patient selection in gastric cancers
- The first large-scale study that **score all three markers on the same tissue slides**
- 344 gastric cancer patients from NUH (1997-2019)
- 97GB images uploaded and scored (fully online) using the HPA Platform

[Raghav Sundar]



Automated quantification of PD-L1 levels of the cells



Subpopulation scores:

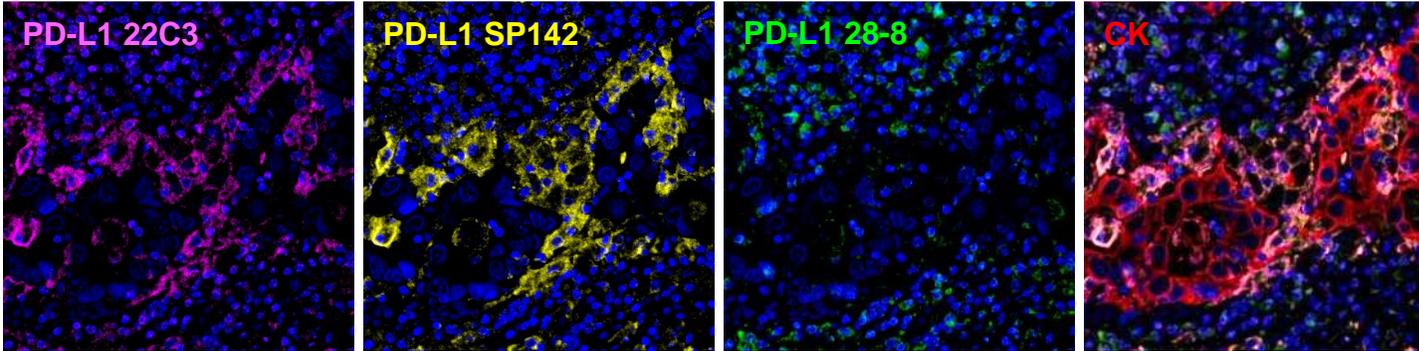
- CK+/PD-L1(28-8)+ (0.92%)
- CK-/PD-L1(28-8)+ (2.79%)
- CK+/PD-L1(28-8)- (59.28%)
- CK-/PD-L1(28-8)- (37.02%)

$$\text{Combined Positive Score (CPS)} = \frac{\text{\# of PD-L1+ cells}}{\text{\# of tumor cells}}$$

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

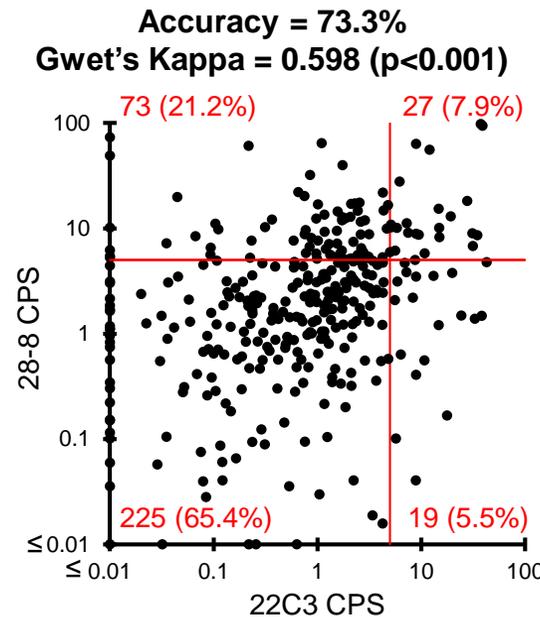
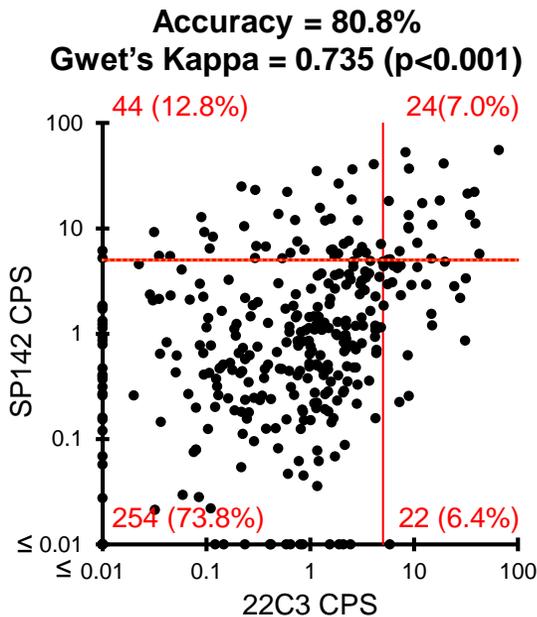
J Yeong, HYJ Lum, CB Teo, BKJ Tan, YH Chan, RYK Tay, JRE Choo, AD Jeyasekharan, QH Miow, **LH Loo**, WP Yong, R Sundar. *Gastric Cancer*, (in press). IF:7.37

[Raghav Sundar]



Assay	CPS ≥1	CPS ≥5	CPS ≥10
22C3	170 (49.4%)	46 (13.4%)	24 (7.0%)
28-8	242 (70.3%)	100 (29.1%)	47 (13.7%)
SP-142	170 (49.4%)	68 (19.8%)	33 (9.6%)

n = 344



- Using HPA, we found that scoring PD-L1 with the 28-8 assay may result in higher PD-L1 CPS scores, and higher proportion of PD-L1 positivity compared to 22C3 and other assays in gastric cancers
- Interestingly, our results are consistent with previous clinical trials: CheckMate 649 using 28-8 (60% patients with CPS>5) and KEYNOTE-061 using 22C3 (31%)
- These assays may not be equivalent, and the results need to be treated with caution
- The first primary research publication based on HPA



# ImmunoAtlas

<https://ImmunoAtlas.org>

## Public portal and references for immuno-oncology phenotypes and diagnostic/prognostic markers

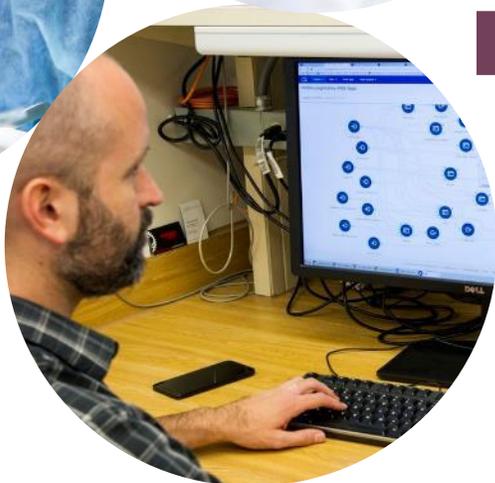


[Lee et al., J. Immunother. Cancer, 2021]

How do tumors with poor outcomes look like?

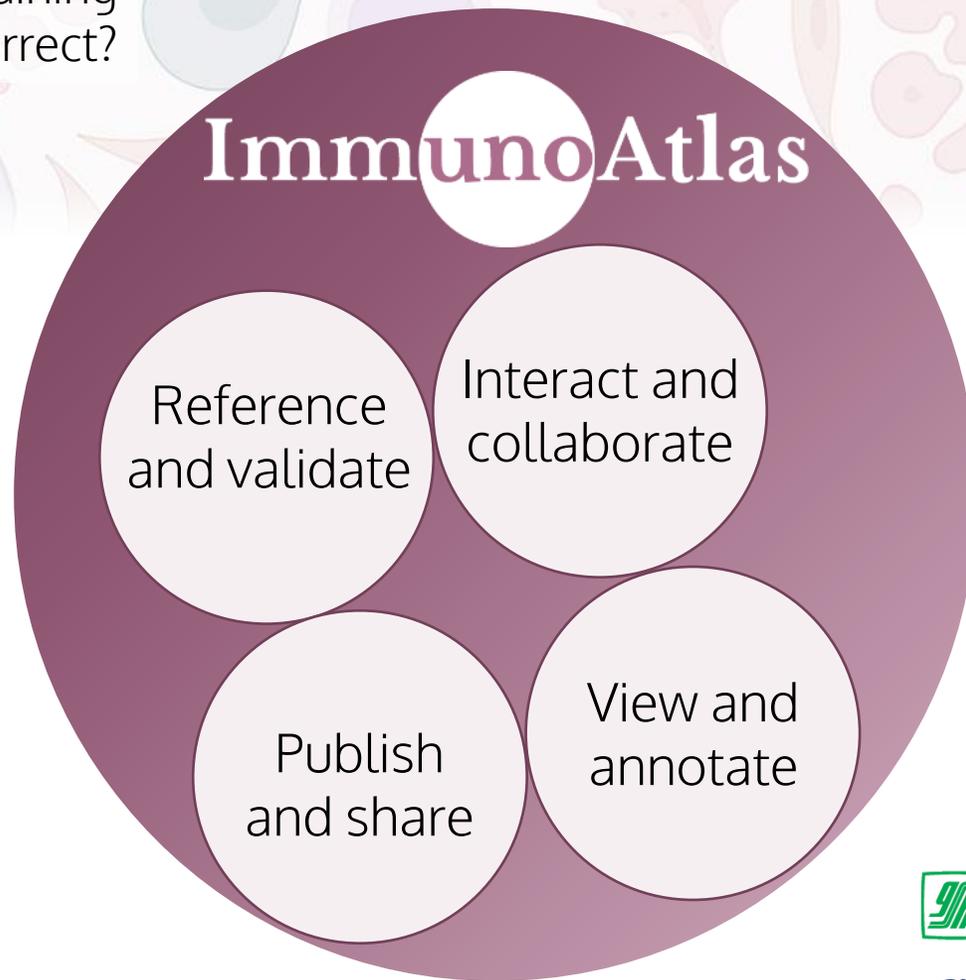
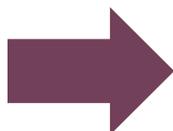


Does this patient have similar phenotypes as the published cases?



How do I train or benchmark my AI algorithms?

Is my staining correct?



Joey Lee



Joe Yeong



Singapore General Hospital  
SingHealth

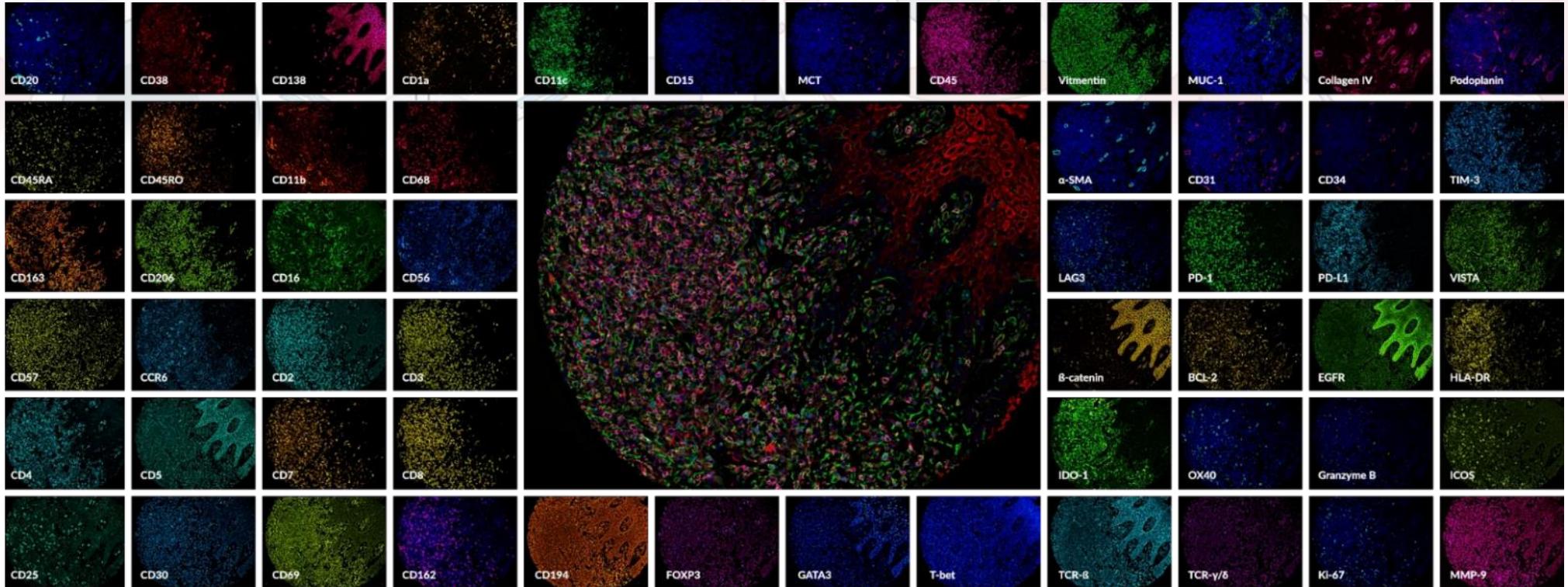


Institute of Molecular and Cell Biology  
IMCB



# Public portal and references for immuno-oncology phenotypes and diagnostic/prognostic markers

- An example of **56-marker** CODEX images of human Cutaneous T-cell Lymphoma from Stanford



[Phillips et al., Front. Immunol., 2021]

**T-cells** – CD2, CD3, CD4, CD5, CD7, CD8, ...  
**Lymphocytes** - CD45, CD45RA, CD45RO  
**B cells and plasma cells** – CD20, CD38, CD138  
**Macrophages** – CD11b, CD68, CD163, CD206  
**Dendritic cells** – CD1a, CD11c

**Epithelia** – CK, MUC-1  
**Smooth muscle** –  $\alpha$ -SMA  
**Vasculature** – CD31, CD34  
**ECM** – Collagen IV  
**Stroma** – Vimentin

<https://immunoatlas.org/NOLN/210614-2/>  
**Proliferation** – Ki67, ICOS  
**Signaling** –  $\beta$ -catenin, EGFR, BCL-2, HLA-DR, Granzyme B  
**Immune checkpoint** – LAG-2, PD-1, PD-L1, Tim-3, VISTA





ImmunoAtlas : ABCM-220216-1 > ABCM22001 > [13761,39743] Lit Hsin

**Specimen information:**

Report ID	<a href="#">ABCM-220216-1</a>				
Report name	<a href="#">BC08118a</a>				
Study name	Abcam Cytolytic T-cell markers panel				
Study authors					
Summary	Markers	Host species	Clone	Supplier	Product Cat #
	DAPI			Akoya Biosciences	<a href="#">FP1490</a>
	Anti-PD-L1	Rabbit	Monoclonal (CAL10)	Abcam	<a href="#">ab251611</a>
	Anti-Granzyme B	Rabbit	Monoclonal (EPR20129-217)	Abcam	<a href="#">ab219803</a>
	Anti-PD1	Rabbit	Monoclonal (CAL20)	Abcam	<a href="#">ab251613</a>
	Anti-pan Cytokeratin	Mouse	Monoclonal (C-11)	Abcam	<a href="#">ab264485</a>
	Anti-EpCAM	Rabbit	Monoclonal (EPR20532-225)	Abcam	<a href="#">ab225894</a>
	Anti-CD8α	Rabbit	Monoclonal (CAL66)	Abcam	<a href="#">ab251596</a>
Anti-FOXP3	Mouse	Monoclonal (236A/E7)	Abcam	<a href="#">ab96048</a>	
Reference					
Specimen type	Human breast tissue				
Specimen ID	<a href="#">ABCM22001</a>				

Submitted by: Case 1/1 : Sample 12/60

Submitter: Justina Lee Powered by HPA and cellXpress 2, © 2019-2022, A\*STAR.

ImmunoAtlas : ABCM-220216-1 > ABCM22001 > [13761,39743] Lit Hsin

Specimen image:

- All
- DAPI ✓
- PD-L1 ✓
- GZMB ✓
- PD-1 ✓
- CK/EpCAM ✓
- CD8α ✓
- FOXP3 ✓

Case 1/1 : Sample 12/60

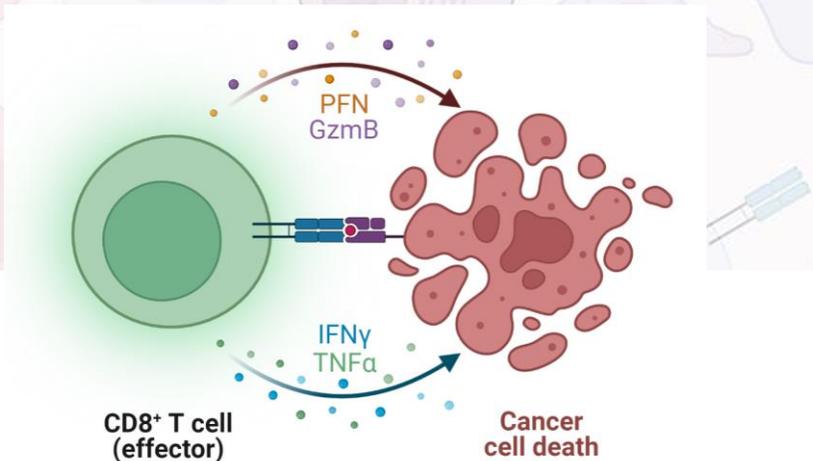
© All rights of the image are reserved by the creators of the image. Please contact them if you are interested in using the image. Powered by HPA and cellXpress 2, © 2019-2022, A\*STAR.

Patient E1 (age 79), breast tissue  
Invasive ductal carcinoma, stage IIIB

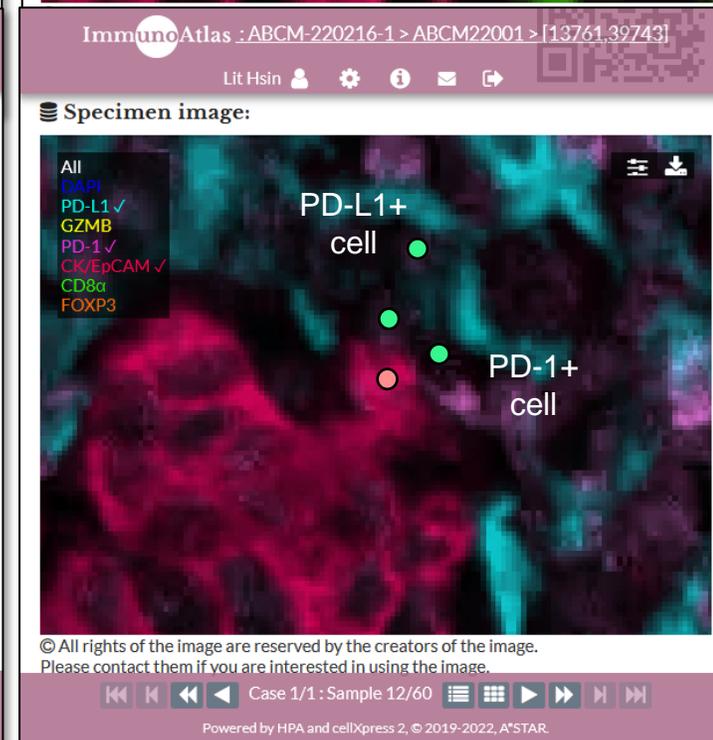
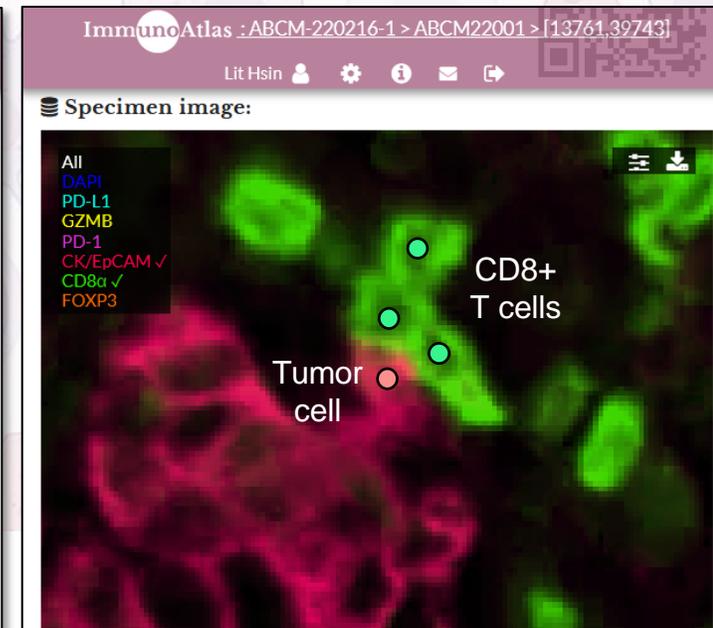
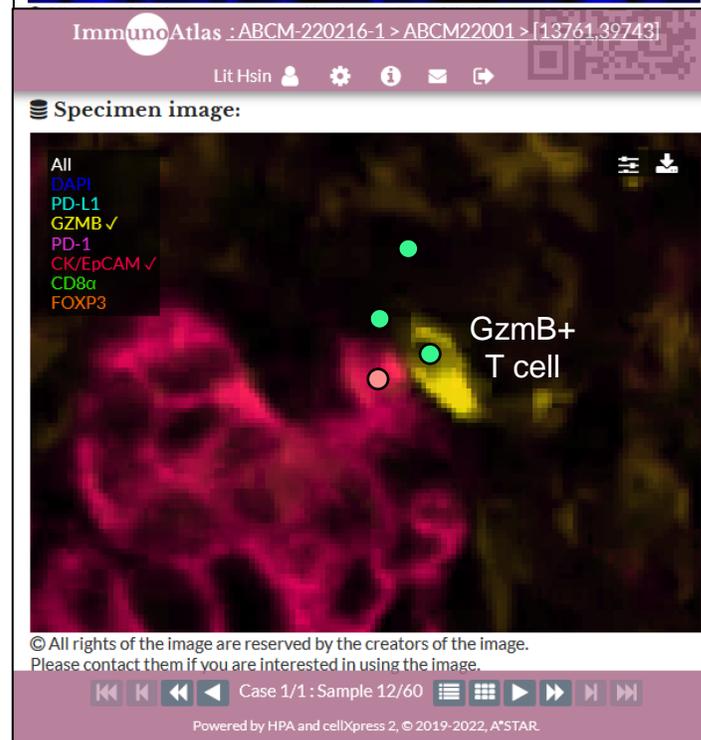
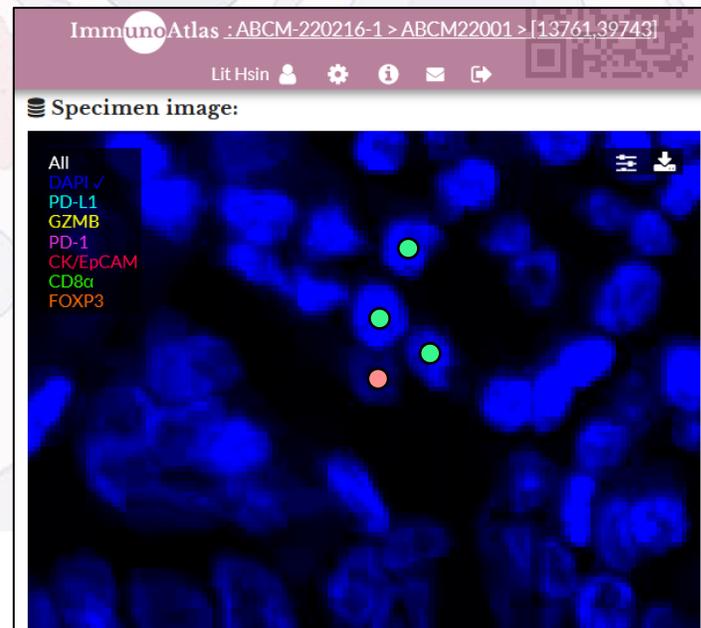




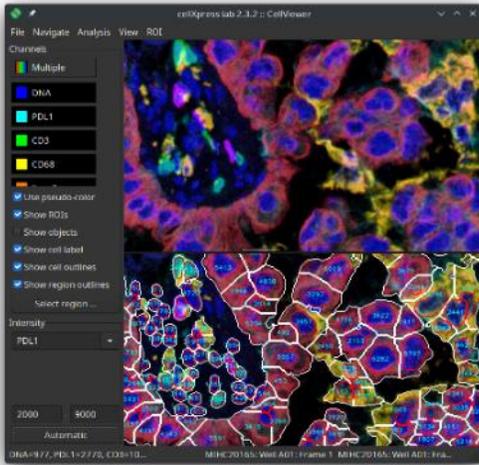
## Cytolytic T-cell Marker Panel



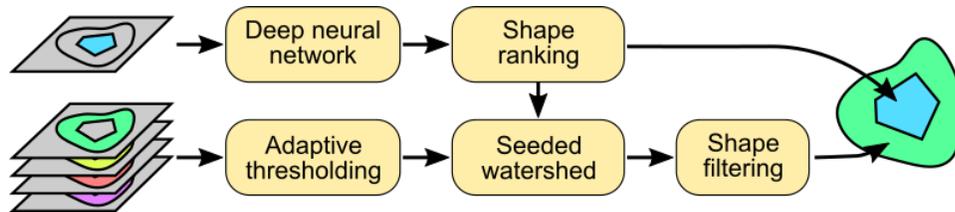
- Public reference for the markers and immune phenotypes (70 breast cancer patients – normal, stages I to III)
- Antibody/marker validation and comparison; and clinical application guidelines
- Training and benchmark data for AI algorithms and tools
- More markers and data are coming!



# cellXpress 2 for next-generation phenotypic profiling

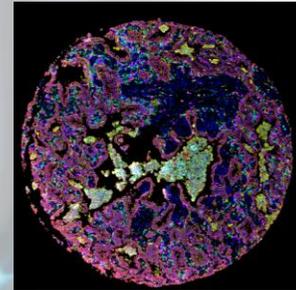


## CellShape AI

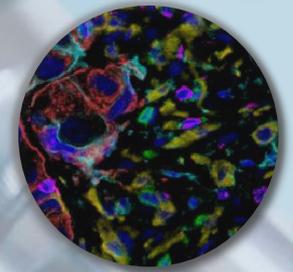


- Point-and-click, no programming needed
- Written in C++
- Support multi-CPU and GPU
- Publicly available soon
- Manuscript in preparation

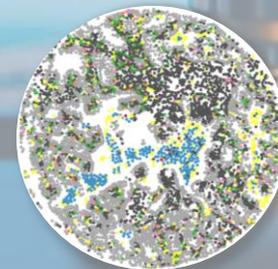
**Large tissue images**  
(>500MB/image)



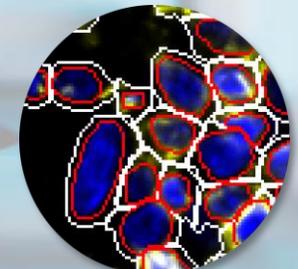
**Hyperplexed markers**  
(>50 markers)



**Spatial and multi-modal profiling**  
(DNA/RNA/  
Protein/Metabolite)



**AI-based cell segmentation**  
(heterogenous and overlapping cells)





# Thank you

New collaborations are welcome!  
looh@bii.a-star.edu.sg

## Complex Cellular Phenotype Analysis (CCPA) Group, BII, A\*STAR

- James Miller
- Su Su Htwe
- Zhong Guorui
- Joey Lee Jia Ying
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