Technology R&D


CRISPR KO, a, i pooled screens


CRISPR KO arrayed screen


siRNA, miRNA arrayed screens


Compound screening


Nucleofection


High Content Imaging

Live imaging


3D characterisation and screening


Metabolism

High throughput, single cell proteomics

PRIME - Project Management Portal

Customised image analysis

VCFG
Victorian Centre For Functional Genomics

innovation | discovery | impact

High throughput screening coupled with Imaging transcriptomics in 2D and 3D models

Prof Kaylene J. Simpson

Peter MacCallum Cancer Centre

Sir Peter MacCallum Department of Oncology, and

Department of Biochemistry and Pharmacology, University of Melbourne

Australia

@VCFGconnect

THE UNIVERSITY OF MELBOURNE

Peter Mac
Peter MacCallum Cancer Centre
Victoria Australia

A core facility needs to evolve, adapt, develop new tools, know what's coming

Data analysis and project management is critical

VCFG
Victorian Centre For Functional Genomics

x3 x1 — lab analyst

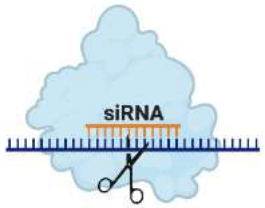
x6 x1

x7 X1.5

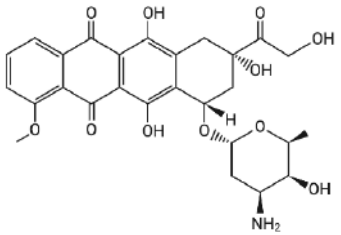
X7.5 x3

X7.5 x5

2008 RNA interference

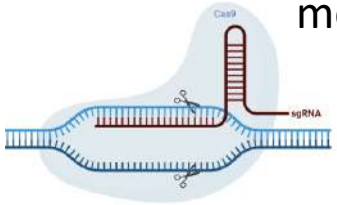


2012 Drug screening



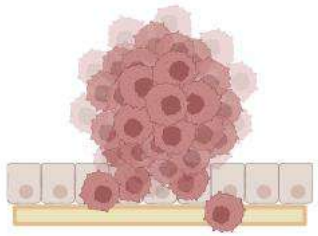
COMPOUNDS AUSTRALIA

2015 CRISPR

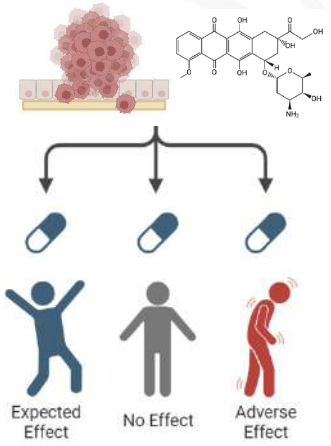


2016 VCCC move

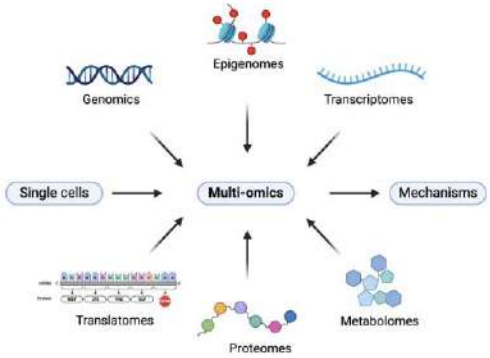
2019 Organoids



2023 Precision medicine

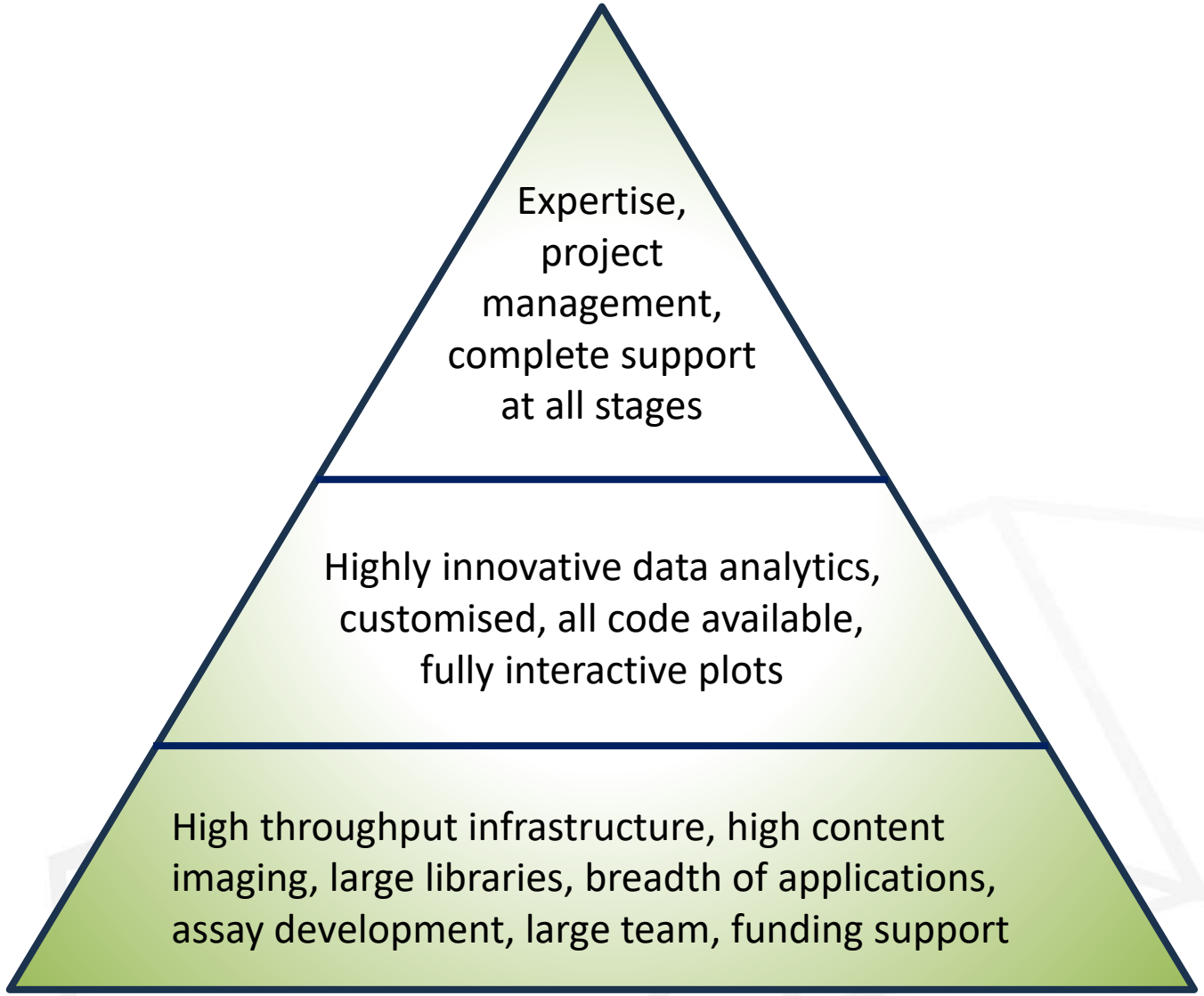


2024 Multi-OMICS



Built with BioRender assistance

VCFG
Victorian Centre For Functional Genomics

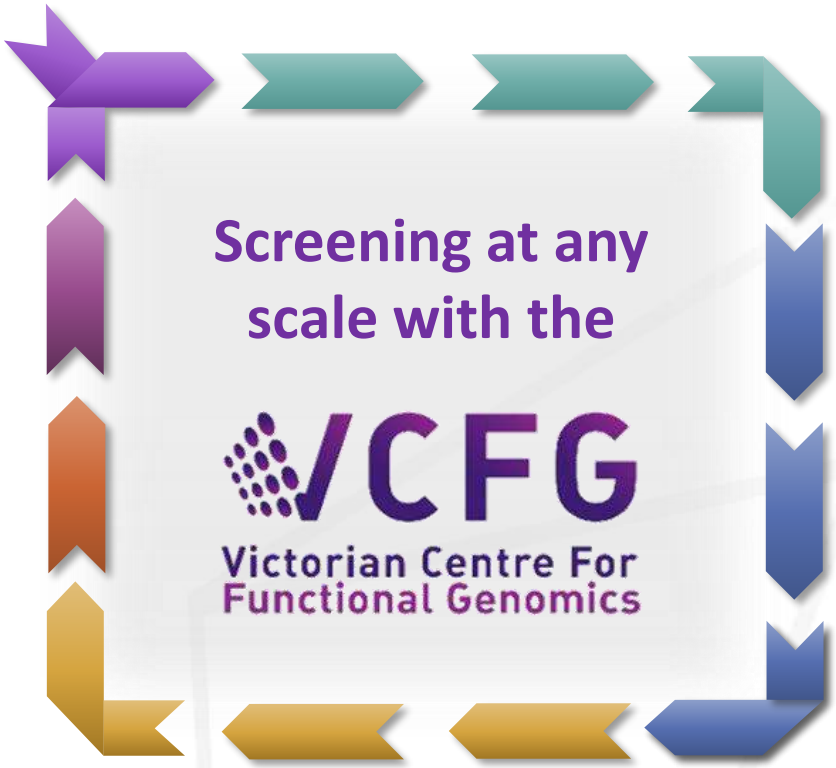


innovation | discovery | impact

Enabling discovery and driving translational medicine with high throughput technologies

Start with a discussion

✓Generate a screen plan
✓Define a grant aim
✓NCRIS voucher award



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Select your model

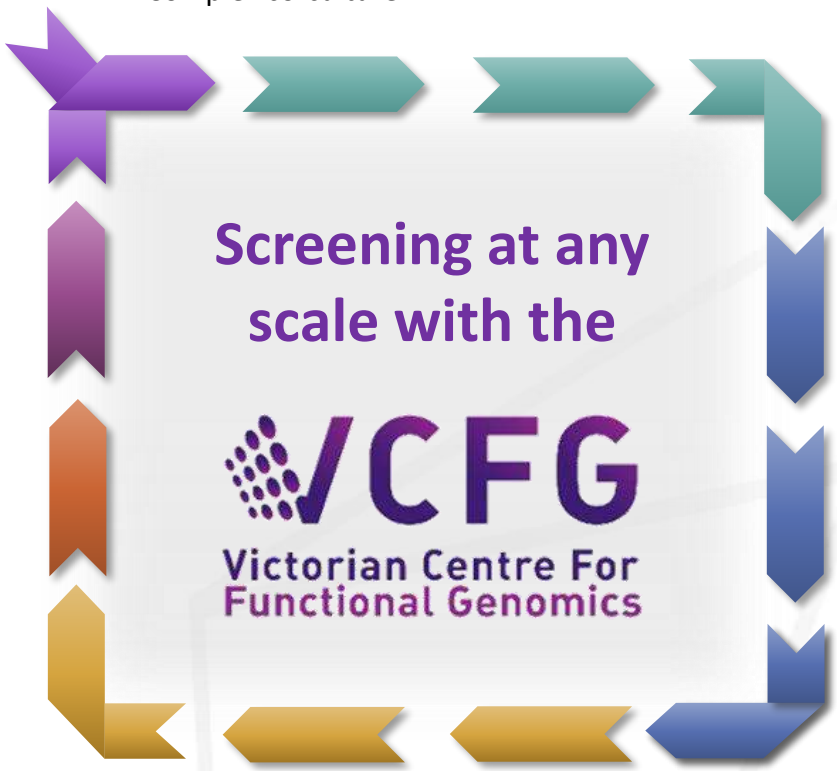
- ✓2D suspension
- ✓2D adherent
- ✓3D spheroids/ organoids
- ✓Complex co-culture

Select your perturbation

- ✓Compound library
- ✓BYO compounds
- ✓RNAi, CRISPR

Define your readout

- ✓Plate reader
- ✓Imaging
- ✓Flow cytometry
- ✓Sequencing (CRISPR)



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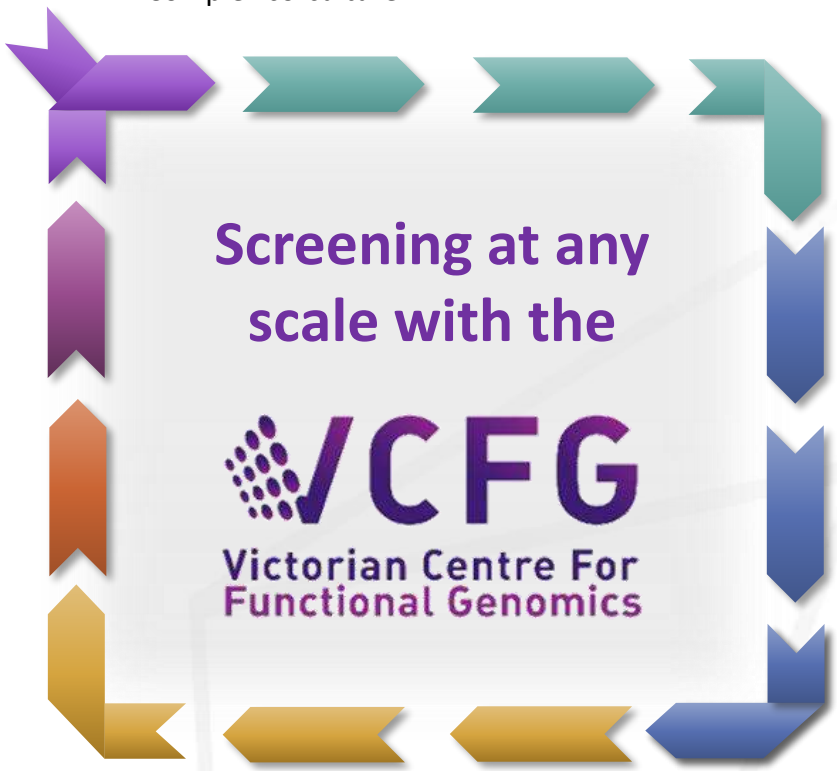

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Introduction to the lab

- ✓Lab induction
- ✓Instrument training
- ✓Protocol development

Optimisation, Assay dev

- ✓Dose-curves
- ✓Cell density
- ✓Live/fixed endpoint
- ✓Reporter assays

Run your screen

- ✓Weeks to months
- ✓Researcher-driven or Fee-for-Service
- ✓Concurrent Quality Control (QC) analyses

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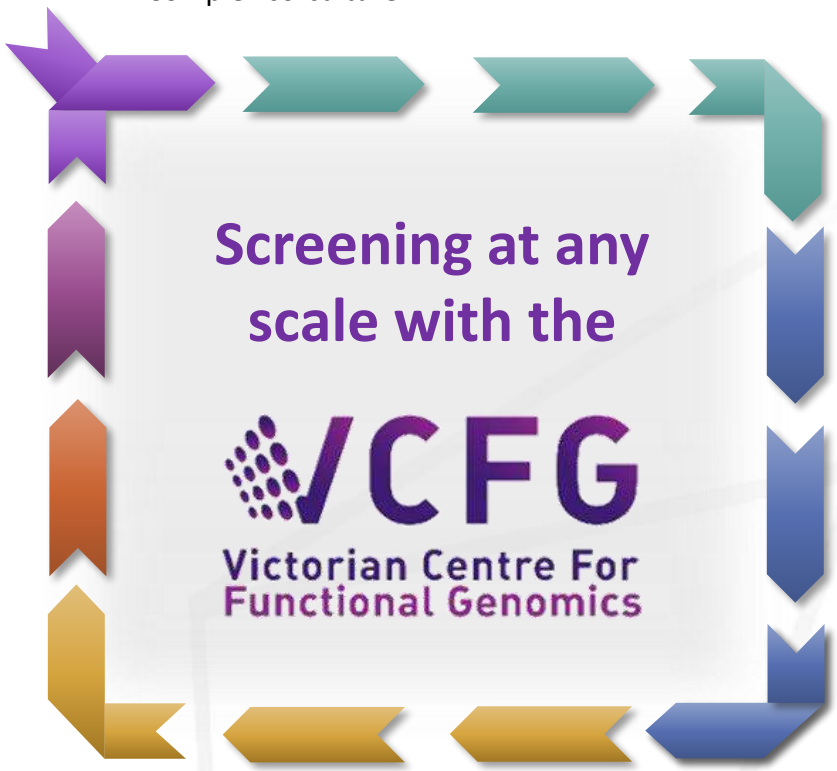

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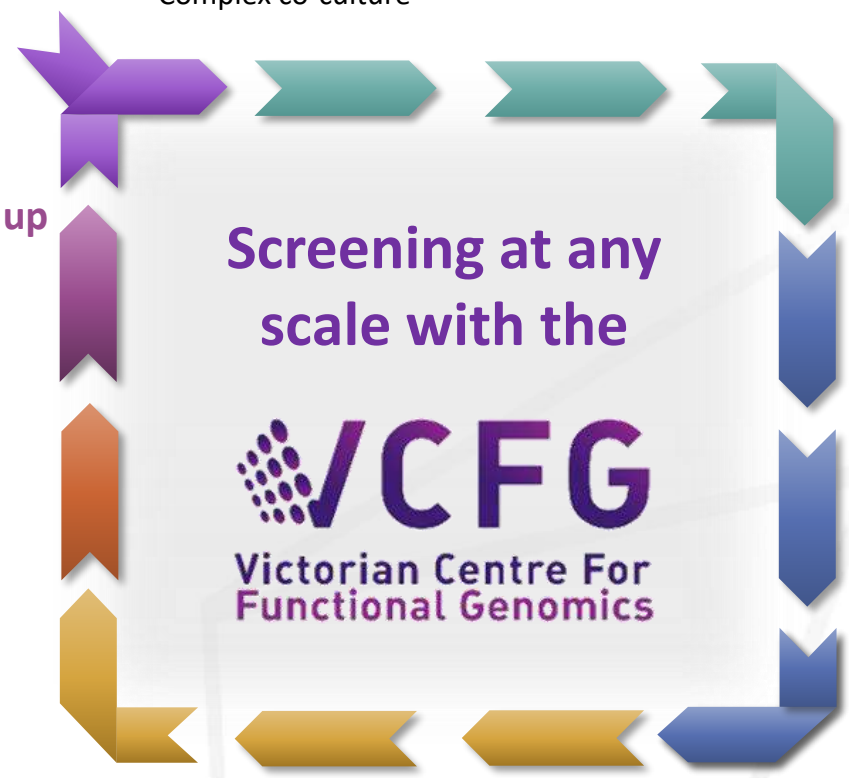
Screen QC output
- ✓Controls (pos/neg)
- ✓Z-prime factor
- ✓Variability (%CV)

Data analytics
- ✓Basic options
- ✓Advanced options
- ✓Customisation
- ✓Machine learning

Hit selection
- ✓Decisions to make
- ✓Plan secondary screen



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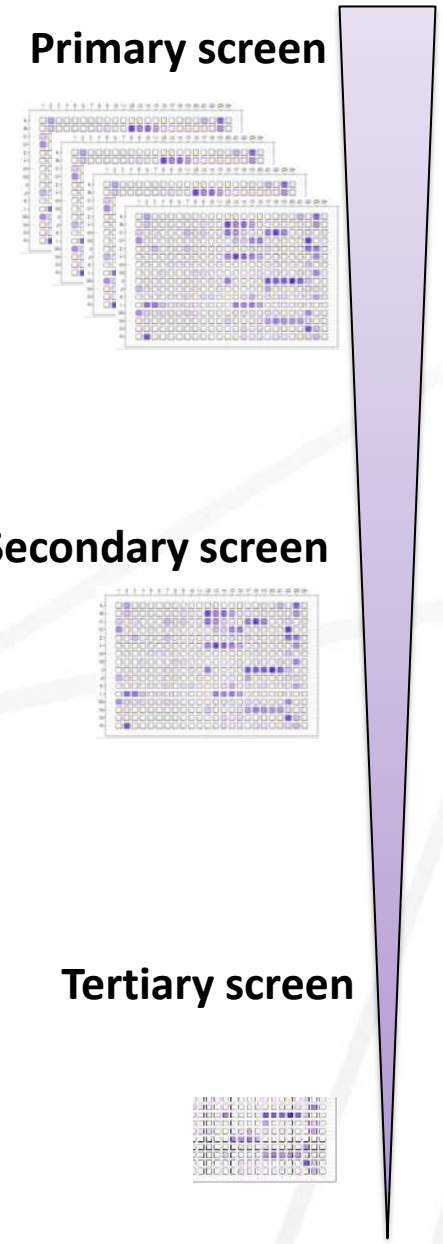
Integrate OMICS
- ✓Metabolomics (MA)
- ✓Transcriptomics (MGC)
- ✓Proteomics (MGC/MSPF)

Detailed target follow up
- ✓Lots of discussion
- ✓Your lab
- ✓Back in VCFG
- ✓Precinct partners

Discovery - high throughput screening concept

- Unbiased interrogation
- Best model to replicate your biology
- Thousands of genes (CRISPR, RNAi)
- Thousands of compounds
- Iterative data analysis to generate ‘hit’ lists

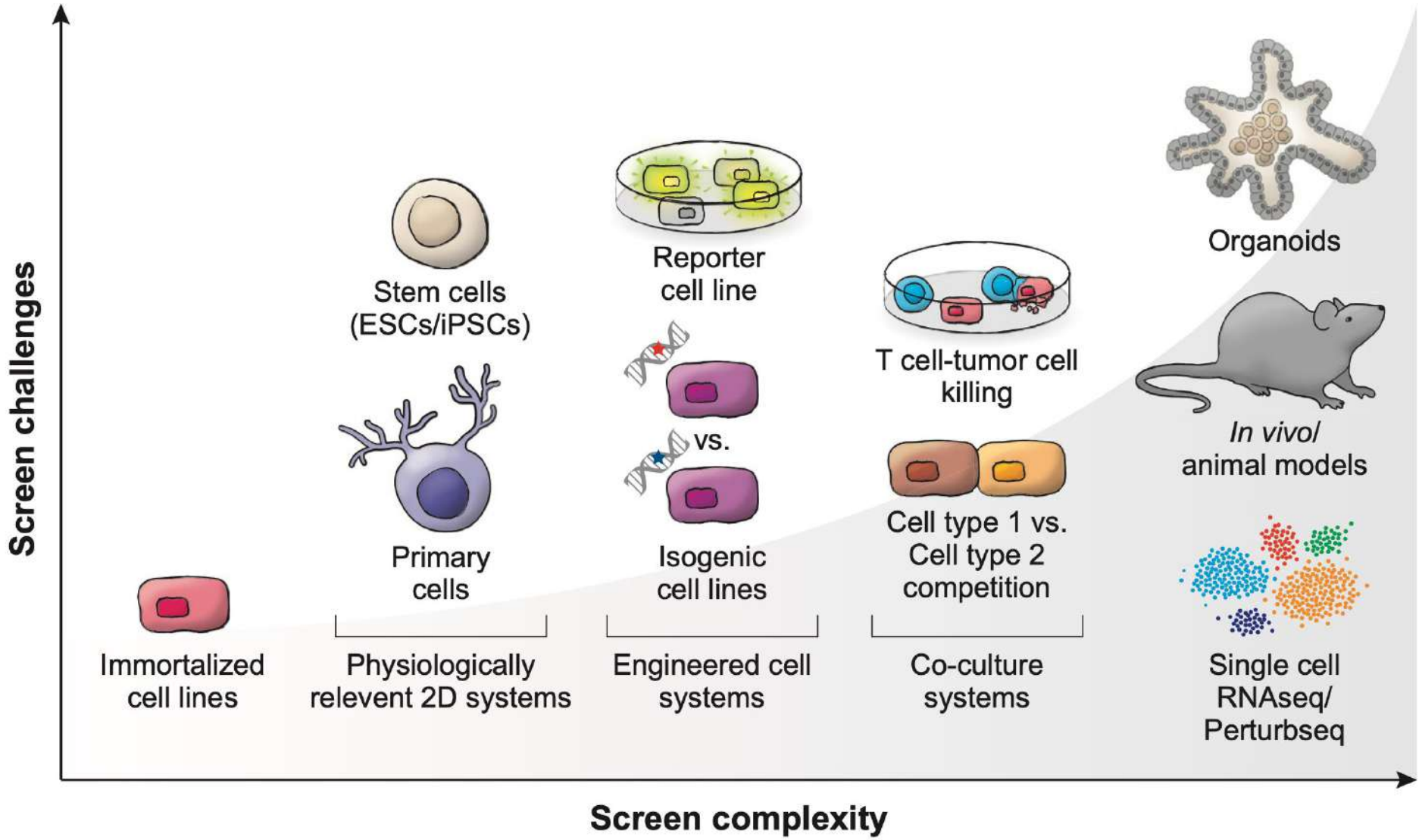


High throughput assay
Adherent or suspension
Multiple lines

Validation of primary screen
More difficult, expensive
Multiplex readouts
Lower throughput

High confidence
Multiple lines
Orthogonal assays

Orders of complexity with experimental approaches



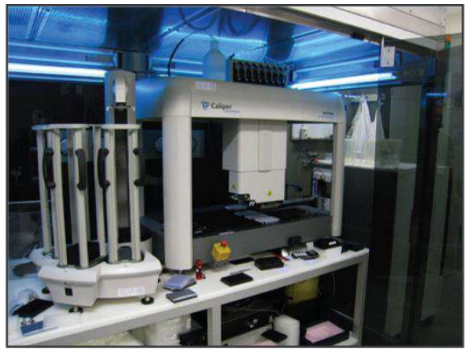
Haley and Roudnicky – Cancer Cell, 2020

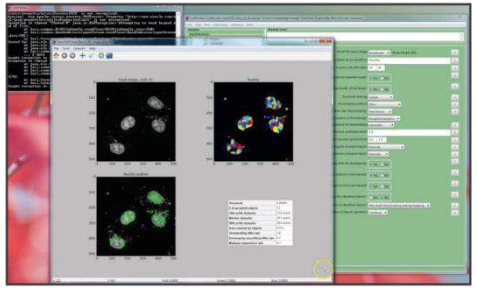
Key infrastructure

- Personal liquid handling workstation – cell dispense, media change, fix/stain
  - BioTek 406 x3, BioTek MFX
- Big robotics – large capacity, flexible movement options with precision small volumes
  - Sciclone, Janus G3
- Plate reader – basic and advanced functionality, kinetic, automated
  - Cytation 3, Cytation 5, Cytation C10
- Microscope – live, widefield, confocal, magnification, speed, throughput, automated
  - CellInsight PRO x 3; Cytation 5 and C10; Incucyte x 2
- Automated plate sealer
  - Plate loc
- Drug dispenser (BYO compounds and controls)
  - Tecan D300e
- Liconics incubator
- Access to Flow through Research Flow Core

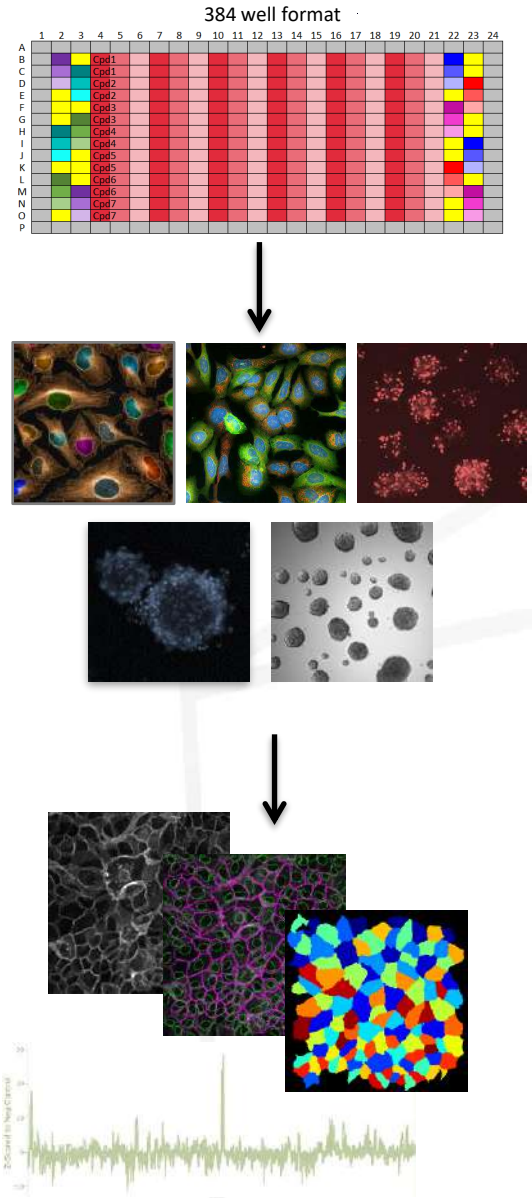
VCFG model 'researcher driven – staff assisted'

Arrayed screening workflow





3-7 days



Library delivery

Compounds (Compounds Australia)
CRISPR and RNAi whole human genome libraries

Data acquisition

Biological relevance - 2D adherent, suspension, 3D matrices

Image, plate reader, flow cytometry

CellInsight PRO’s
Cytation 5 & C10
Novocyte

Data analysis

Fundamental QC
Basic to advanced options
Machine learning innovation
Fully customised

Cell painting to quantify cell biology

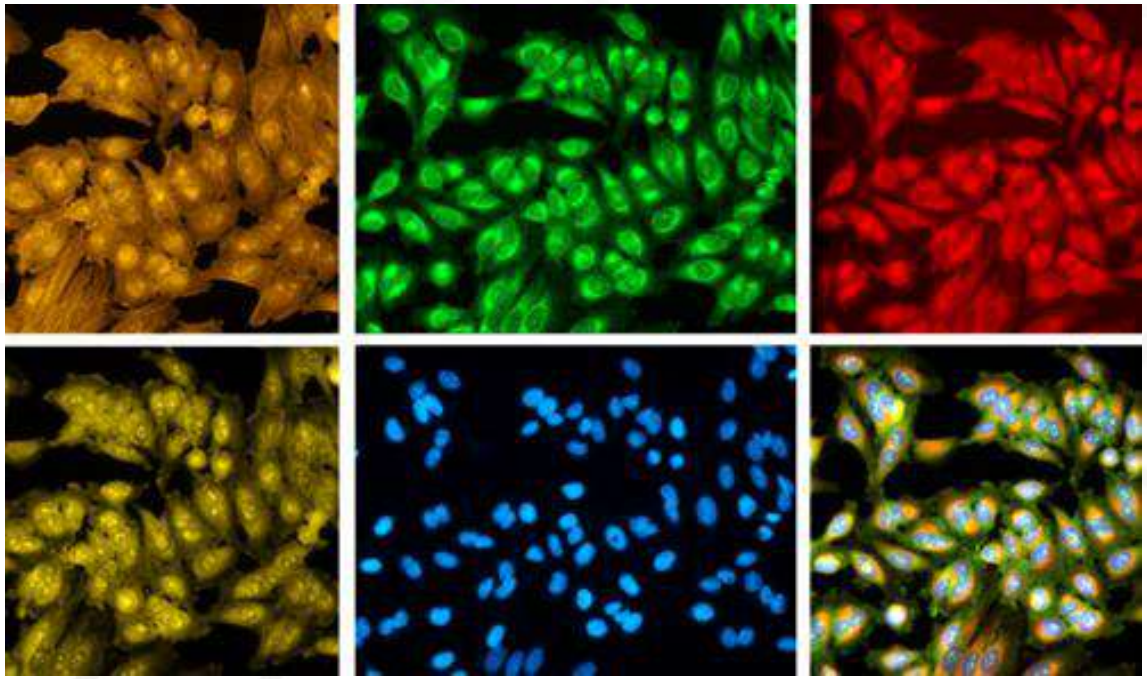
Victorian Centre For Functional Genomics

Cell Painting is a high-content, multiplexed image-based assay used for cytological profiling.

Up to six fluorescent dyes are used to label different components of the cell including the nucleus, endoplasmic reticulum, mitochondria, cytoskeleton, Golgi apparatus, and RNA.

Imaging and analysis extracts thousands of features to profile cells and elucidate behaviour.

Reference compounds/controls required



@Broad Institute

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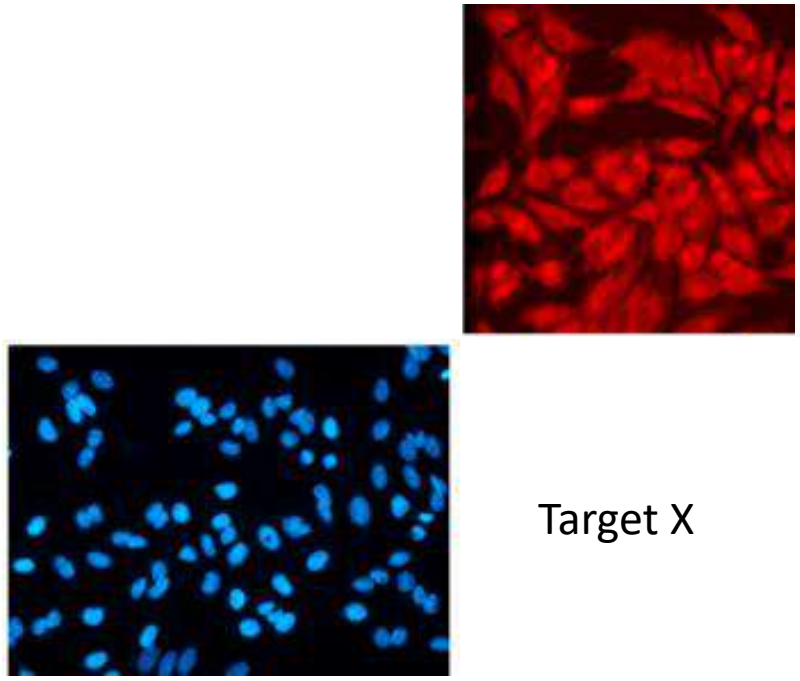
Depending on scale – cell painting may be a secondary screen

Still powerful to do less and include biological targets of interest

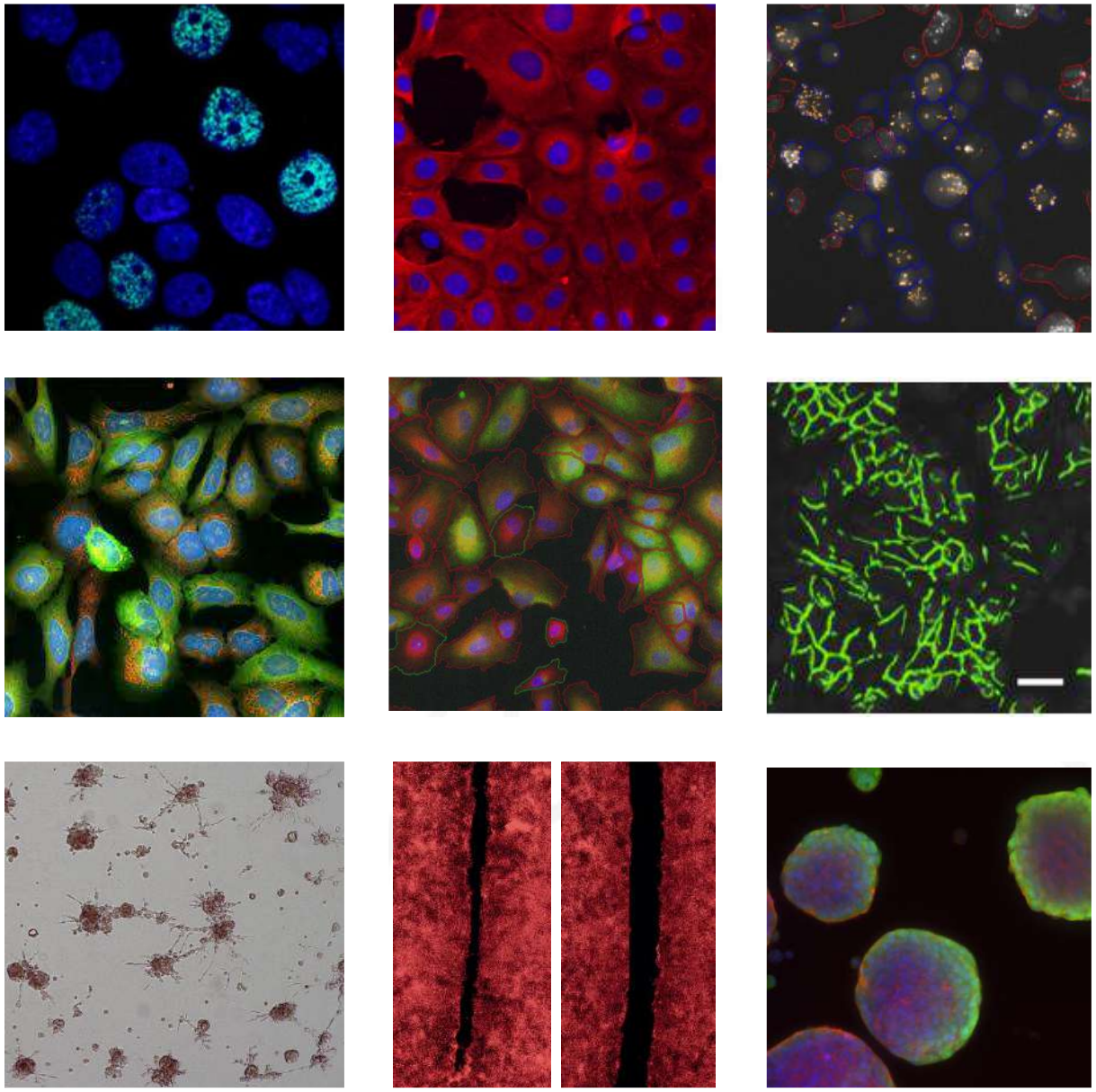


Target X

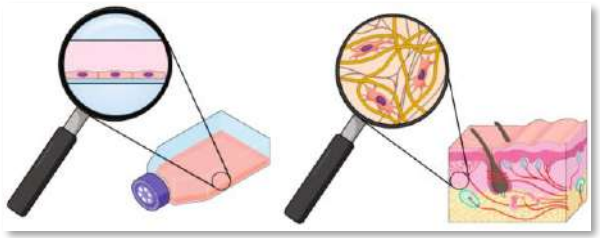
@Broad Institute

HCS screens – great diversity possible

drug resistance/sensitivity
cell death
cell size (senescence)
cell cycle
cell morphology
cell motility and metastases
host-pathogen interactions
3D morphogenesis
complex co-cultures
multi-parametric readouts
complex single cell phenotyping
protein modification
protein location
reporter assays, transcriptional activation
internalization/secretion



Working in a more translatable model system

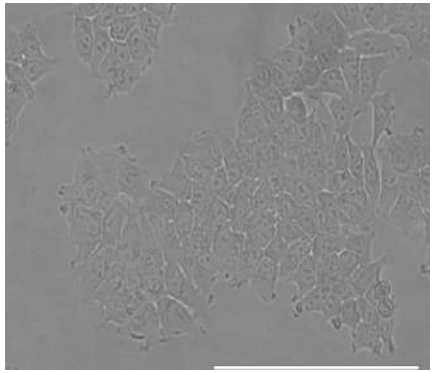


2D cells grown on flat plastic – not very physiological

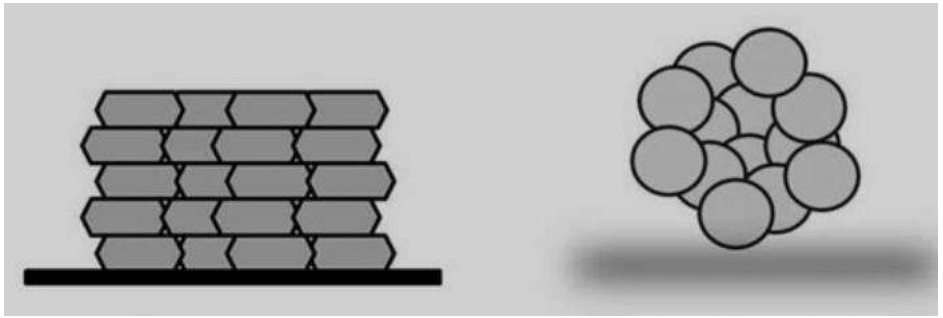
- drug discovery in 2D can fail at clinical trials

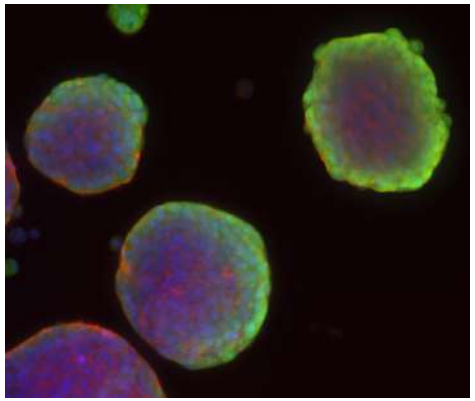
3D cells cultured in a matrix full of growth factors and supporting scaffolds

- more closely replicates the microenvironment in the body
- allows cell growth in all planes
- allows us to co-culture different cell types that more closely model a disease
- ideal setting for cells derived directly from a patient
- or cells cultured from a patient in a mouse



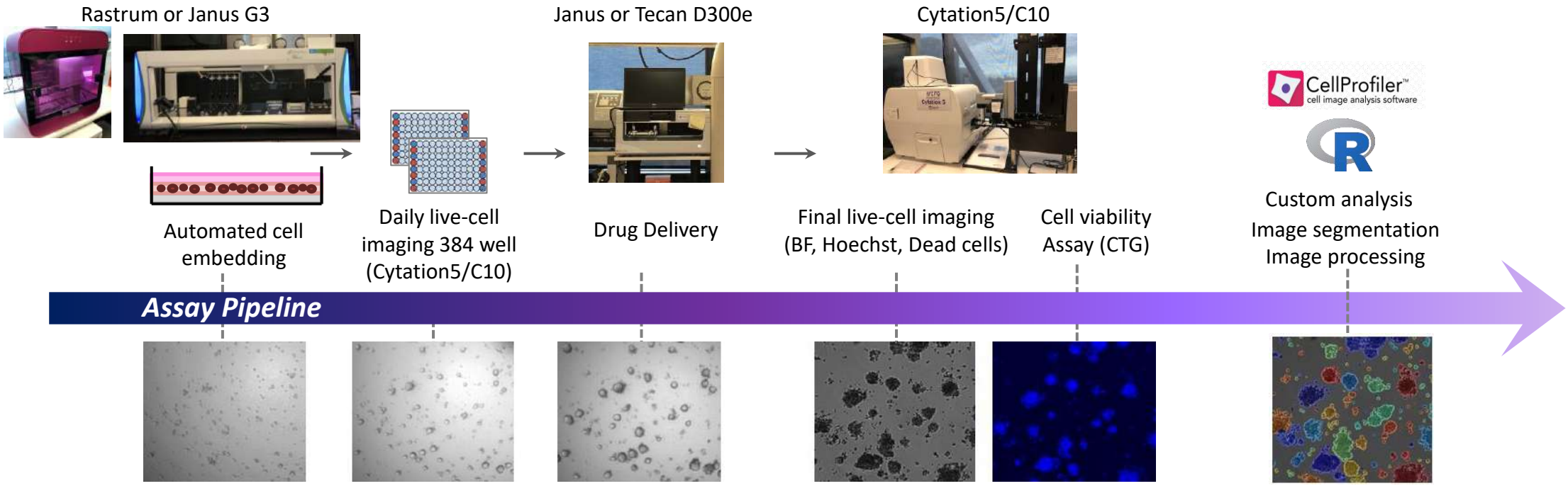
MCF7 cells grown in 2D





MCF7 cells grown in 3D

Automated 3D pipeline



N Choo, ***S Ramm, J Luu****, …, R Taylor, GP Risbridger, MG Lawrence,* ***KJ Simpson****.* ***SLAS Discovery*** *(2021)*

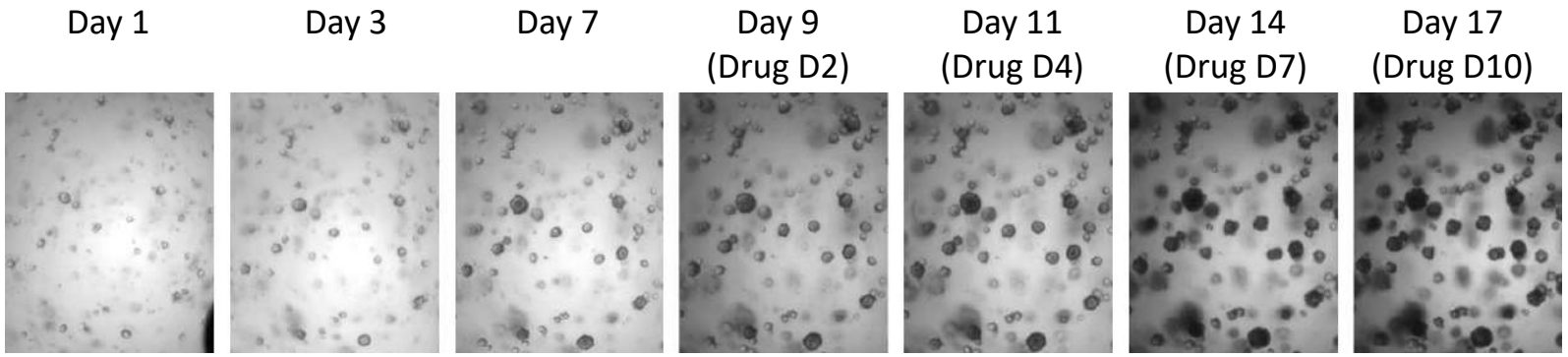
Automated 3D pipeline

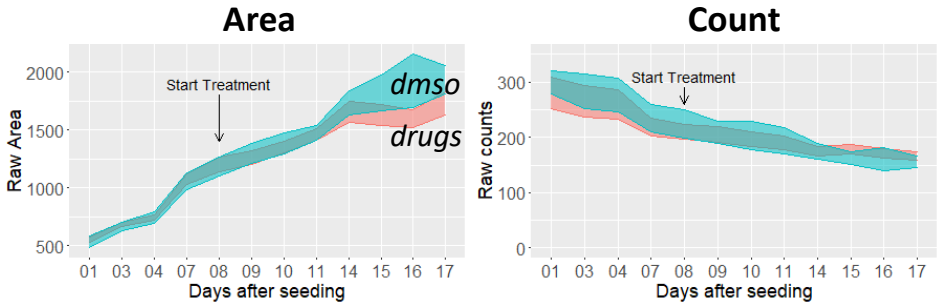
- Growth kinetics
- Drug screens
- Immune cell killing/ infiltration
- Single organoid population data
- Phenotypic clustering

Basic characterisation
- organoid area
- organoid number

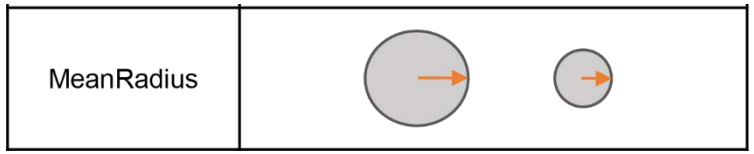
PDX/PDO/cell lines
Pancreatic
Colorectal
Prostate
Ovarian
Oesophageal
Genito-urinary
Cancer unknown primary
+ Cell lines (breast, prostate)

Prostate PDX – growth and death time course

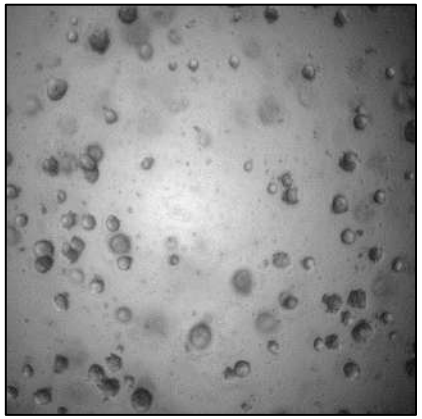


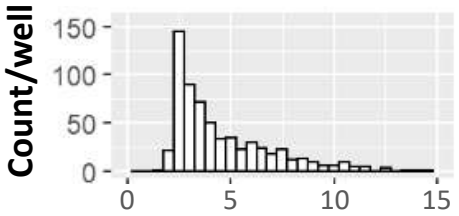


Quantify structure dynamics, organoid size in a whole population

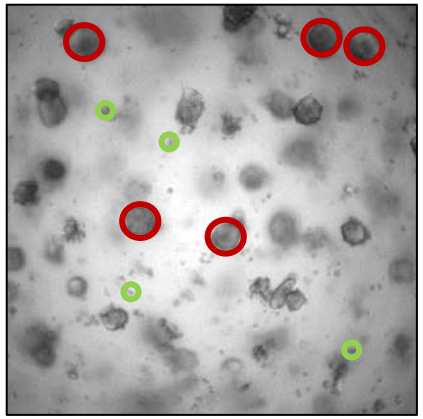


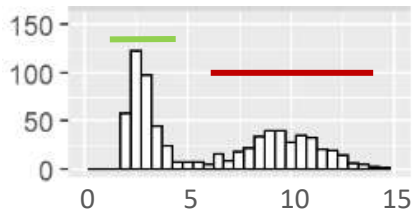
Prostate PDX 1



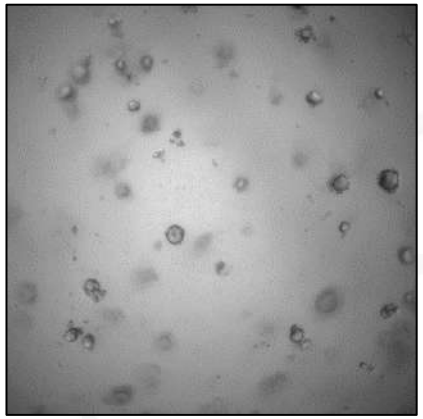


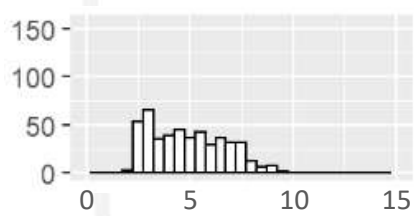
Prostate PDX 2





Prostate PDX 3





Cancer of Unknown Primary

Day 5 (drug Day 0) | Day 10 (drug Day 5)

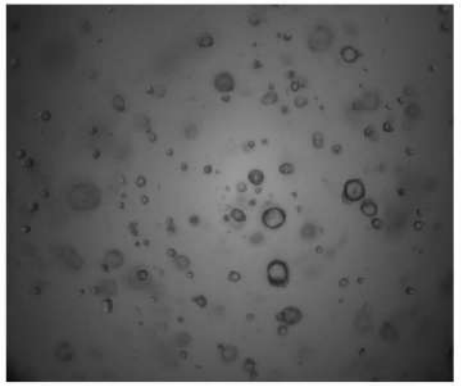


Plate2 Growth-D05_Drug-D00

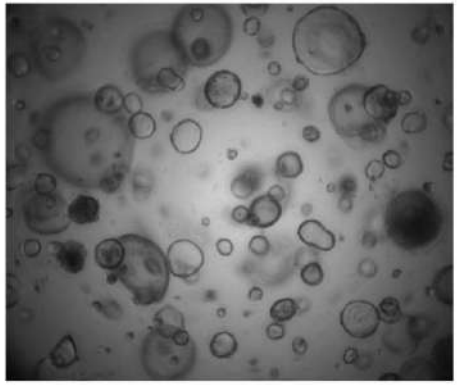


Plate2 Growth-D10_Drug-D05

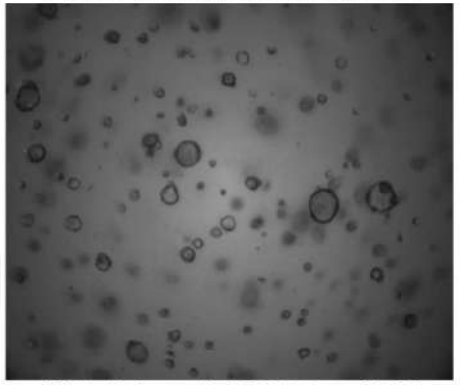


Plate1 Growth-D05_Drug-D00

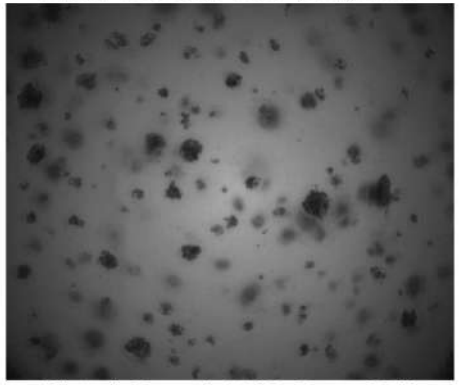


Plate1 Growth-D10_Drug-D05

*N Choo et al., **SLAS Discovery** (2021)*

Tothill, UoM

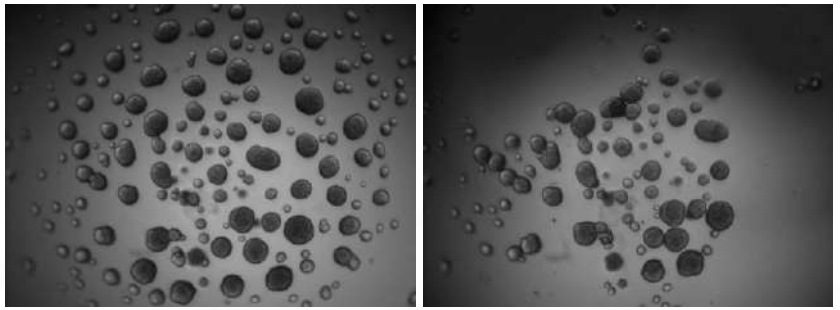
Fixing and staining in Matrigel in situ - a grand challenge

Victorian Centre For Functional Genomics

<u>Problem 1 - fixing</u>

- PFA deploymerises Matrigel
- Fixing changes structural integrity (shrink, swell)
- Shift in position means no relational imaging quantitation
- Total structure loss/disintegration

MCF7 2% embedding



unfixed fixed

<u>Solution:</u>

Tried numerous agents, settled on 0.3% glutaraldehyde, 10 mins, RT

Peter Mac
Peter MacCallum Cancer Centre
Victoria Australia

Fixing and staining in Matrigel in situ - a grand challenge

Victorian Centre For Functional Genomics

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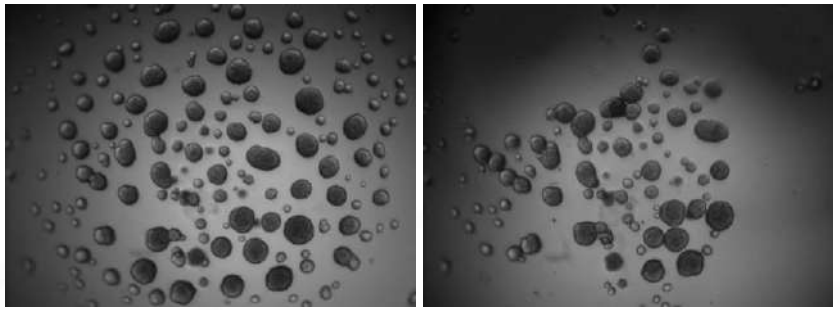

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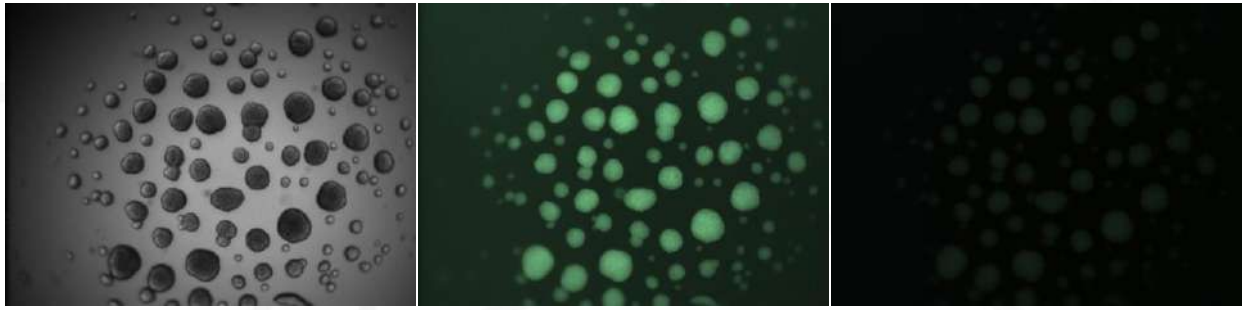
Problem 2- background

Fixing in matrigel leaves high autofluorescence



fixed not quenched quenched

Solution:

Quenching – identify agent: Sodium Borohydride

- creates hydrogen gas
- modify concentration
- length of incubation
- temperature of incubation

Time post fixation – must be less than 1 week

Ramm et al., Organoids, 2023

Peter Mac
Peter MacCallum Cancer Centre
Victoria Australia

High multiplex parameter staining – discriminates drug response

MCF7 cells – culture endpoint
20X magnification
Single structure, single cell

Suite of markers available

PI (dead)

Image IT Dead

Phalloidin (actin)

Ki67 – (proliferation)

Caspase 3/7

Concanavalin A (membrane)

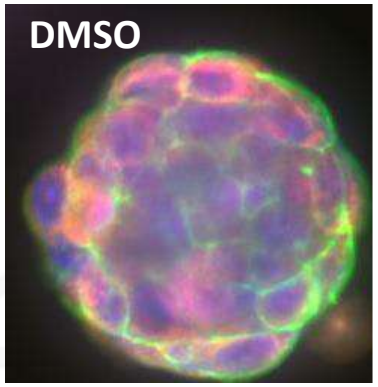
SYTO14 – (nucleic acid stain)

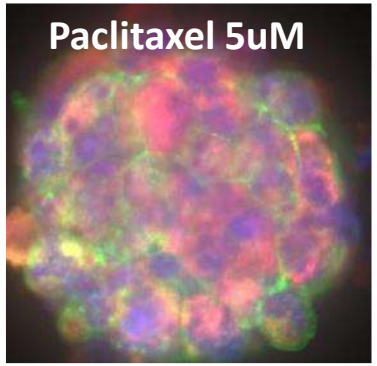
WGA – (membrane, Golgi)

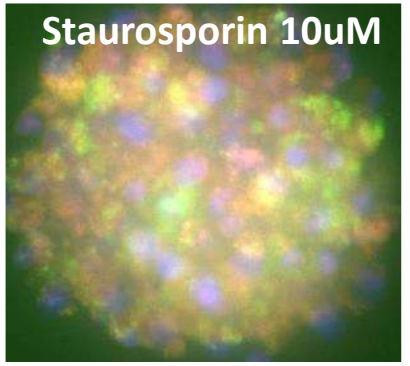
Histone H3 – (chromatin)

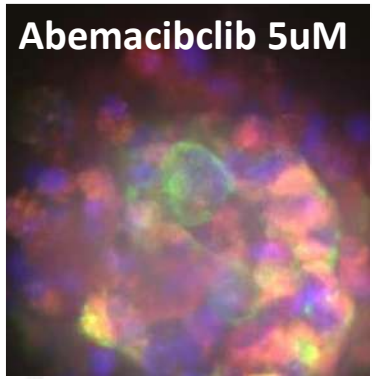
Mitotracker (mitochondria)

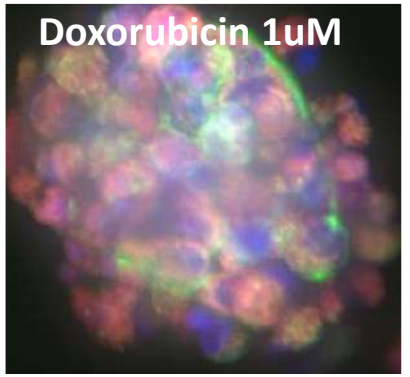
Cell painting











DAPI (Nuclei)

Phalloidin (Actin)

pH3 (mitosis)

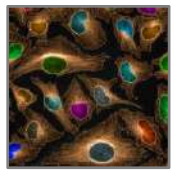
Mitochondria

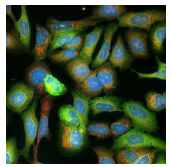
Ramm et al., Organoids, 2023

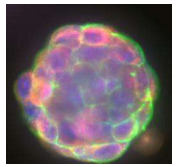
Data analytics – Cellular phenotyping and hit identification

Images → Viability/toxicity/cell cycle

→ Cell features → Clustering Phenotypic signatures → Biological information



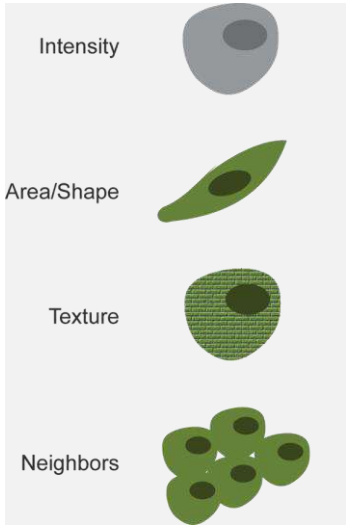




CellProfiler™ cell image analysis software

Cell Painting

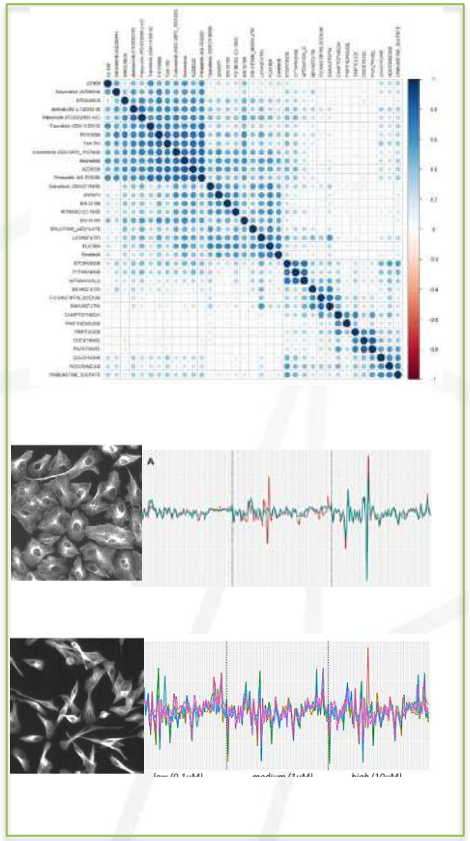
2D



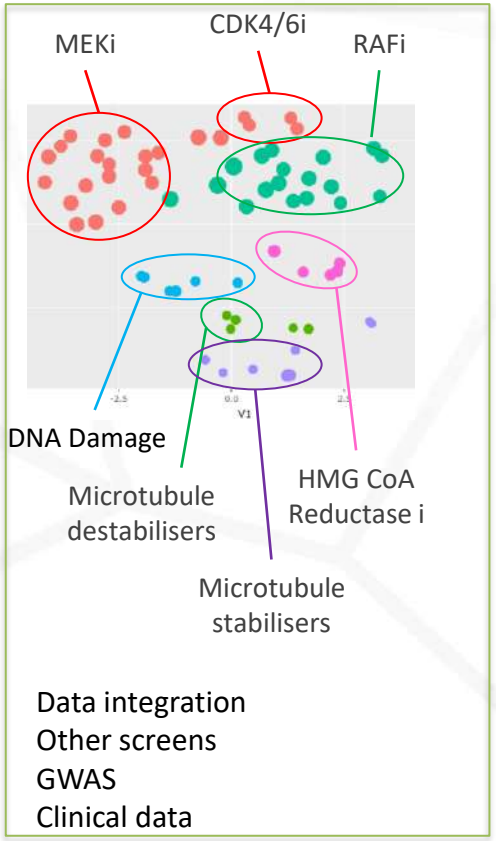
3D

Imaging feature	Schematic representation
Area	
MeanRadius	
Eccentricity	
FormFactor	
MeanIntensity	
Texture	

Extraction of cell features (~1,000 per image)



Feature reduction

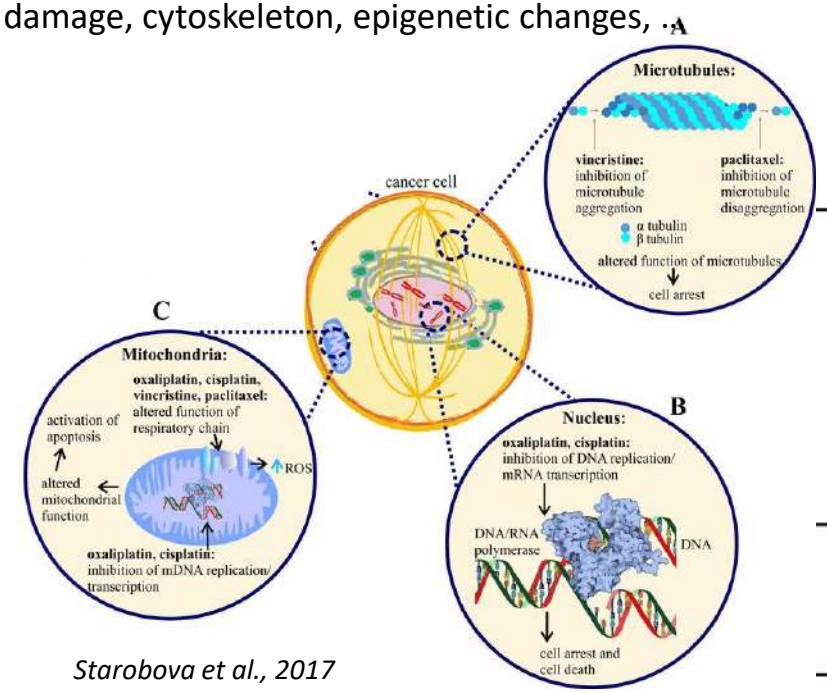


Data integration
Other screens
GWAS
Clinical data

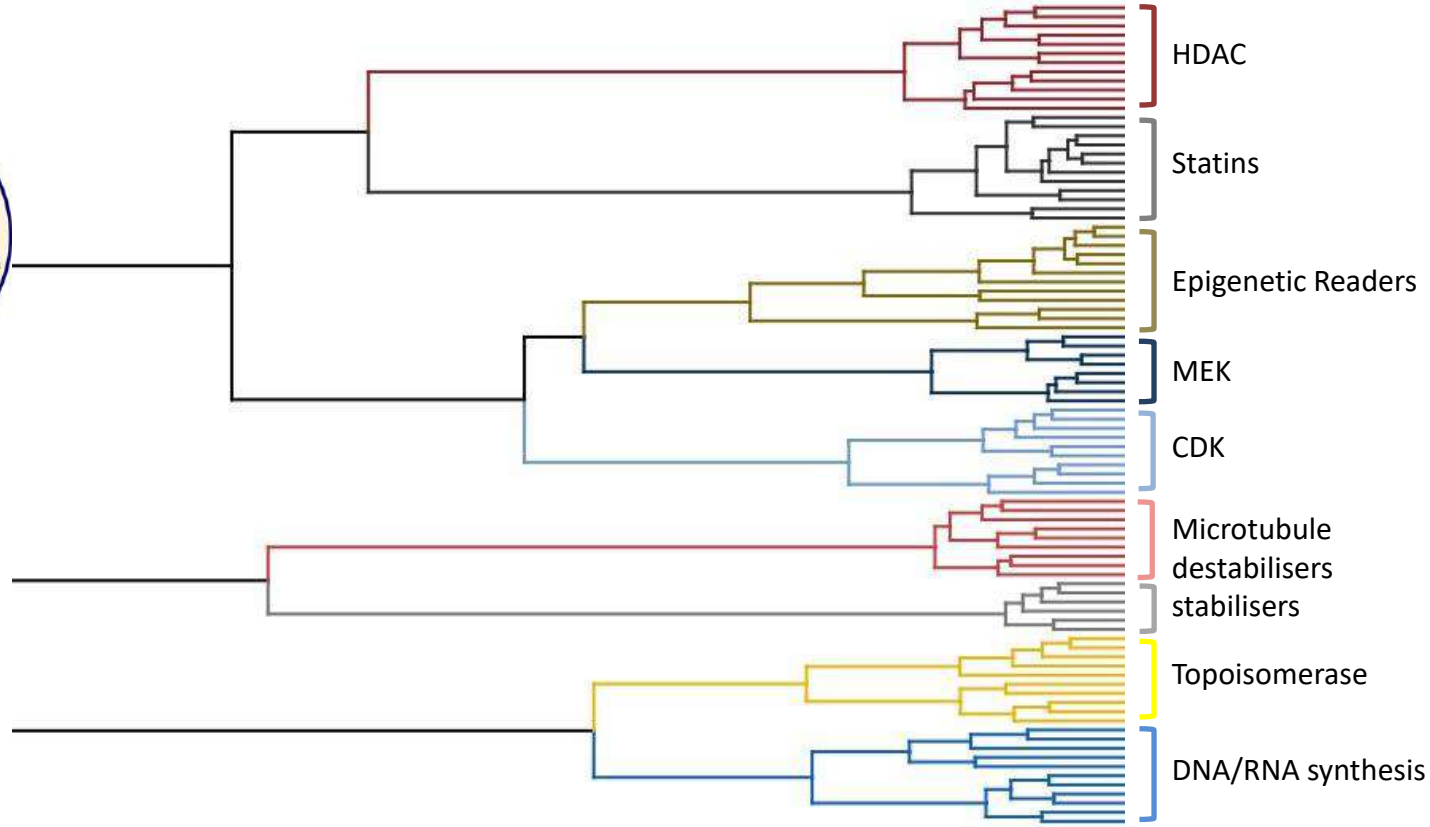
Data integration

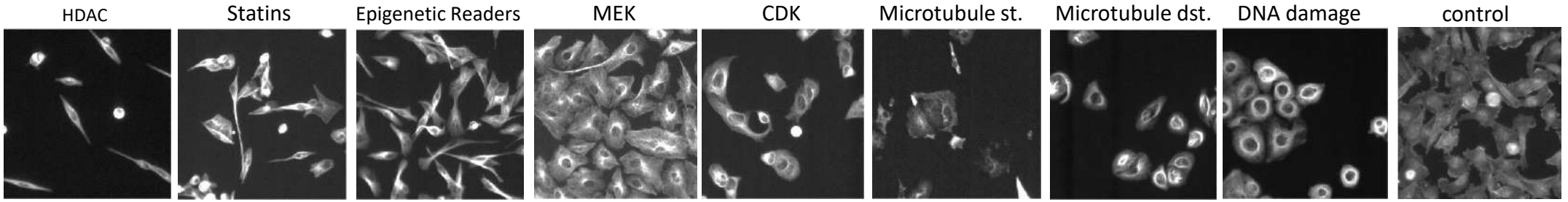
Phenotypic clustering

Mechanism of action cancer reference compound library:
69 compounds – multiple doses
DNA damage, cytoskeleton, epigenetic changes, ..



Starobova et al., 2017



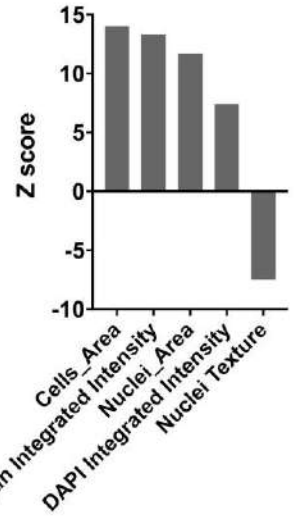


MDA-MB-231

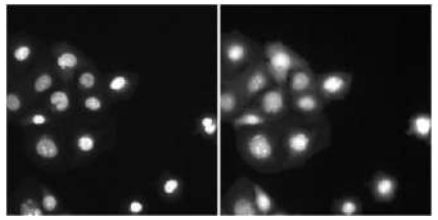
Top features identifying each cluster – towards mechanism of action

Victorian Centre For Functional Genomics

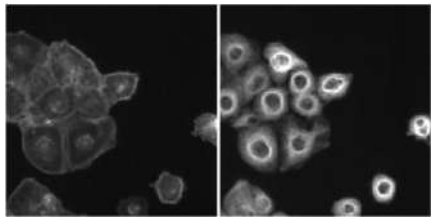
DNA Damage inducers



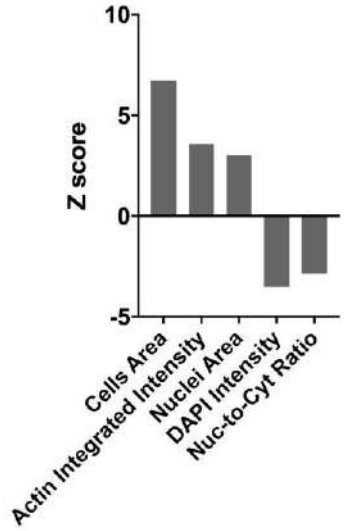
DAPI CellMask



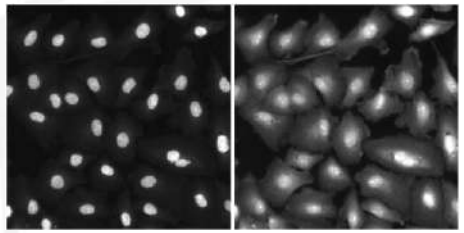
Actin Tubulin



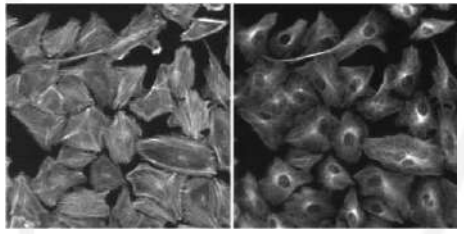
MEK inhibitors



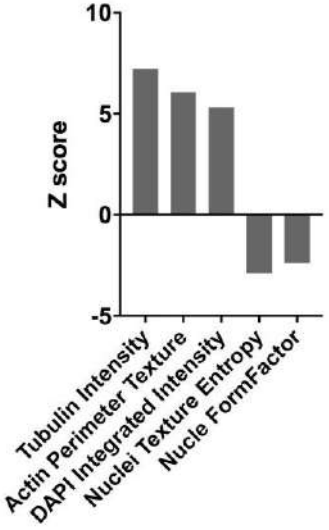
DAPI CellMask



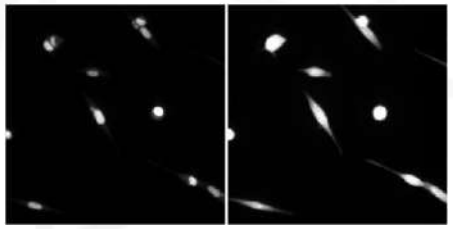
Actin Tubulin



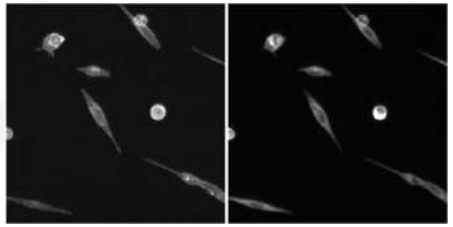
HDAC inhibitors



DAPI CellMask

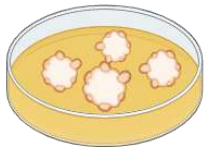


Actin Tubulin

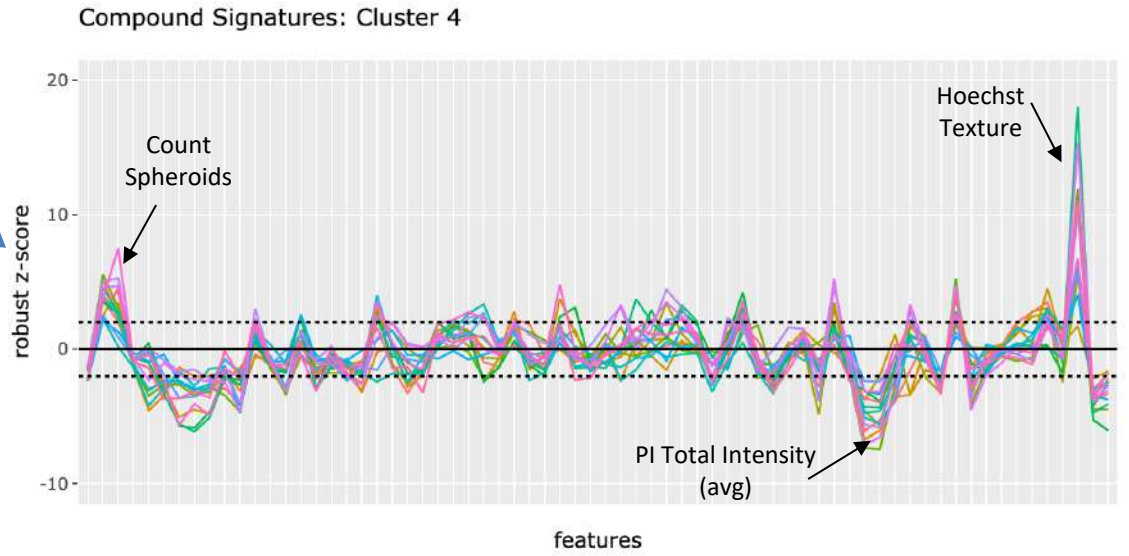


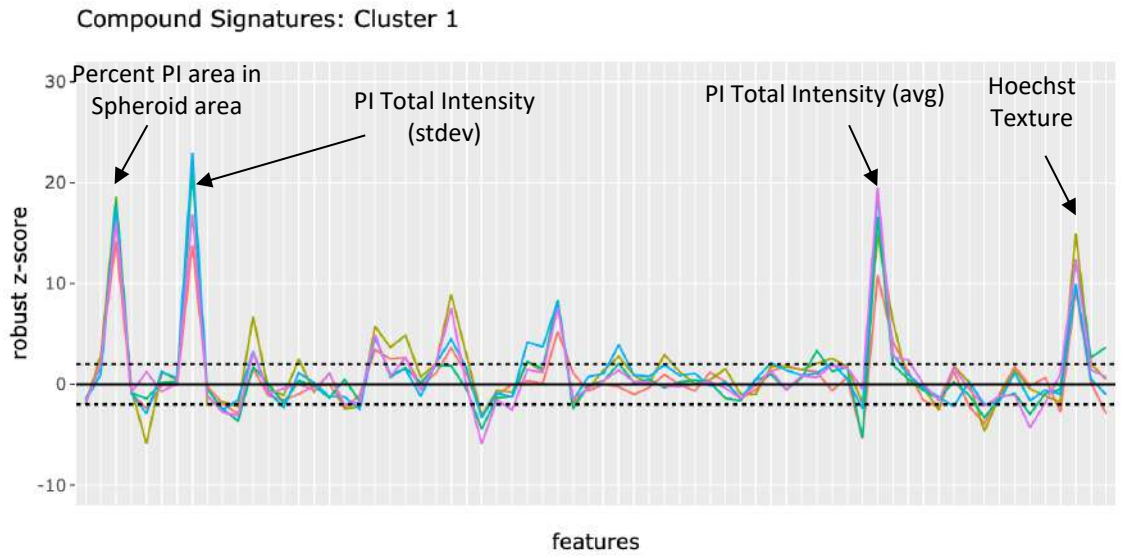
Peter Mac
Peter MacCallum Cancer Centre
Victoria Australia

3D compound screen - clusters similar phenotypes



MCF7

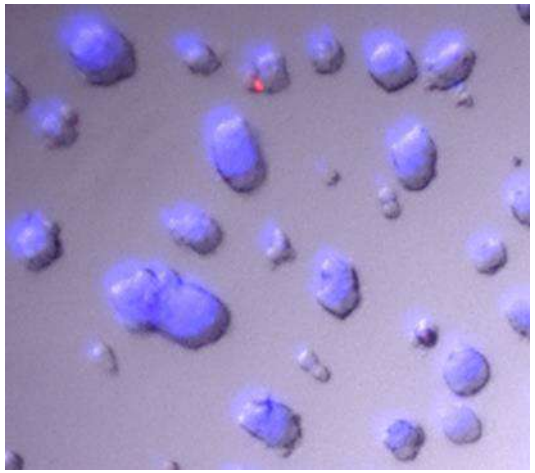


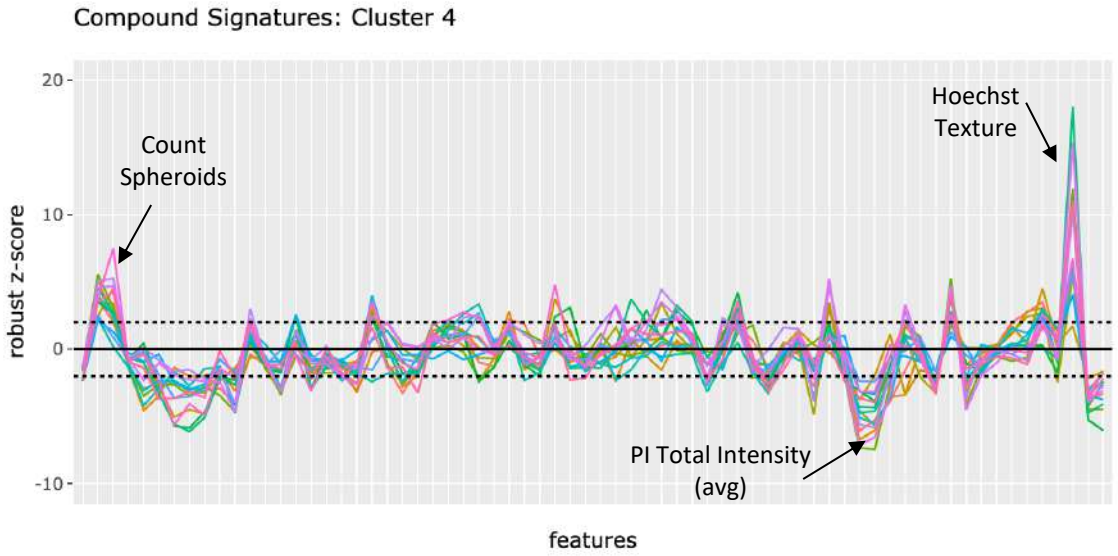


VCFG

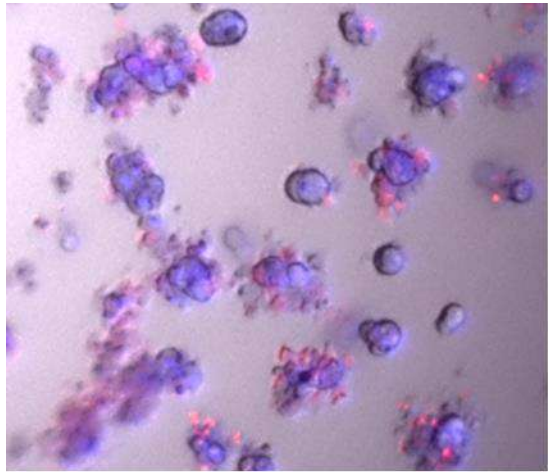
3D compound screen - clusters similar phenotypes

Compound X1 (C4)

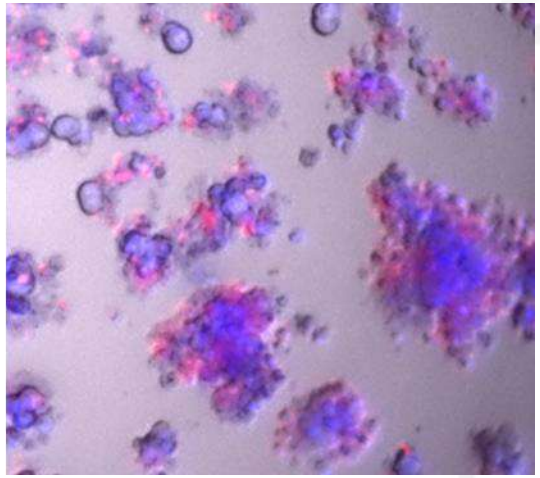


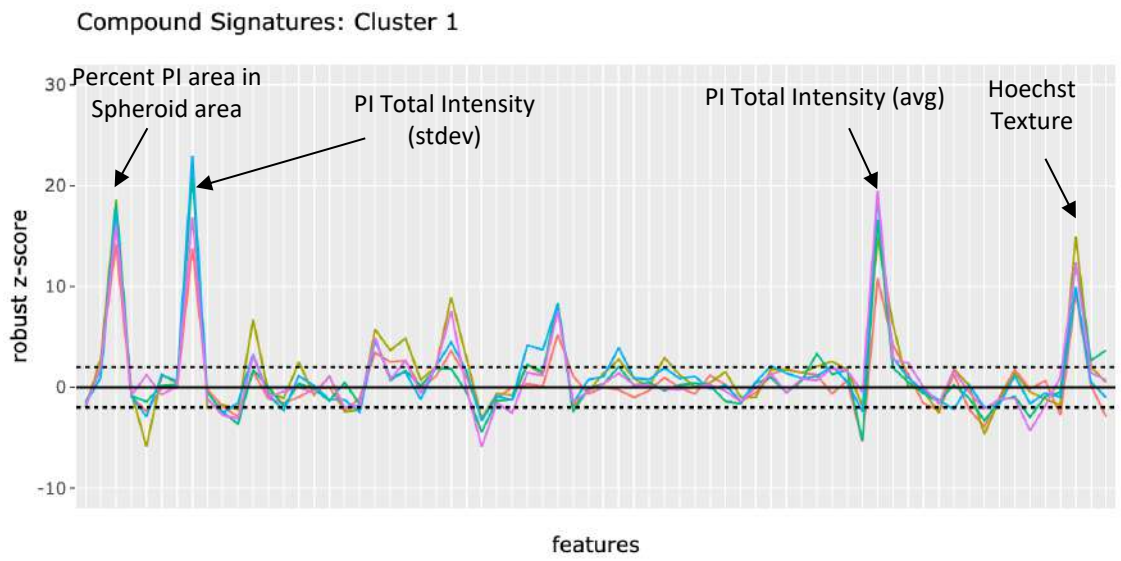


Compound X2 (C1)



Compound X3 (C1)



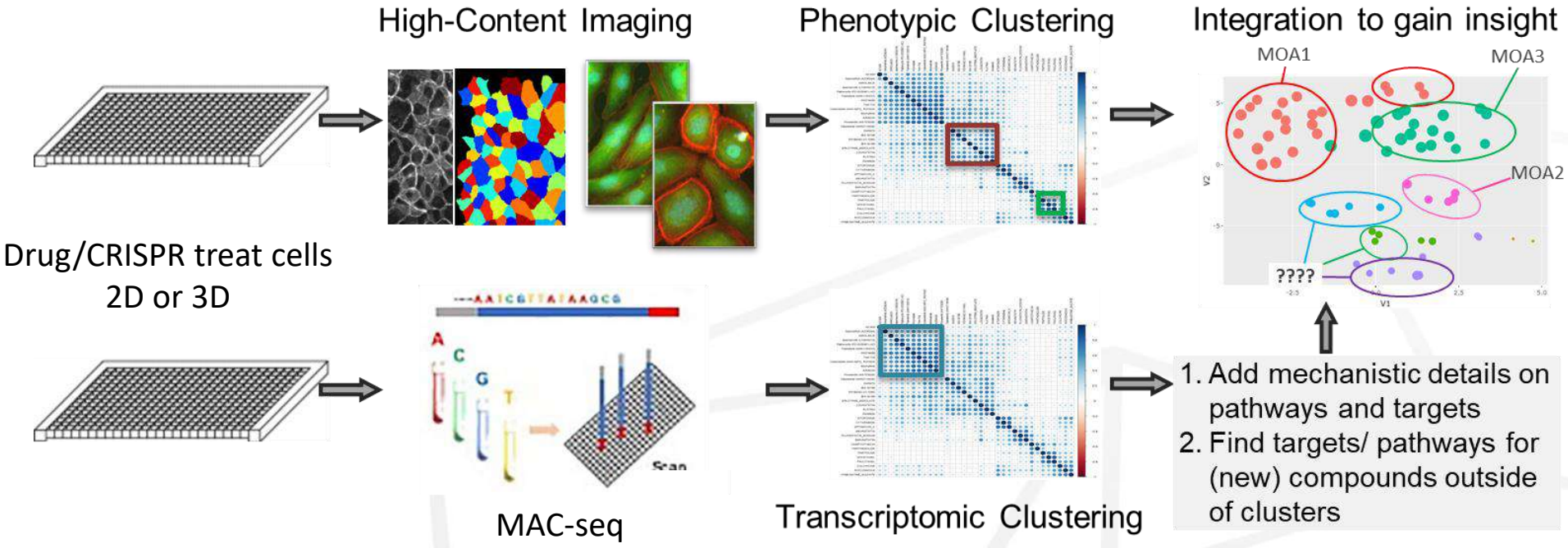


Hoechst, PI

VCFG

High-throughput transcriptomics (MAC-seq – multiplexed analysis of cells)

384 well format
In situ RNA extraction with barcoded wells
Bulk RNA seq



Multi-OMICS
Bulk or single cell proteomics
Metabolomics
Cytokine profiling
Cell lineage tracing
Other applications

Li et al., Methods in Molecular Biology, 2023

Screen vignettes

1 Drug screen for low grade ovarian carcinoma using high content imaging

- Dr Kathleen Pishas, Dr Dane Cheasley

2 Drug screen for immunomodulatory targets using flow cytometry

- Emily Derrick, A/Prof Paul Beavis, Eva Orlowski-Oliver

3 Organoids in personalised medicine – colorectal patients

- Dr Anshini Jain, Prof Rob Ramsay, A/Prof Alexander Heriot, A/Prof Nick Clemons

4 Immune cell 3D complex co-culture screening – profiling CAR-T cells

- Dr Milton Mui, A/Prof Nick Clemons

We are sharing unpublished work

"Enabling discovery and driving translational medicine with high throughput technologies"

1- High-throughput strategies to discover synergistic drug combinations for low-grade serous ovarian carcinomas.

Victorian Centre For Functional Genomics

Scaled discovery

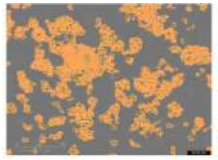
Single agent screening
6760 compounds
- FDA library
- Kinase inhibitors
- Epigenetics

Focus set of targets

Combinatorial screening
2D and 3D

Cell lines
12 LGSOC
1 normal line

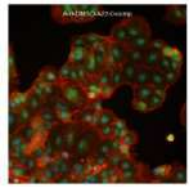
Characterisation
Cell banking; DNA pellets, IHC
Growth kinetics
Pilot screen



- Day 1: Plate cells
- Day 2: Add compounds
- Day 5: Fix/Stain Cells
- Day 5+: Image Cells
- Analyse Data
- Hit Selection

Screening
72 hours treatment
0.1; 1; 10uM

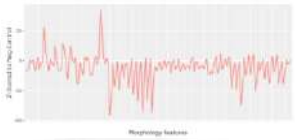
Staining
DAPI nuclei
Cell mask – membrane
Phallodin – actin cytoskeleton



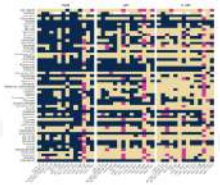
Imaging
10x and 20X

Custom analysis pipelines

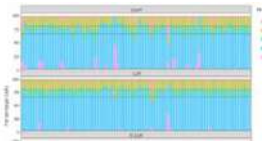
Cell Morphology



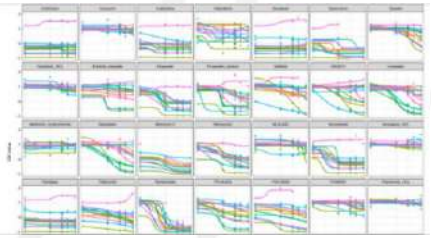
Viability



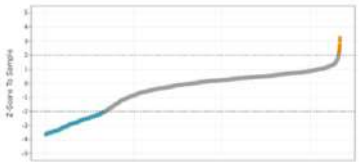
Cell Cycle



Dose response curves



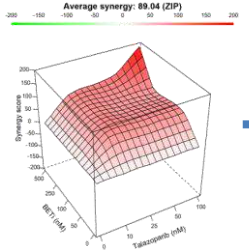
Z-score



Data integration
Molecular data
Clinical data

Hit ID
79 targets

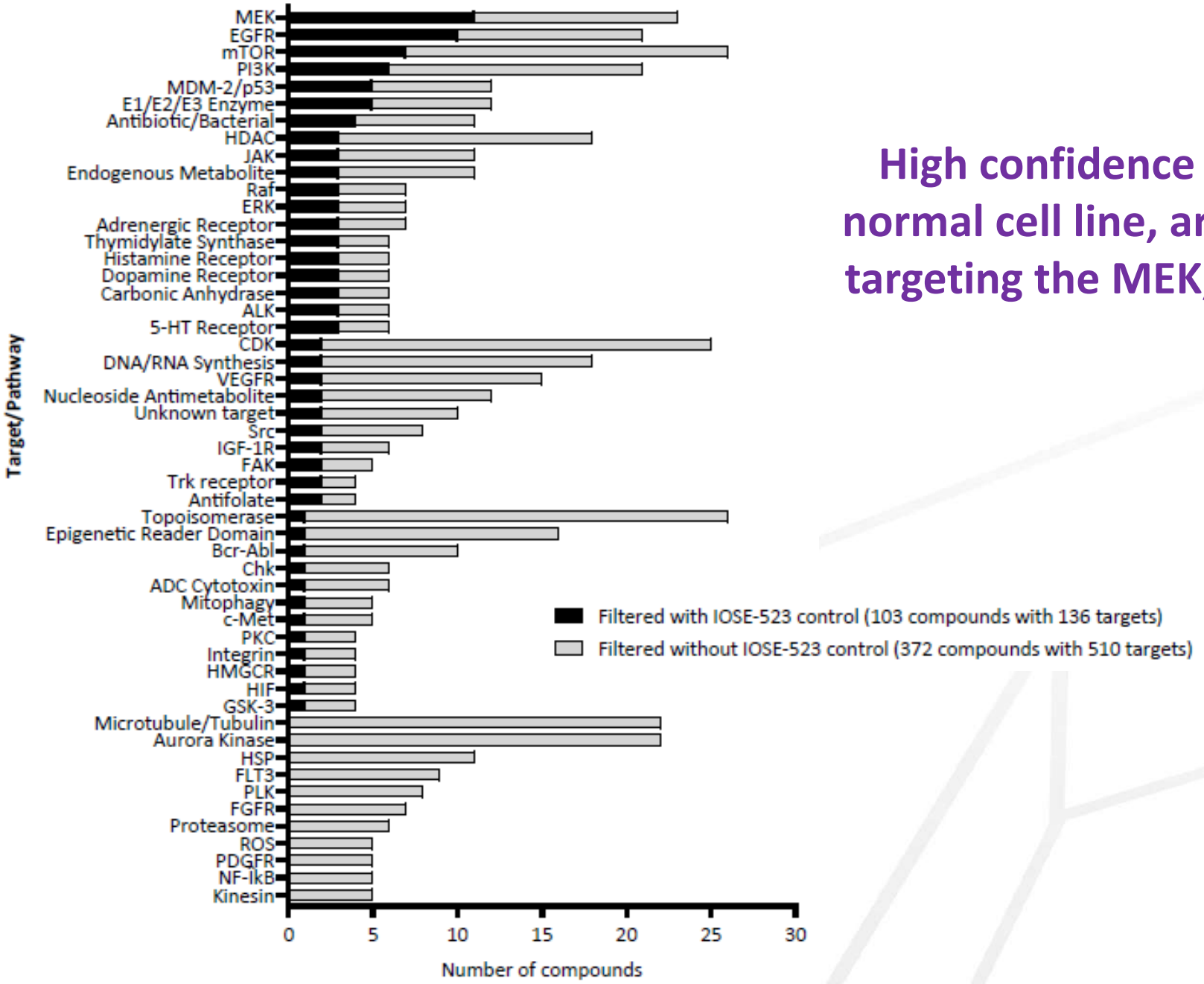
Synergy with Standard of care



MAC-seq

Mark Carey (Vancouver General Hospital)
Kathleen Pishas, Dane Cheasley (Peter Mac)
Jennii Luu, Robert Vary, Karla Cowley, Mark Li (VCFG)

Peter Mac
Peter MacCallum Cancer Centre
Victoria Australia

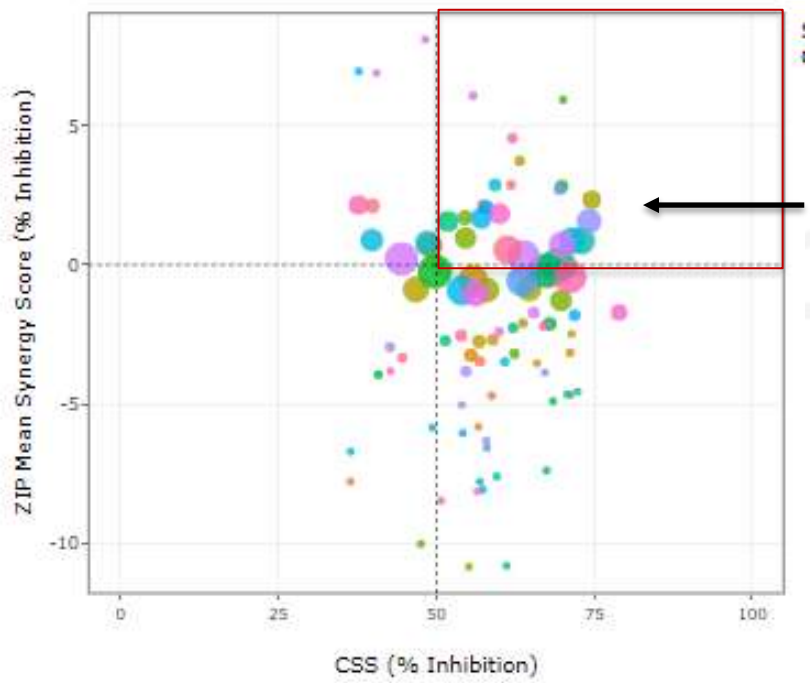


High confidence hits with no effect in the normal cell line, are enriched for compounds targeting the MEK, EGFR and mTOR pathway

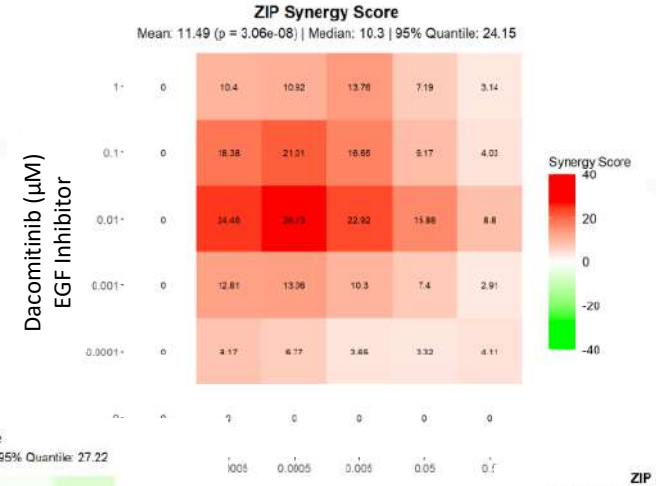
EGFR and MEK inhibitors synergise

Pair-wise synergy interaction study
- 6x6 dose grid
- 6 anchors (standard of care + experimental) in combo with 79 hits
- screening 4 cell lines

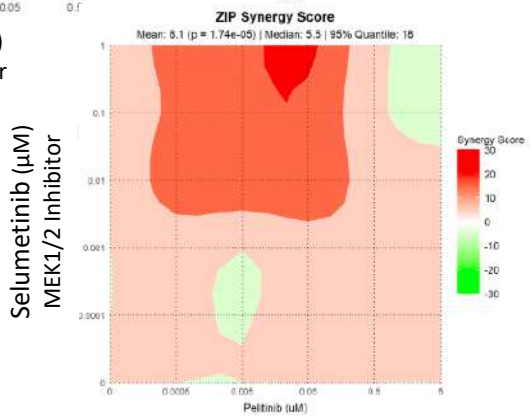
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A																								
B																								
C		DMSO						5						5						5	DMSO	Dox 10uM	Dox 0.1uM	
D		Media			Cobimetinib + Drug 1			4			Mirdametinib + Drug 1			4			Pelitinib + Drug 1			4	DMSO	Staur 10uM	Stauro 0.1uM	
E		DMSO						3						3						3	DMSO	Mito 10uM	Mito 0.1uM	
F		Dox 10uM						2						2						2	Media	Cobimetinib_10uM	Cobimetinib_10uM	
G		Dox 0.1uM						1						1						1	DMSO	Mirdametinib_10uM	Mirdametinib_10uM	
H		DMSO	5	4	3	2	1	0	5	4	3	2	1	0	5	4	3	2	1	0	Media	Pelitinib_10uM	Pelitinib_10uM	
I		Staur 10uM						5						5						5	Media	Trametinib_10uM	Trametinib_10uM	
J		Stauro 0.1uM						4						4						4	DMSO	Paclitaxel_10uM	Paclitaxel_10uM	
K		Mito 10uM			Trametinib + Drug 1			3			Paclitaxel + Drug 1			3			Carboplatin + Drug 1			3	Media	Carboplatin_10uM	Carboplatin_10uM	
L		Mito 0.1uM						2						2						2	Media	DMSO	DMSO	
M		DMSO						1						1						1	DMSO	DMSO	DMSO	
N		DMSO	5	4	3	2	1	0	5	4	3	2	1	0	5	4	3	2	1	0	Media	Drug 1_10uM	Drug 1_10uM	
O		Media	Media	Dox 0.01uM	Stauro 0.01uM	Mito 0.01uM	DMSO	Media	Media	Media	Media	Media	Media	Dox 0.01uM	Stauro 0.01uM	Mito 0.01uM	Media	Media	DMSO	Media	Media	Media	Media	
P																								



Pelitinib (EGFR small molecule) synergistic hits



Trametinib (μM)
MEK1/2 Inhibitor

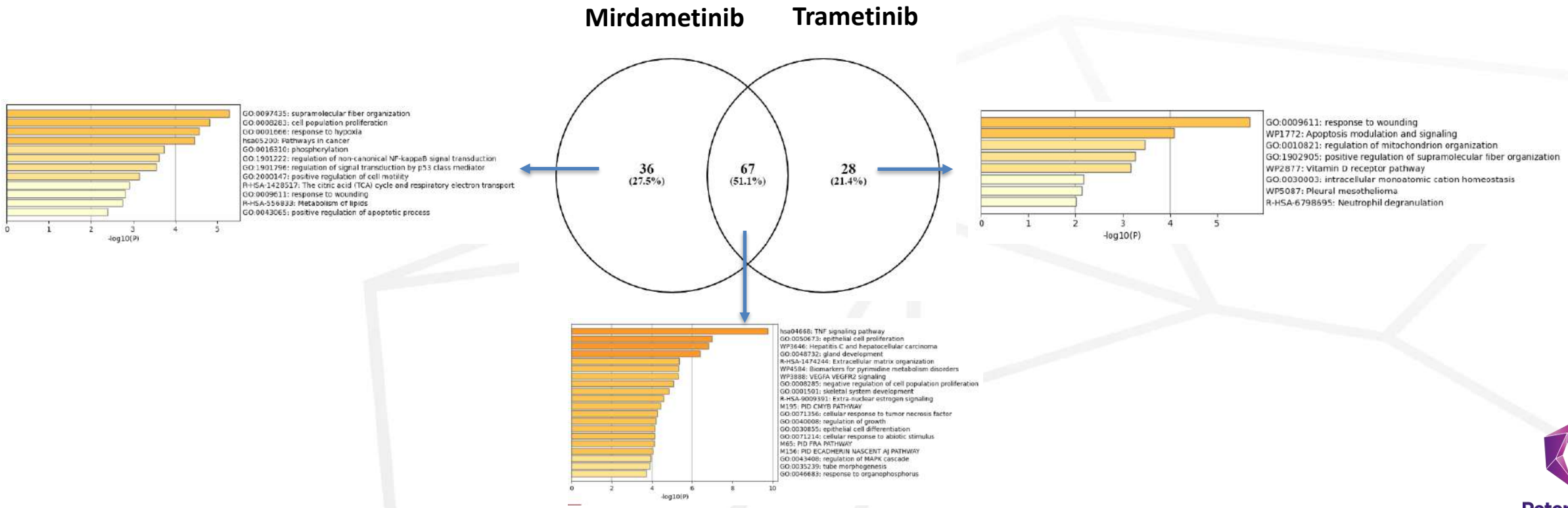


EGFR Inhibitor

MAC-seq reveals transcriptional programs driving drug sensitivity

Comparing transcriptional profiles driving drug sensitivity between a series of i) EGFR and ii) MEK inhibitors

VOA-4698 cells, 1µM, 24hrs



Dr Kathleen Pishas – record holder! Largest screen in VCFG

Pishas et al., Scientific DATA 2024

13 cell lines screened with >3500 compounds
685 x 384 well plates = 263040 wells of data

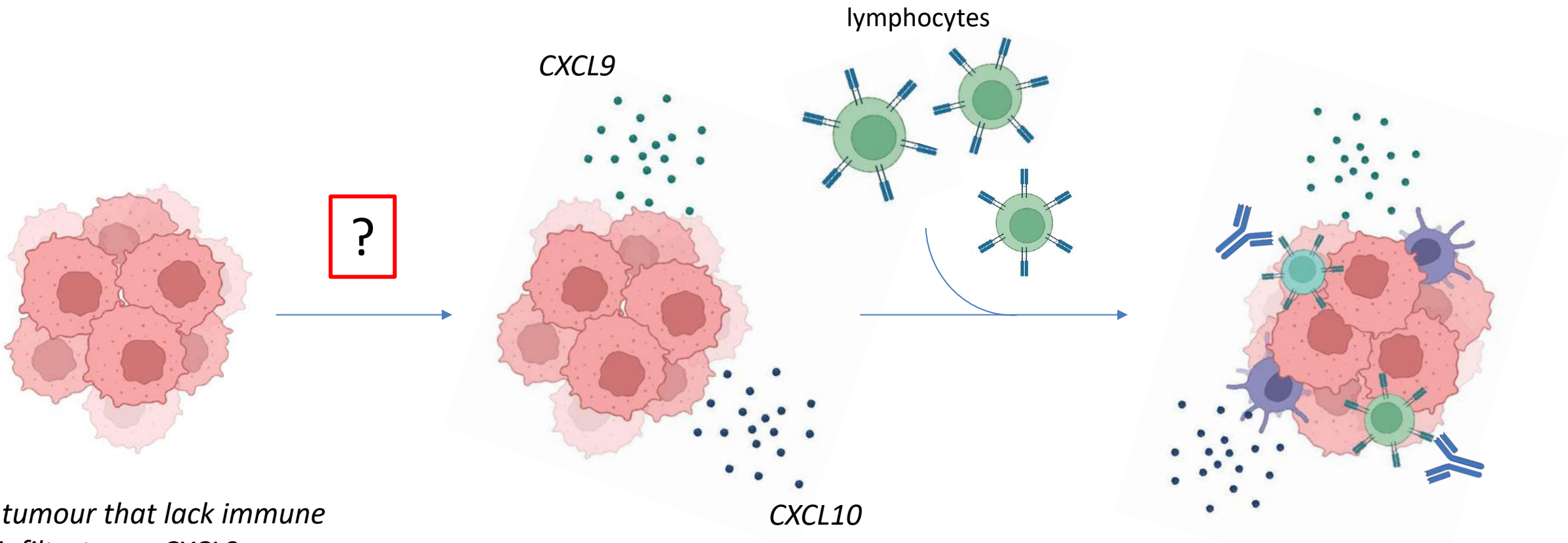
~2.5 hours imaging per plate
1712.5 hours = 72 days!
Feb 2021 start and still going

PMC codes

Pilot	**Primary**		**Validation/3D/MAC-seq**
PMC37	PMC101	PMC122	PMC220
PMC78	PMC102	PMC137	PMS28
PMC83	PMC103	PMC146	PMS29
PMC88	PMC104	PMC147	PM3D94
PMC95	PMC107	PMC150	PMC3D104
PMC96	PMC108	PMC152	PM3D110
	PMC109	PMC155	PM3D113
	PMC110	PMC156	PM3D130
	PMC113	PMC184	
	PMC114	PMC185	
	PMC117		
	PMC118		

2- T cell infiltration model – mouse macrophage

CXCL9 and CXC10 are chemokines induced by IFNγ
Play a role to induce chemotaxis, promote differentiation and multiplication of leukocytes, and cause tissue extravasation.



Cold tumour that lack immune cell infiltrate are CXCL9$_{low}$

Not responsive to Immune Checkpoint Blockade (ICB)

Hot tumour are CXCL9$_{high}$

Responsive to ICB

What agents can drive tumours make more CXCL9/10?

Emily Derrick, A/Prof Paul Beavis, Peter Mac

Identify novel immunomodulatory drugs to enhance CXCL9/10 expression

Victorian Centre For Functional Genomics

Day 1 AM

Plate cells
@ 10K/well
BioTek 406

Day 1 Midday

Add compound library
(20480 compounds)
Janus G3

Day 1 PM

Add IFNγ (0.625ng/mL,
prepared at 10X)
BioTek 406

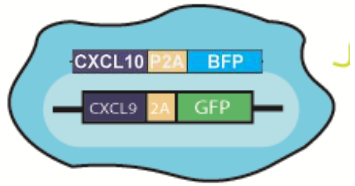
Day 2 AM

Lift cells,
fix with 2% PFA
BioTek 406

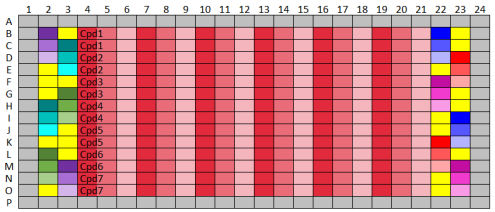
Day 3++

Flow cytometry

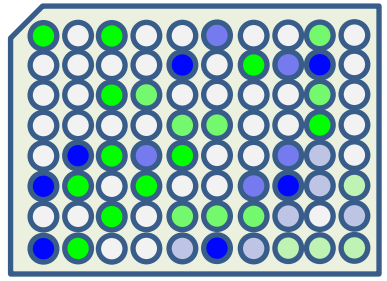
Read MFI of
GFP and BFP
3 systems



CXCL9-P2A-GFP
CXCL10-T2A-BFP



72 x 384 well plates
Single dose compound 10uM



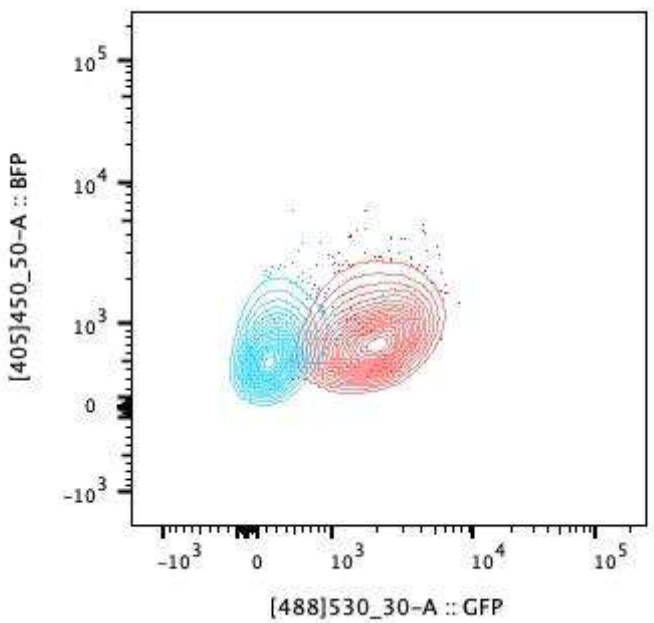
High viability
Exclude toxic targets

www.alid.com.au Australian Drug Discovery Library

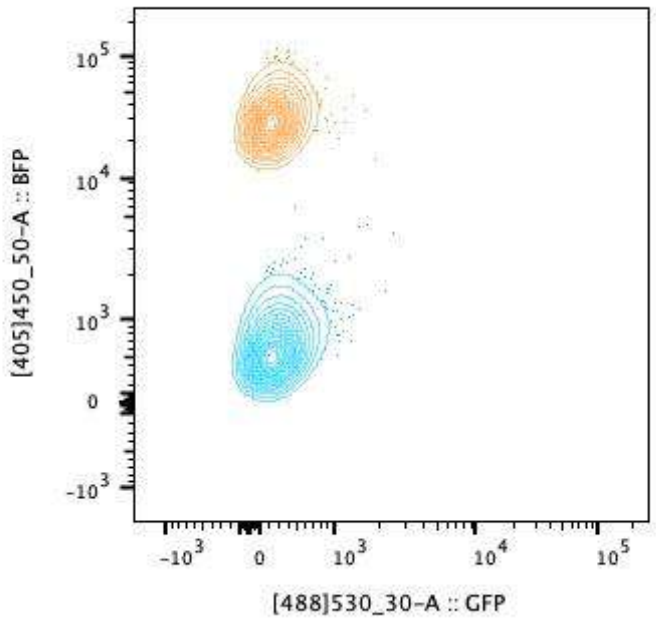
Example compounds

Blue = IFNγ-stimulated DMSO control.

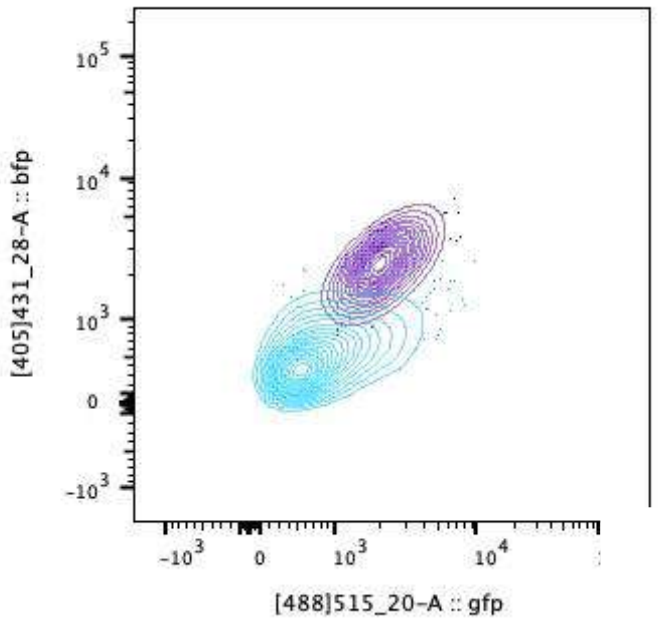
Modifies CXCL9 expression



Modifies CXCL10 expression



Modifies CXCL9&10 expression



CXCL10

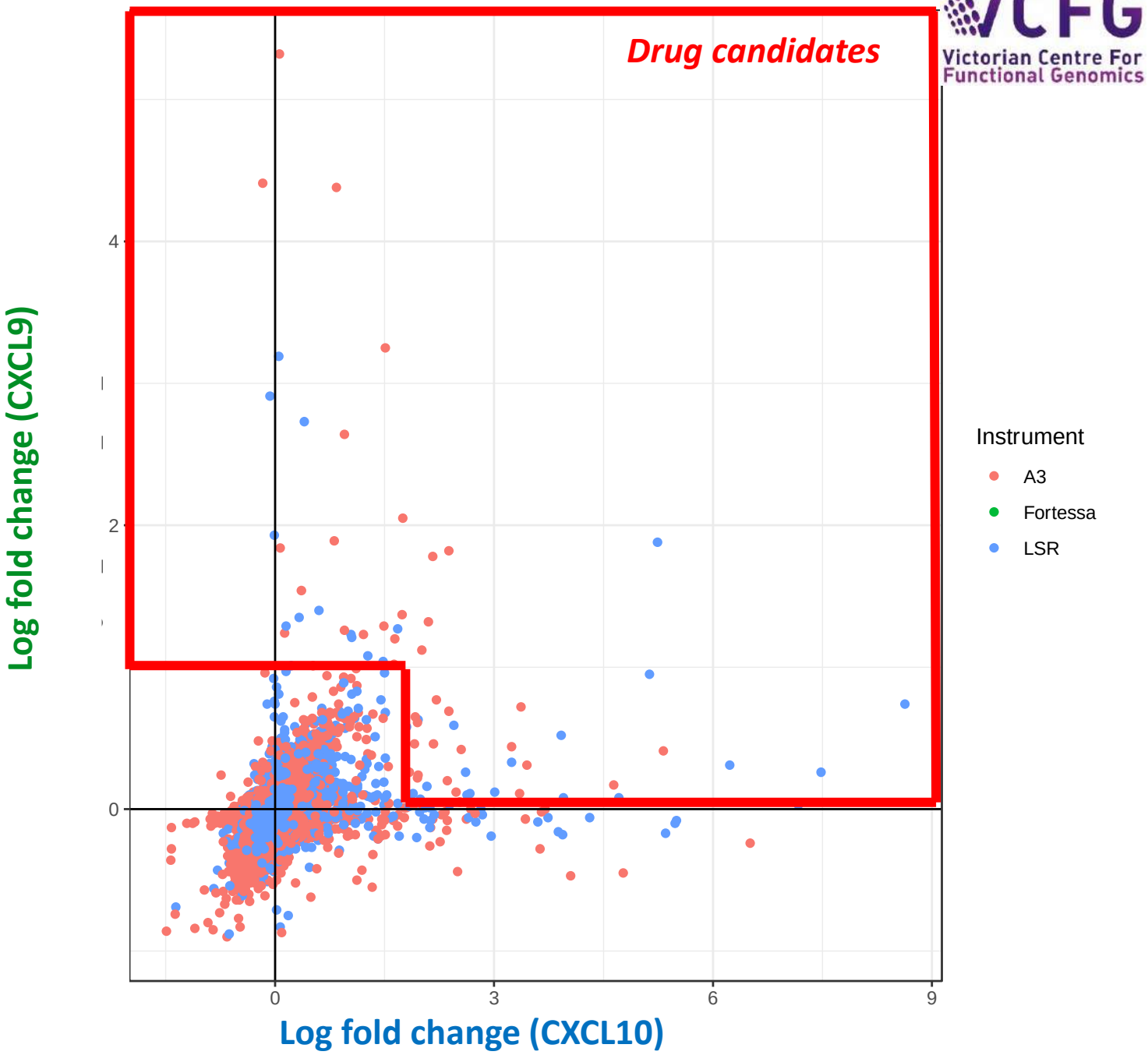
CXCL9

Primary screen outcomes

20480 compounds screened

- CXCL10 log fold change > 1.85
- CXCL9 log fold change > 0.7
- Counts > 100 (toxicity)

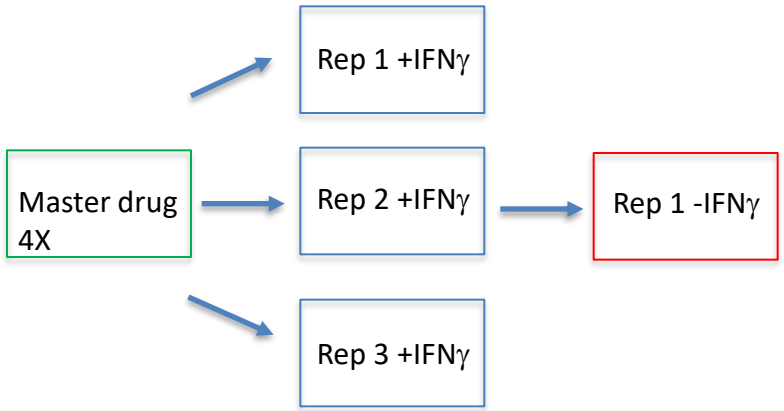
- Short listed 138 compounds



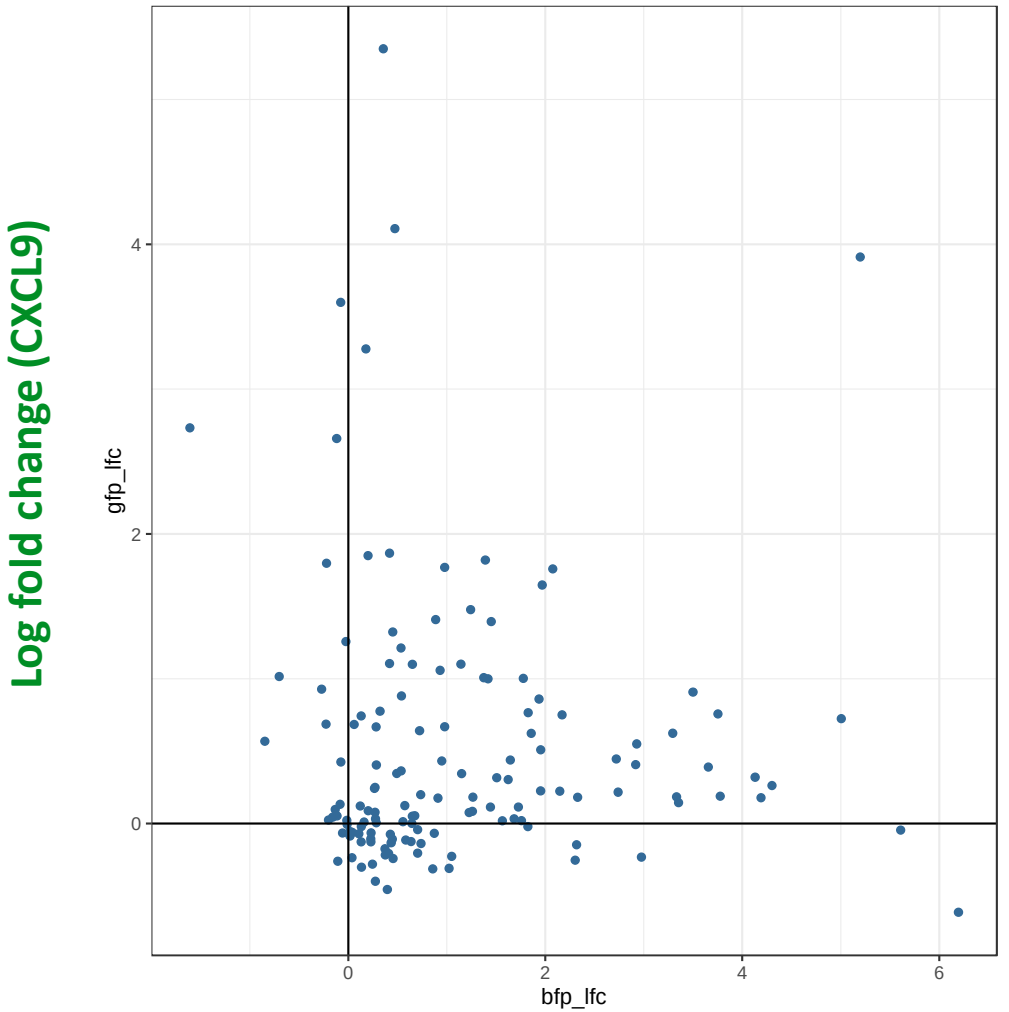
Drug candidates – validation screen

Secondary screen containing 138 compounds

4-point dose curve (10, 3, 1, 0.1 uM, ± IFNγ) in triplicate



10uM only



80/138 Drugs validated

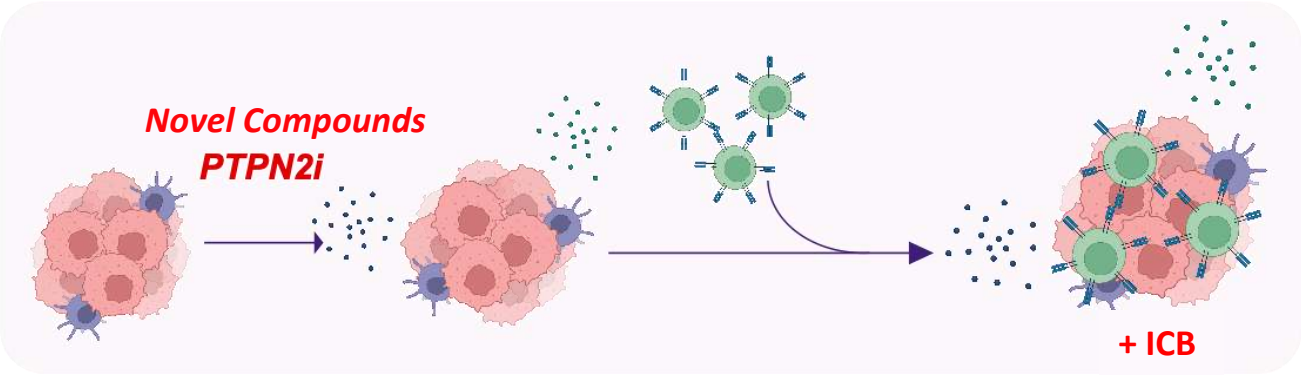
High confidence targets for detailed mechanistic study – clinical relevance

- Selecting best 15-20 drugs:
- ~5 CXCL9 only
- ~5 CXCL10 only
- ~5 modulate CXCL9 & CXCL10
- Some drugs modulating CXCL9/10 expression **without** IFNγ

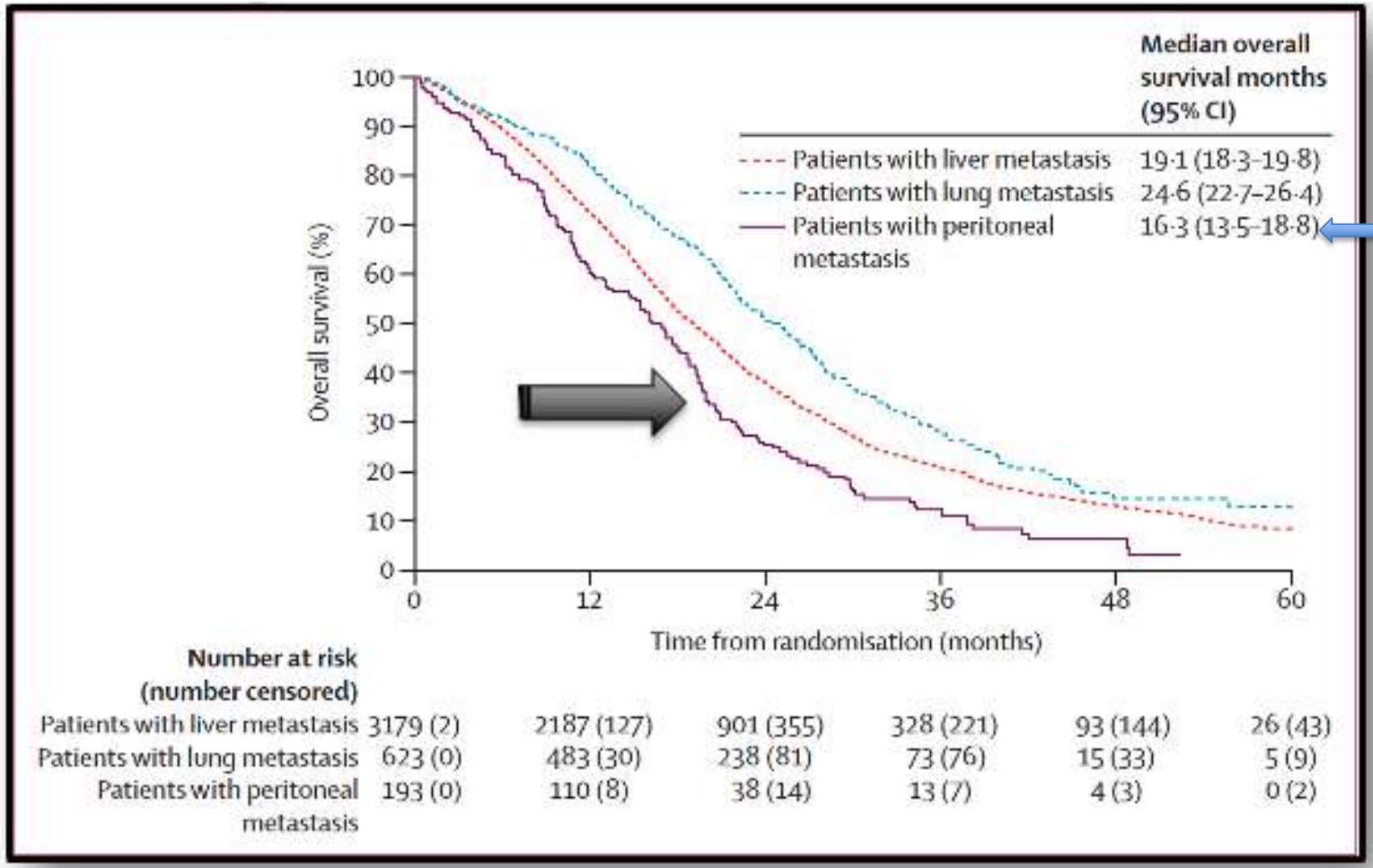
- Validate in human primary macrophages.
- Chemokine production
- RNAseq for mechanistic insight

Novel compounds improve CXCL9/10 expression in tumors, and subsequently make them more responsive to ICB.

These may be more specific in modulating chemokine production than PTPN2 inhibitors (less adverse reactions).



3- Personalised approach to Colorectal Peritoneal Metastases (CRPM)



Franko J et al. *Lancet Oncology* 2016. 17 (12):1709-19

Worse prognosis compared to nonperitoneal metastases (liver, lung)

Supportive care → 3-5 months

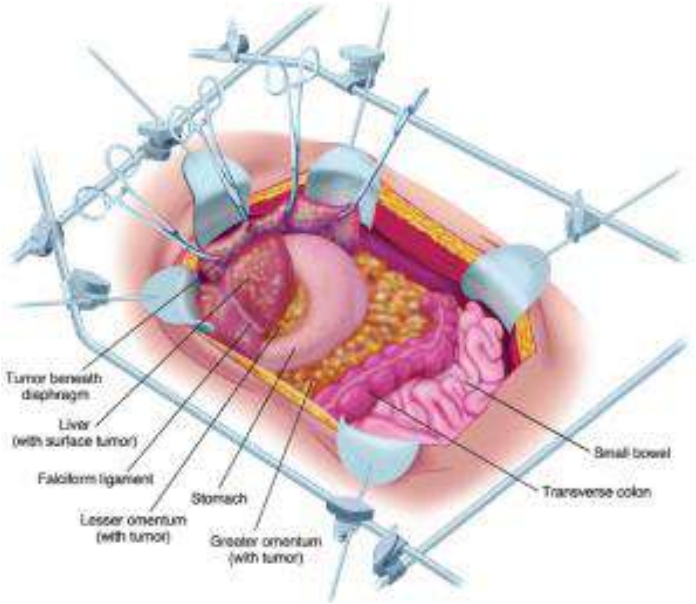
Systemic Chemotherapy → 16 months

Limitations

- *Tumour biology*
- *Poor drug penetration*
- *Drug Resistance*

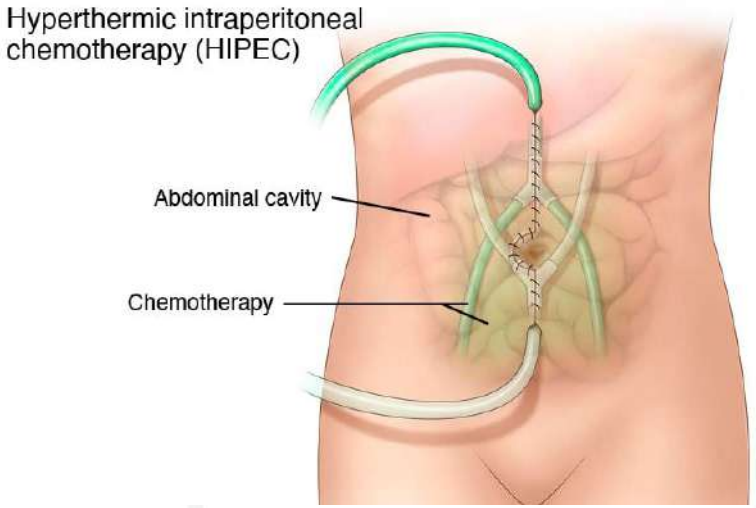
Treatment options for Colorectal Peritoneal Metastases

Cytoreductive Surgery (CRS)



Sugarbaker PH. 2016 Jul;48:42-9.

Heated Intraperitoneal Chemotherapy (HIPEC)



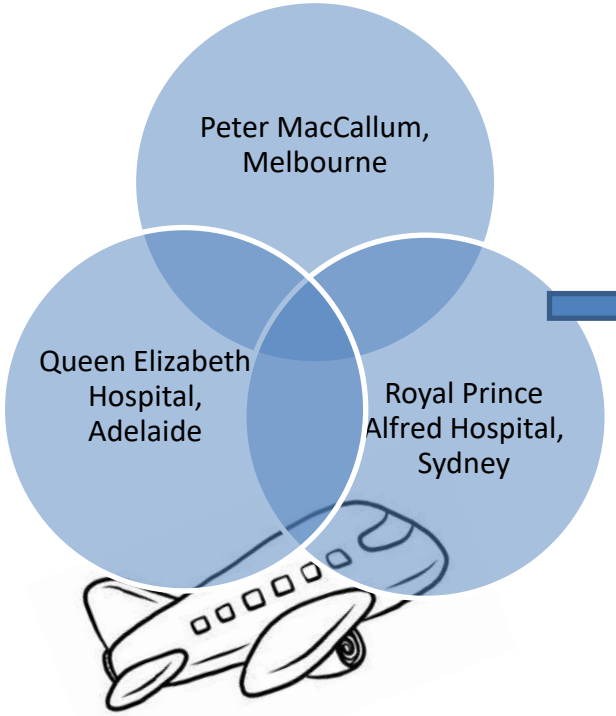
© Mayo Clinic

Well selected patients with good cytoreductive surgery

→ can improve outcomes

But does HIPEC provide oncological benefit in addition to the surgery?

Feasibility study design

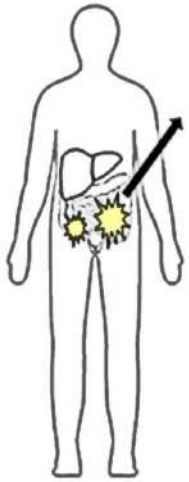


Peter Mac
Peter MacCallum Cancer Centre
Victoria Australia

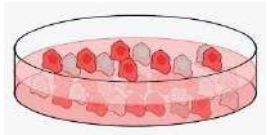
Biopsied, transported and organoids cultured and drug screened at PMCC

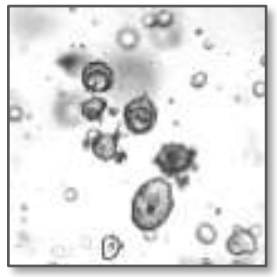
Image analysis and generation of Reports

Determine feasibility of inter-site transfer and success rate of organoid culture and drug screening within **4 weeks**



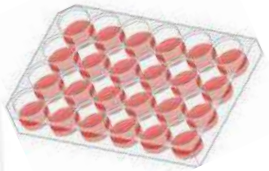
Biopsy of Colorectal peritoneal metastases

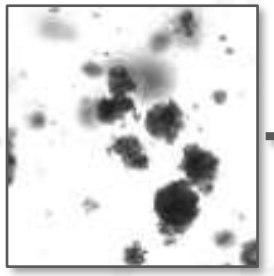




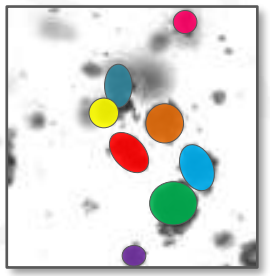
Viable Cells

Drug-screening





Cell Death



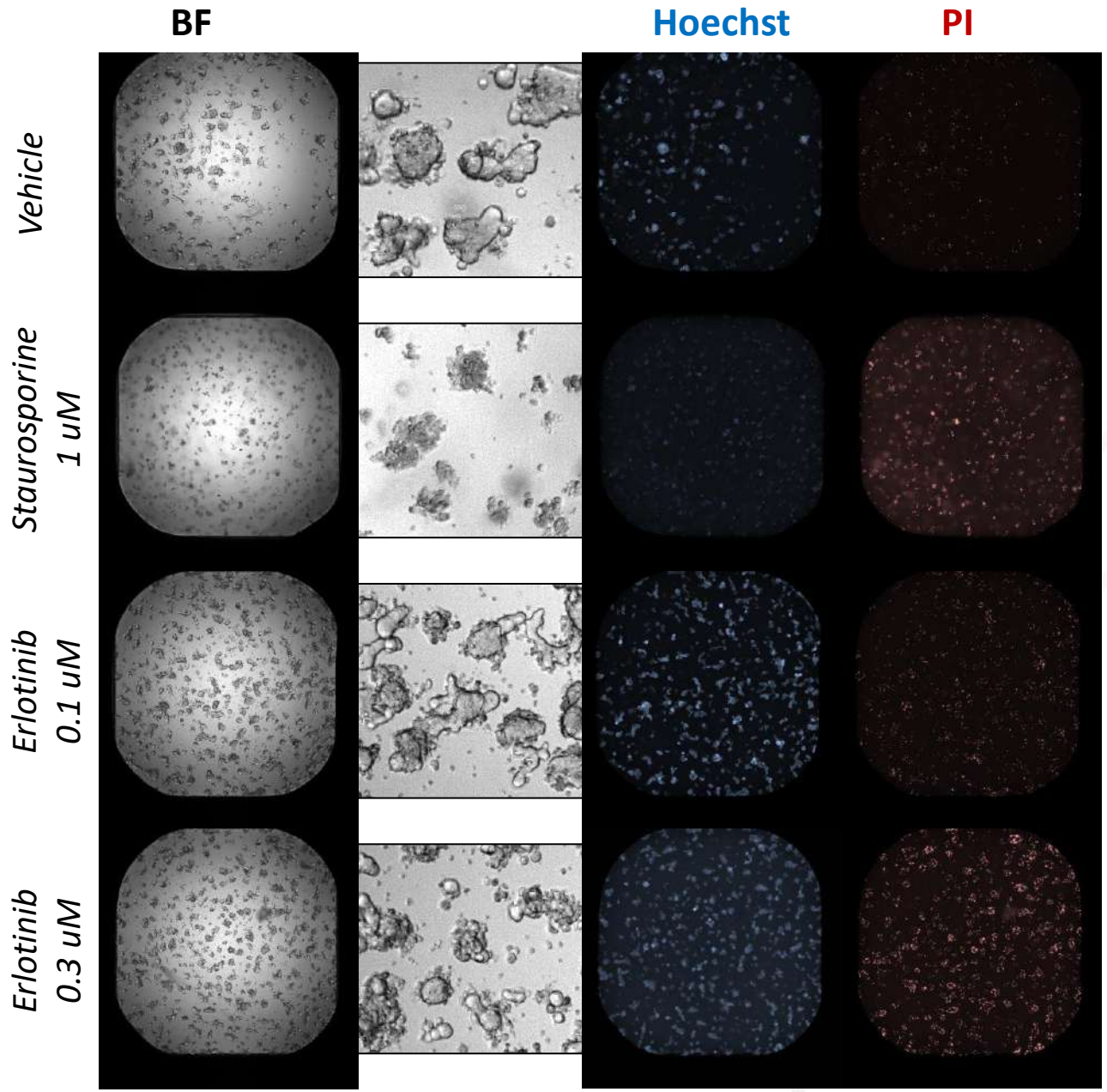
Single organoid classification (Image-based ML)

Individual organoid/ patient drug sensitivity

Reports for Clinician

Anshini Jain, Susi Ramm, Rob Ramsay, Sandy Heriot, Nick Clemons, VCFG

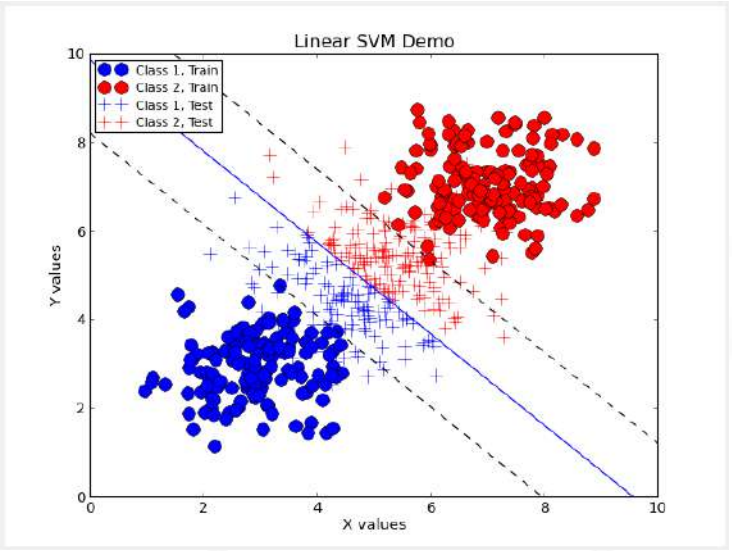
Support Vector Machine learning based on imaging data



100% alive

100% dead

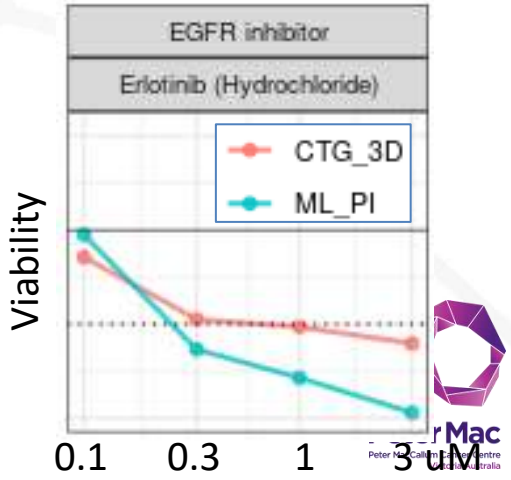
Build SVM algorithm for every patient



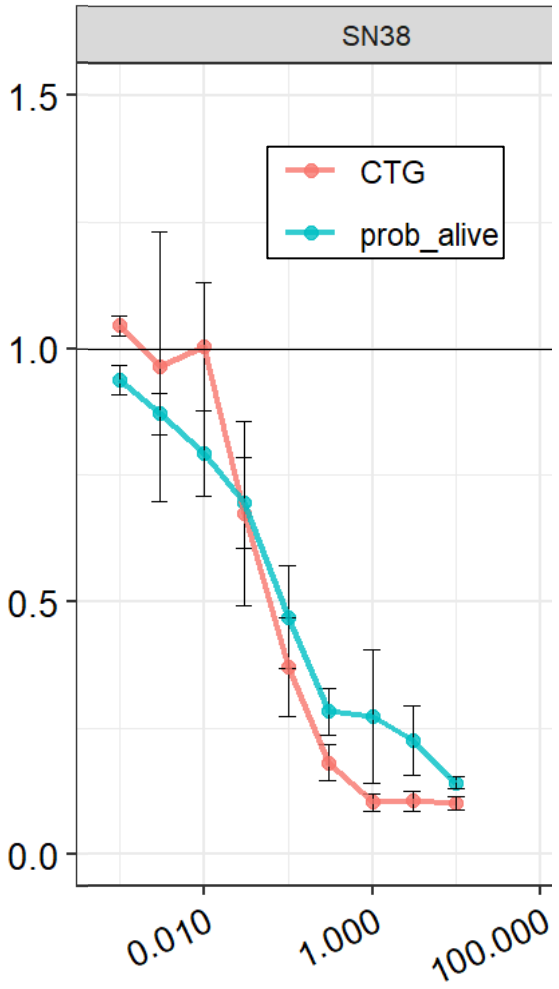
?

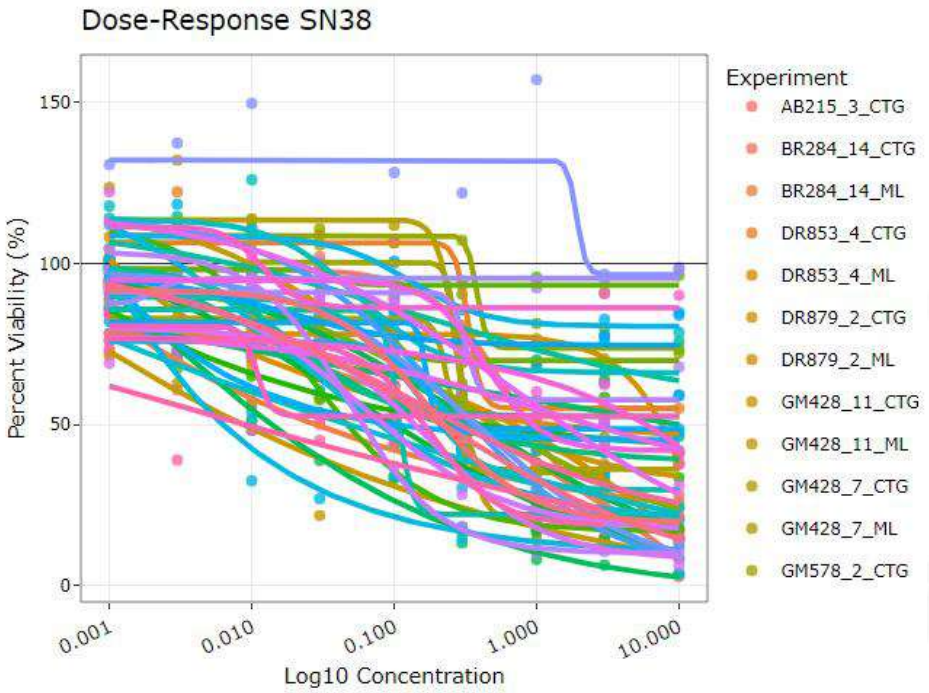
Predict unknown conditions

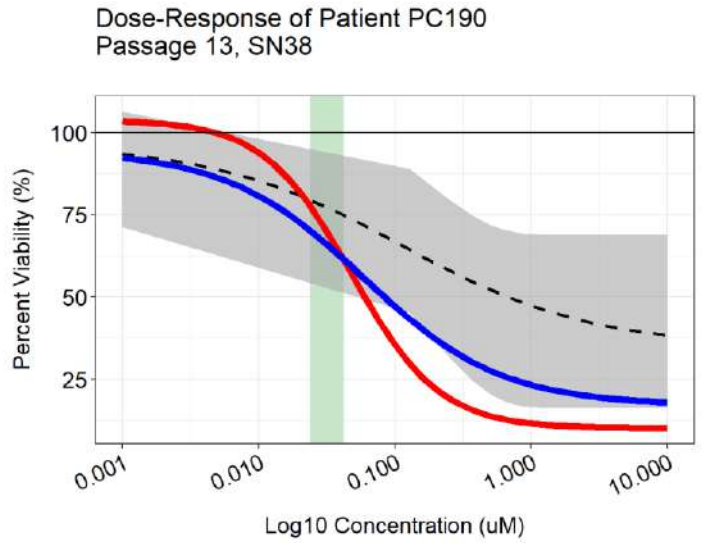
?



How do we benchmark a patient's response?







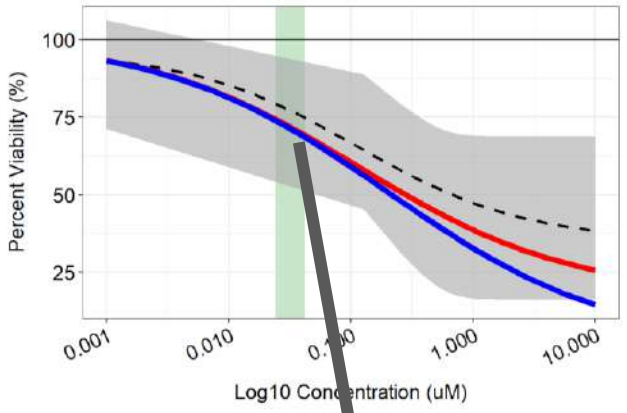
Dose response curve range of all samples in the existing database of Colorectal Cancer Organoids

Clinically relevant concentration ranges determined in the clinical cohort

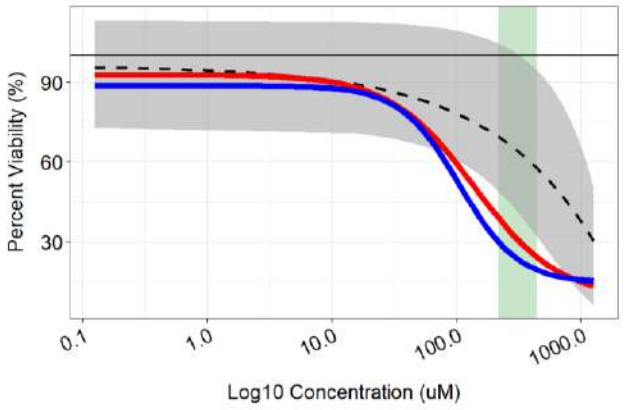
Colorectal Cancer Cohort's Mean Dose-response curve

How do we benchmark a patient's response?

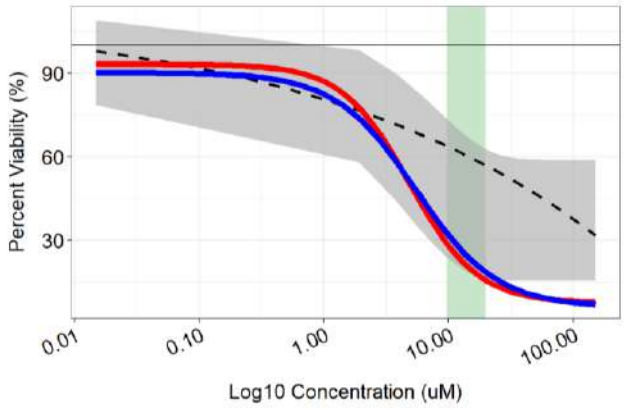
Irinotecan (SN38)

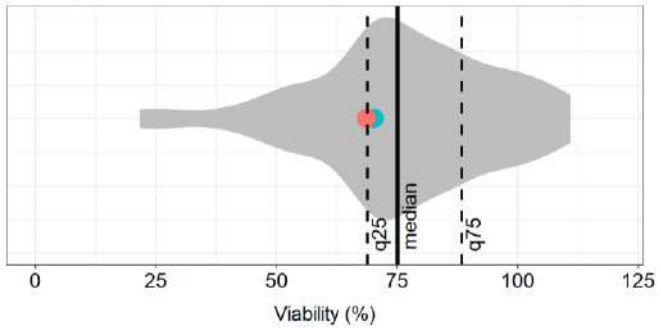


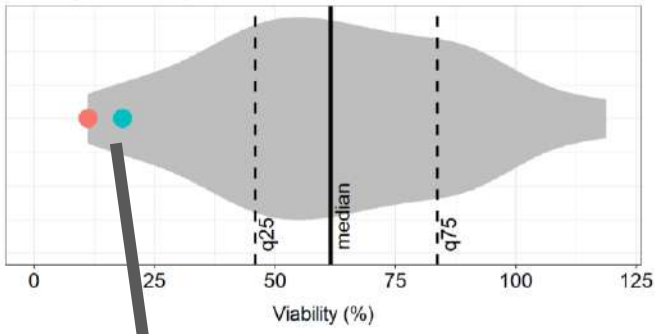
Oxaliplatin

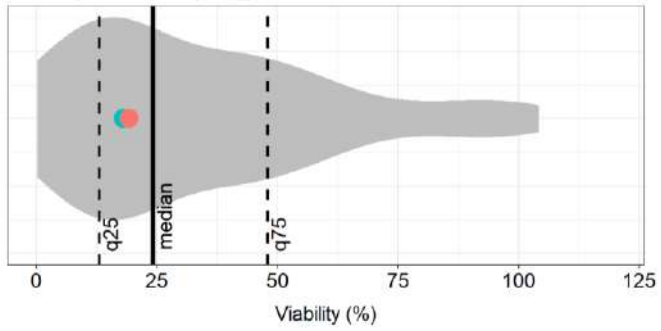


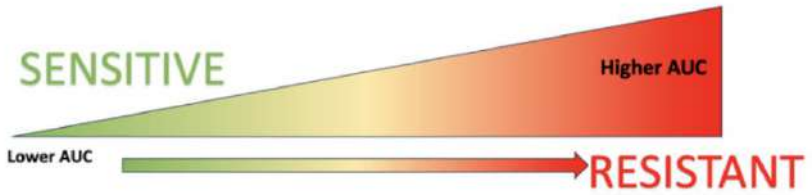
Mitomycin C











More sensitive to Oxaliplatin than 98% of patients in our database

Awaiting clinical response data now

4- Modelling immunotherapy in organoids

Immunotherapy efficacy *in vitro* have been assessed using cell lines with engineered reporters such as luciferase in ***2D***, or luminescent based whole well assay.

There is a lack of translational tool and a read out that encompasses the cellular heterogeneity to apply to primary cells.

Most endpoint assays don't capture kinetic information of cell therapy, a late stage end point will likely include non-specific cytokine killing.

Complex co-culture immuno-oncology

MCF7 spheroids with activated NK92 cells - two different effector:target ratios

Cytation 5

Victorian Centre For Functional Genomics

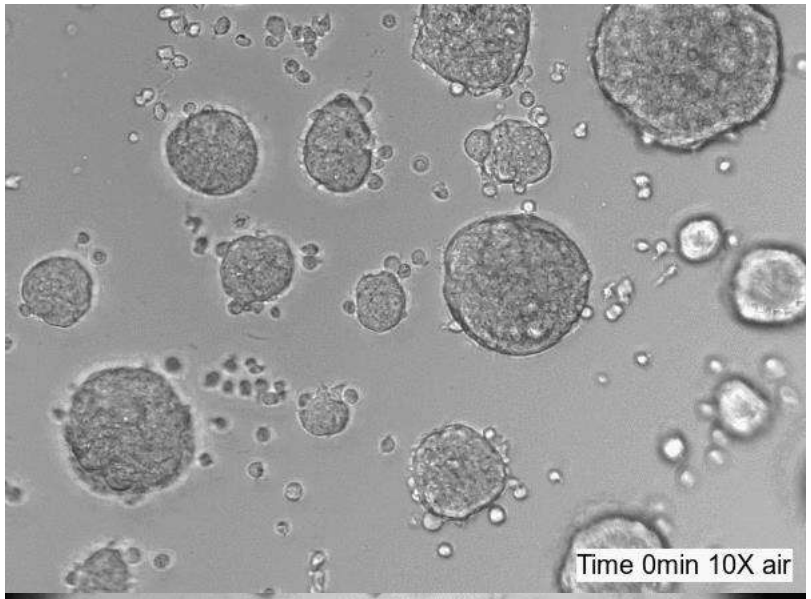
Day 4 MCF7 cells

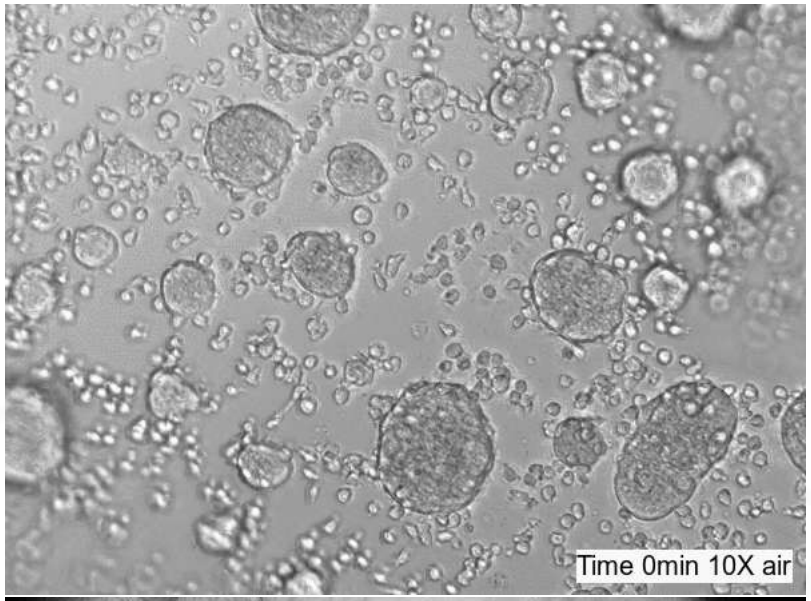
30 minute intervals for 30 hours

300 cells per well

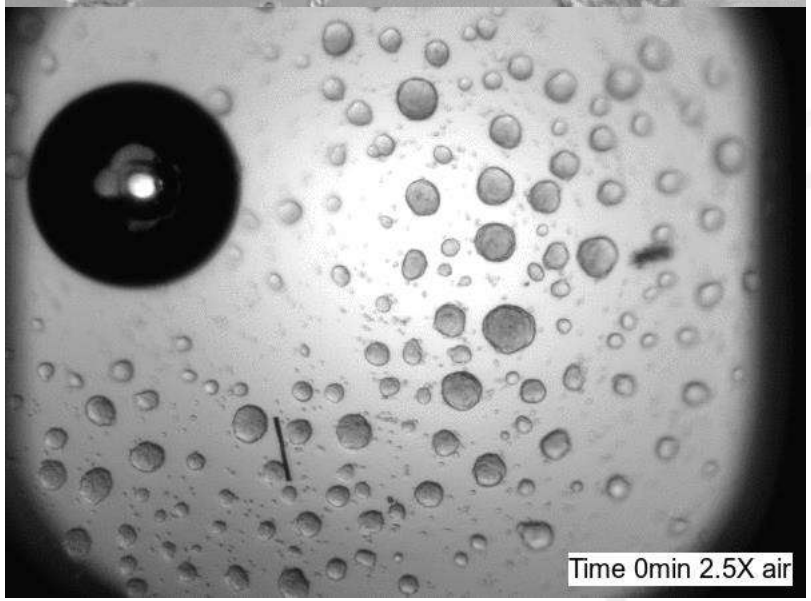
2000 cells per well

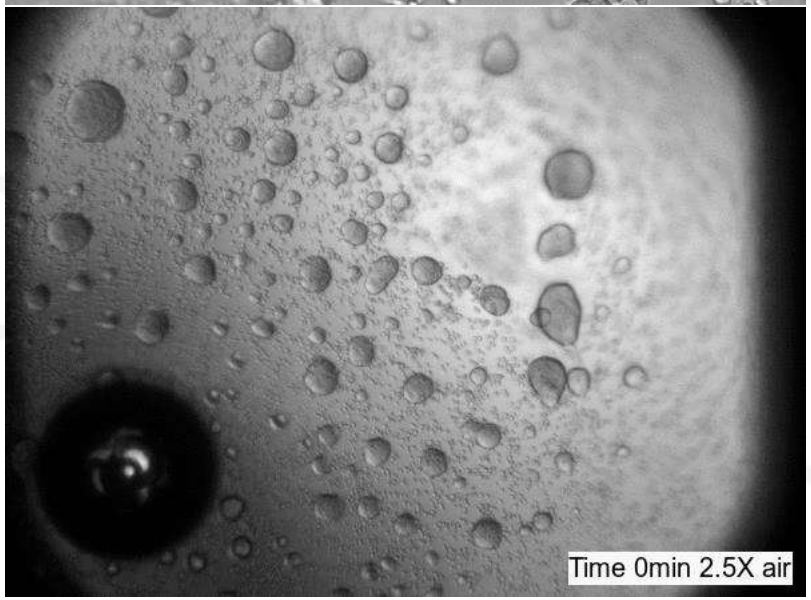
10x





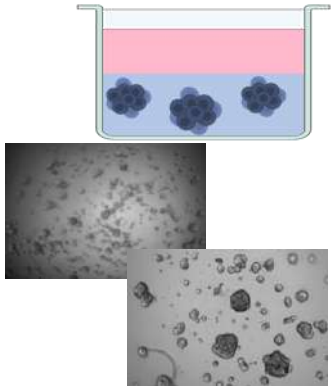
2.5x



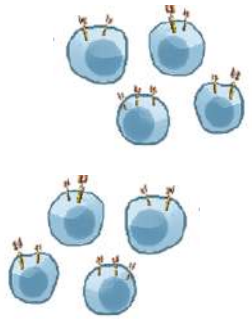


High-throughput 3D Immune Co-culture Screening Platform

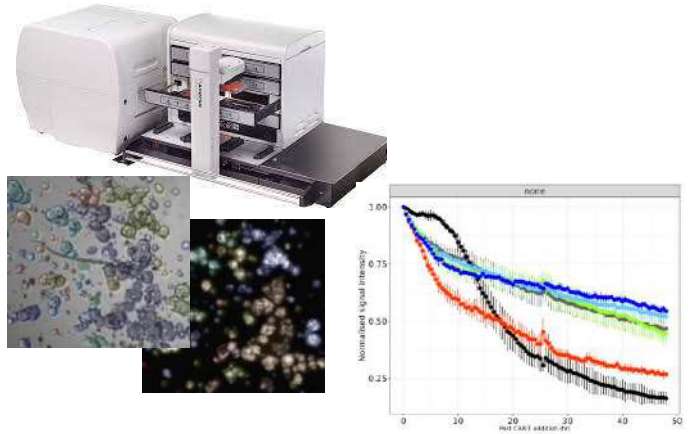
Automated Cell Seeding in 3D Matrigel
in 384-well format
~ 2000 cells/well



Tumour 3D Spheroids



Introduction of CAR-T
Or other immune cells



Time-lapse live cell imaging with TMRE
(**hours** to **days**) and image analysis

Key points

- **Minimal cell material requirement**
- **High-throughput with real kinetic resolution**
- **Tumour heterogeneity in 3D**
- **Scalable and expandable platform for multi-omics assay**

Live-image tracking over time ✓

Endpoint parallel readout such as CellTiter-Glo ✓

Flow ✓

MAC-seq (deconvolution of tumor and CAR-T transcriptome) ✓

A bottom-up approach to reconstruct miniaturised models for "**micro-environment**" for *in vitro* high-throughput screening

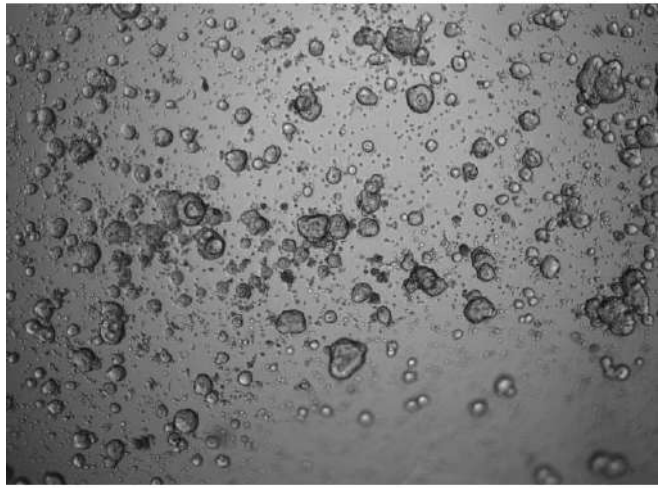
CAR-T induced cell death of colorectal spheroids

Using different ratios of CAR-T cells

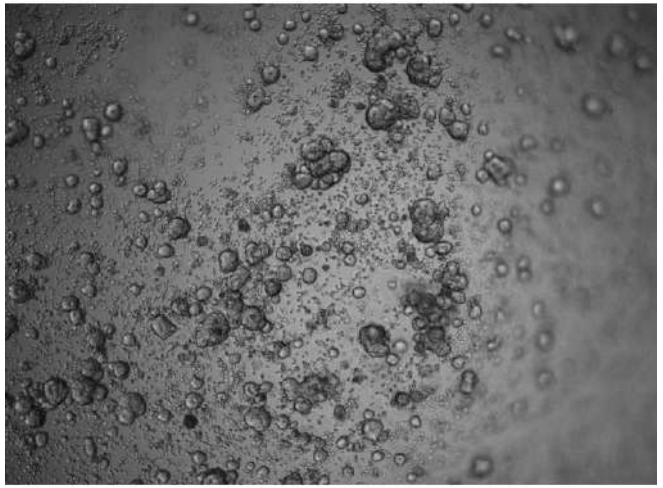
Loss of TMRE is “dose” dependent.

We can measure each individual spheroid in the well for a whole well population heterogeneity readout

Lews-Y CAR-T 1:1 CRC **Patient JF016** Lews-Y CAR-T 5: 1 CRC

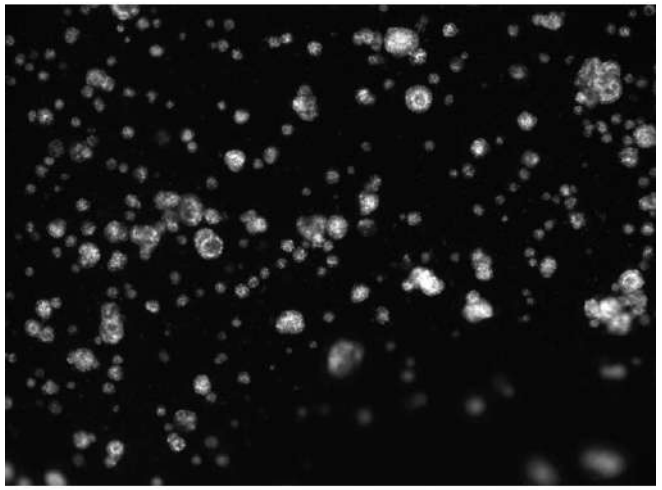


4X_JF016_CAR-T_20000_cells_none_0hr

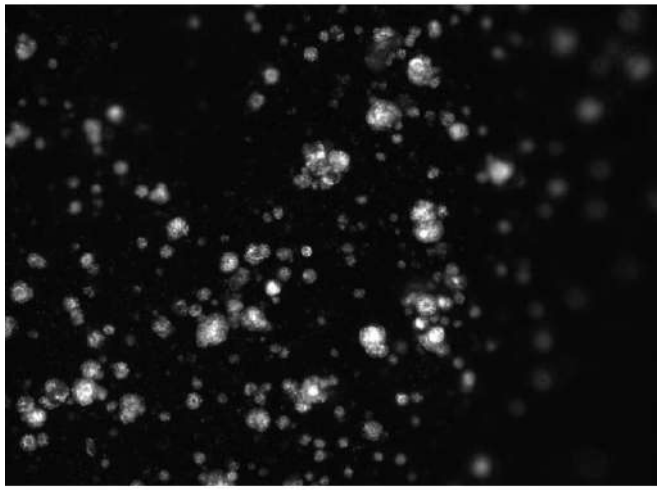


4X_JF016_CAR-T_100000_cells_none_0hr

Bright Field



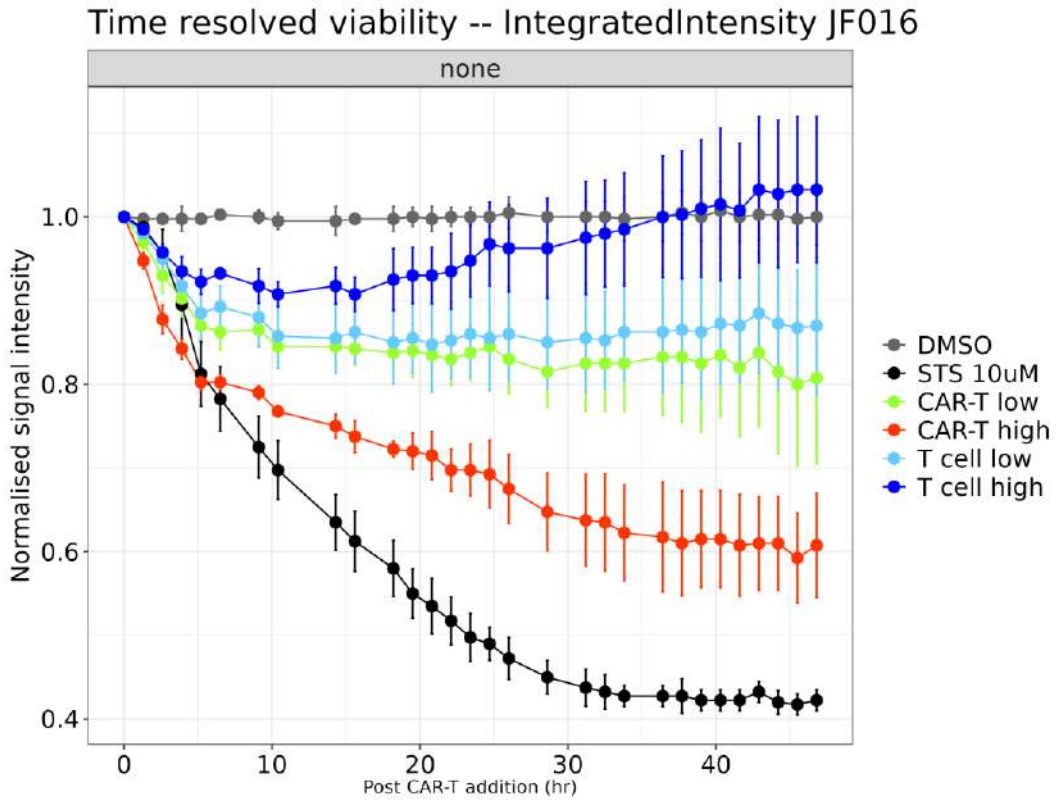
4X_JF016_CAR-T_20000_cells_none_0hr

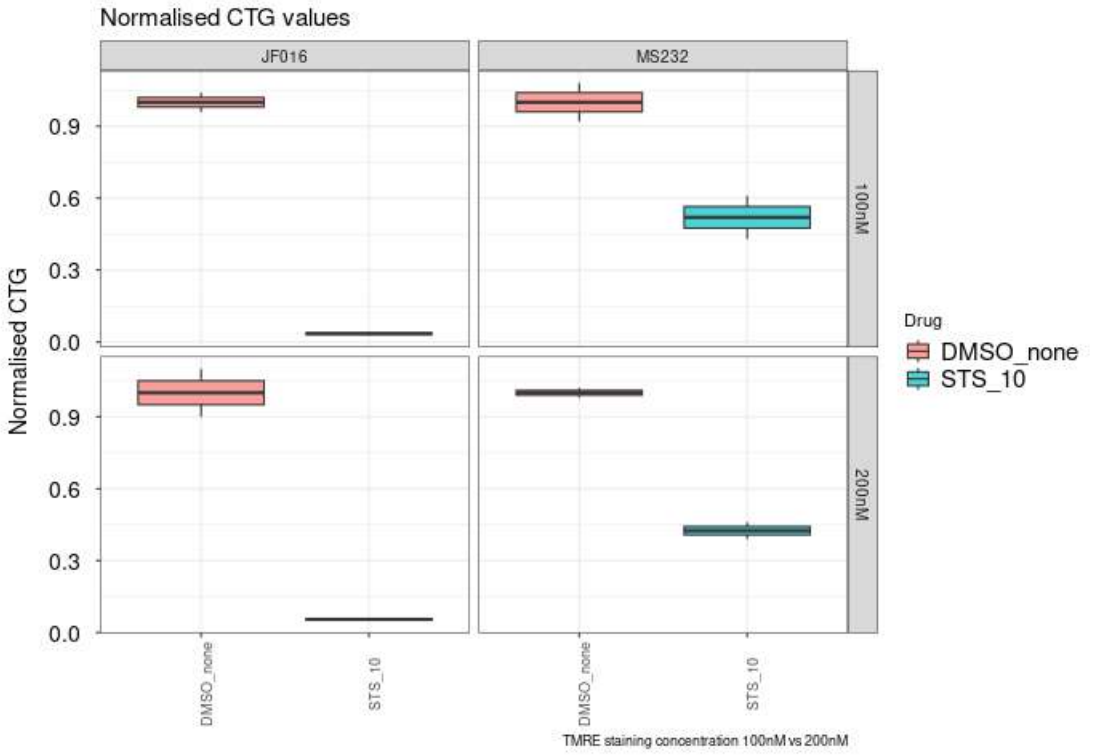


4X_JF016_CAR-T_100000_cells_none_0hr

TMRE

Using TMRE to quantify spheroids over time with Cell titre glo endpoint viability



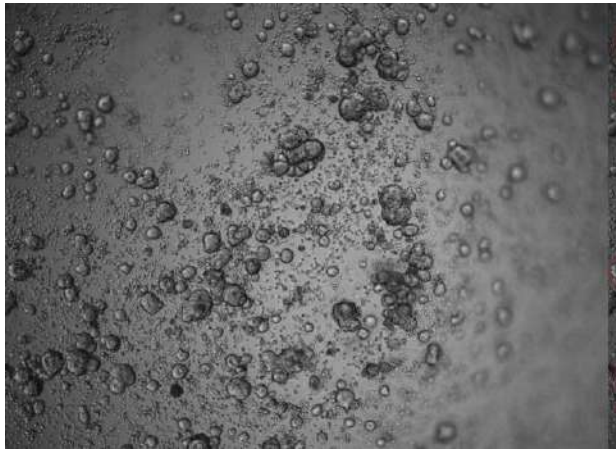
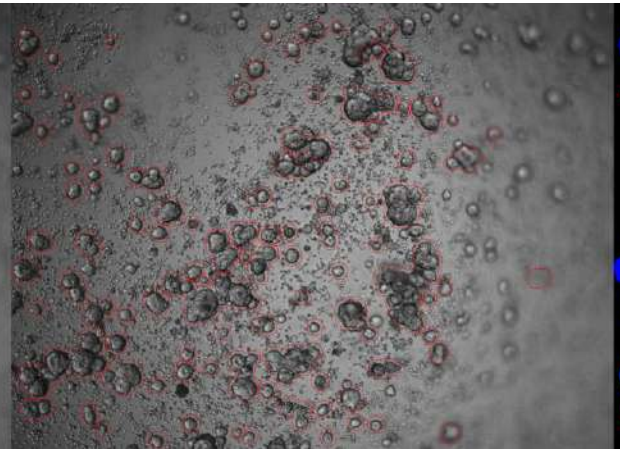
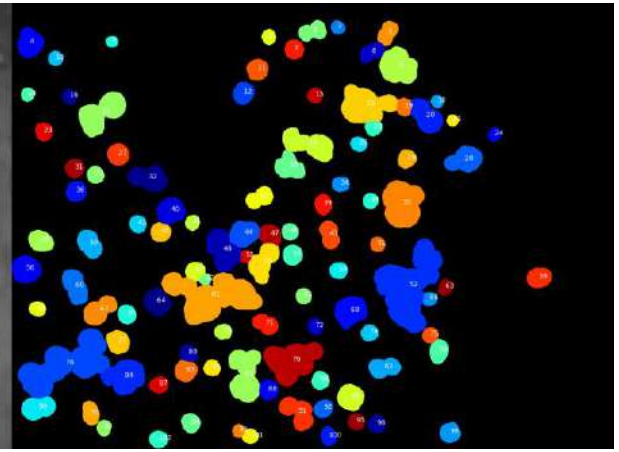
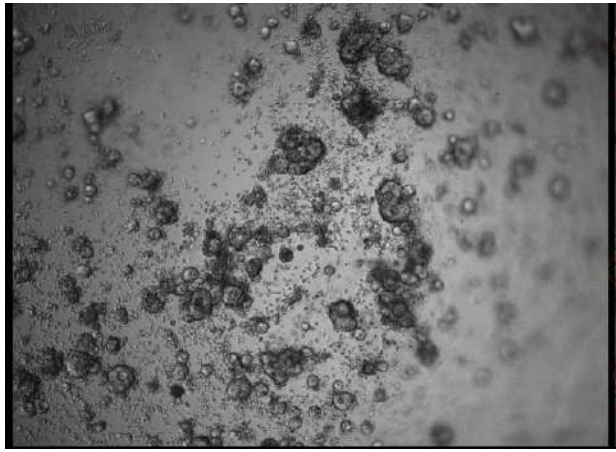
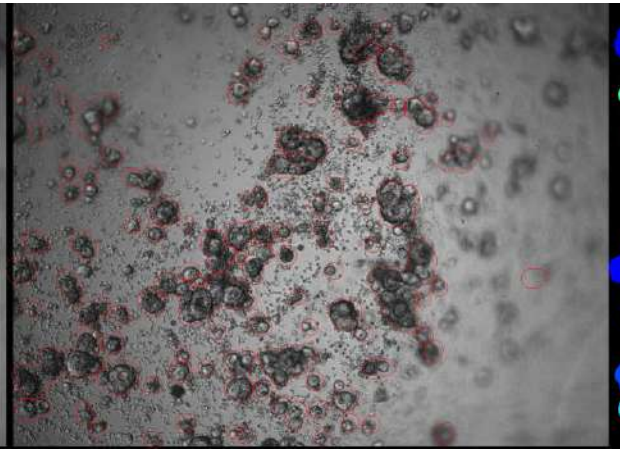
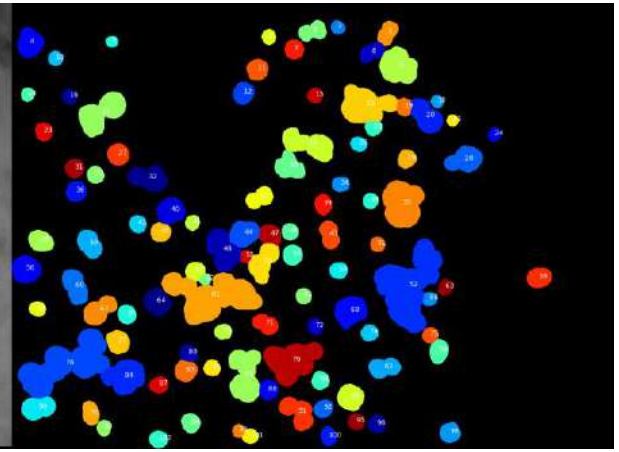


Different ratios of CAR-T and untransduced T cells
Staurosporine 10uM killing agent

Milton Mui, Robert Ramsay, Nick Clemons, Mark Li, CART from Dr Jessica Li CoE Cellular Immunotherapy

Single spheroid level image analysis and object tracking

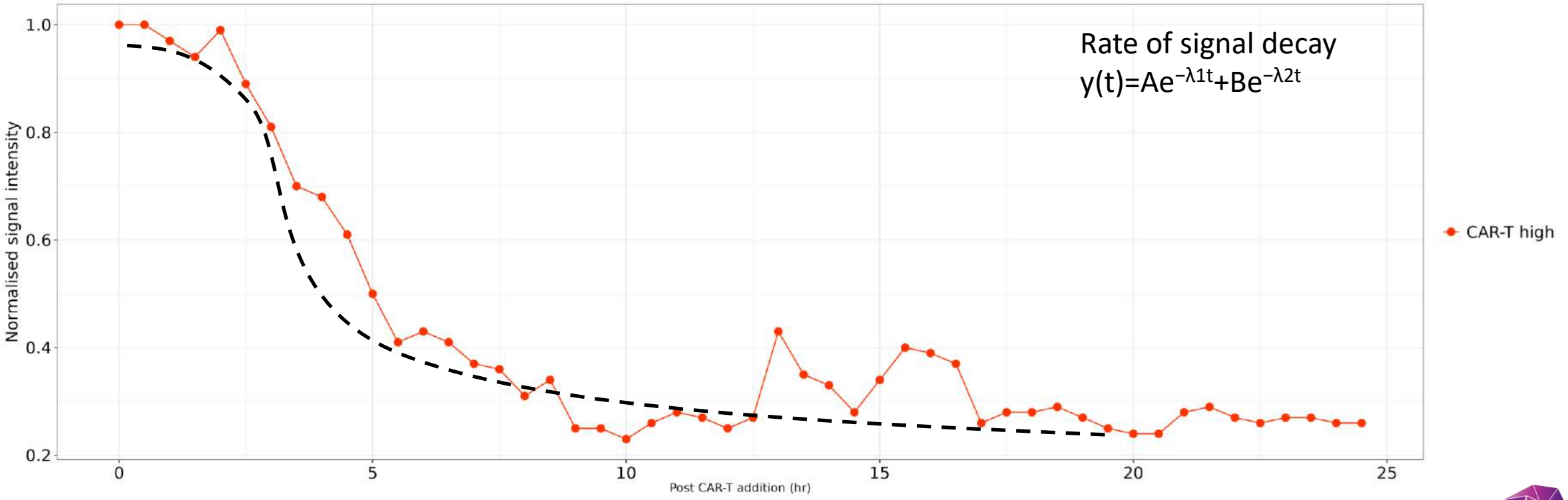
Victorian Centre For Functional Genomics

	Bright Field	Object detection	Object Tracking
Time 0			
Time 6hr			

Peter Mac
Peter MacCallum Cancer Centre
Victoria Australia

Single spheroid level image analysis and object tracking

TMRE signal change for a given single spheroid over time
Summarise heterogeneity into a single number

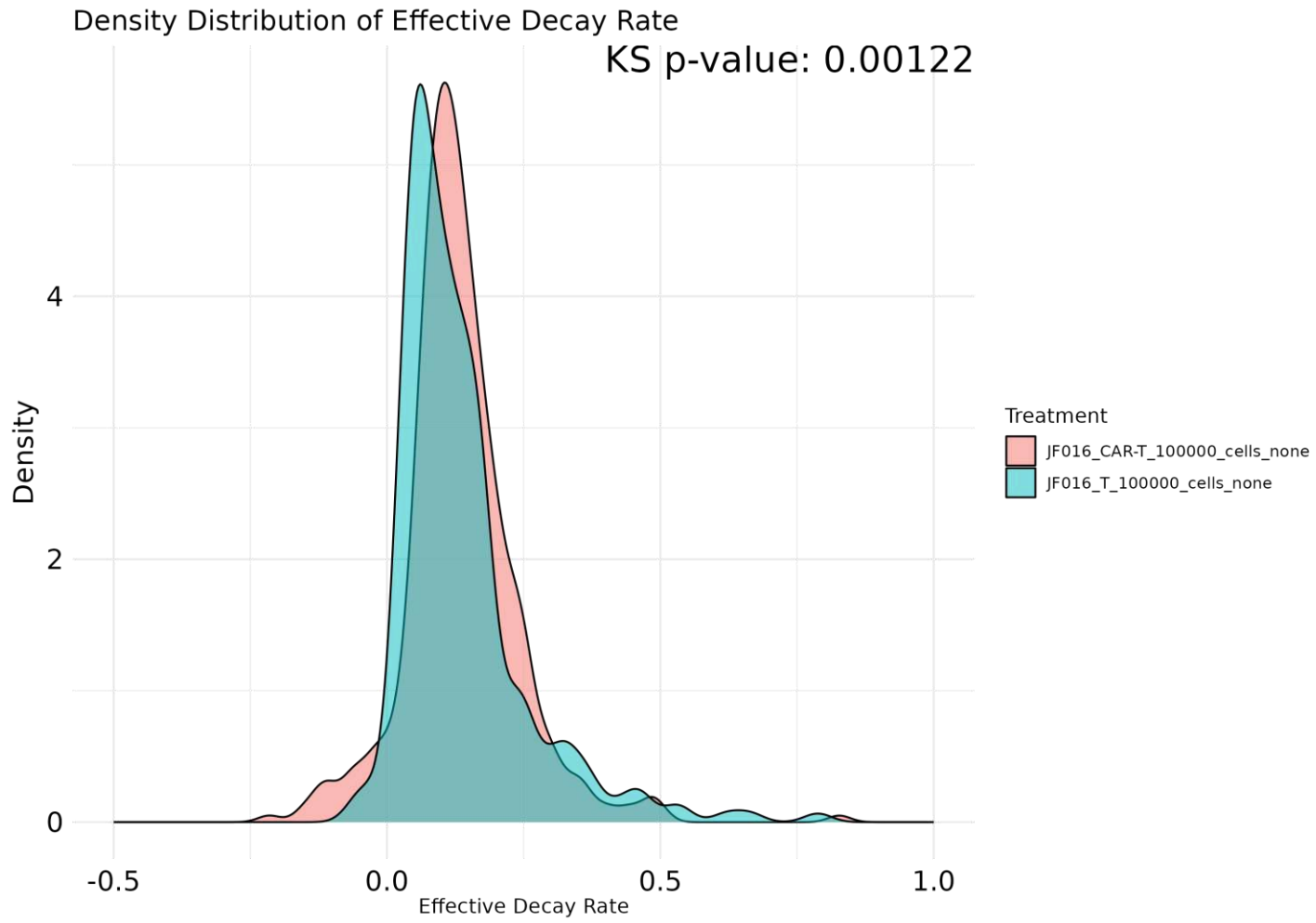


Comparison between 2 samples aggregating single spheroids

plot out the decay rate for every structure per treatment.

compare the 2 distributions using a Kolmogorov-Smirnov test.

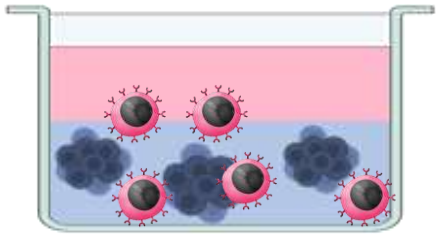
CAR-T high dose **significantly** faster death.



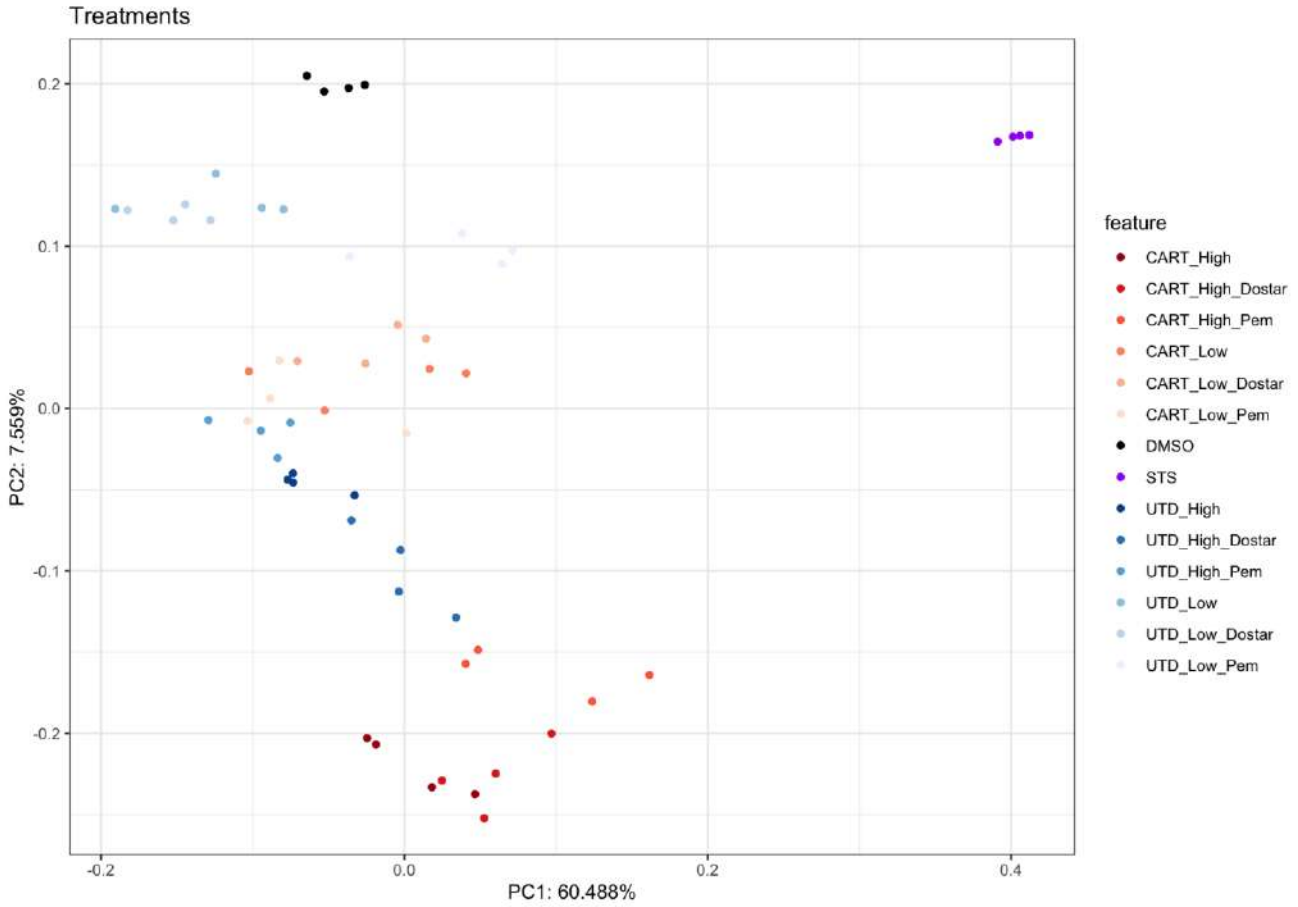
Can we use MAC-seq to quantify signaling differences in presence of CAR-T cells?

Using JF016 samples 24hr timepoint

CRC alone



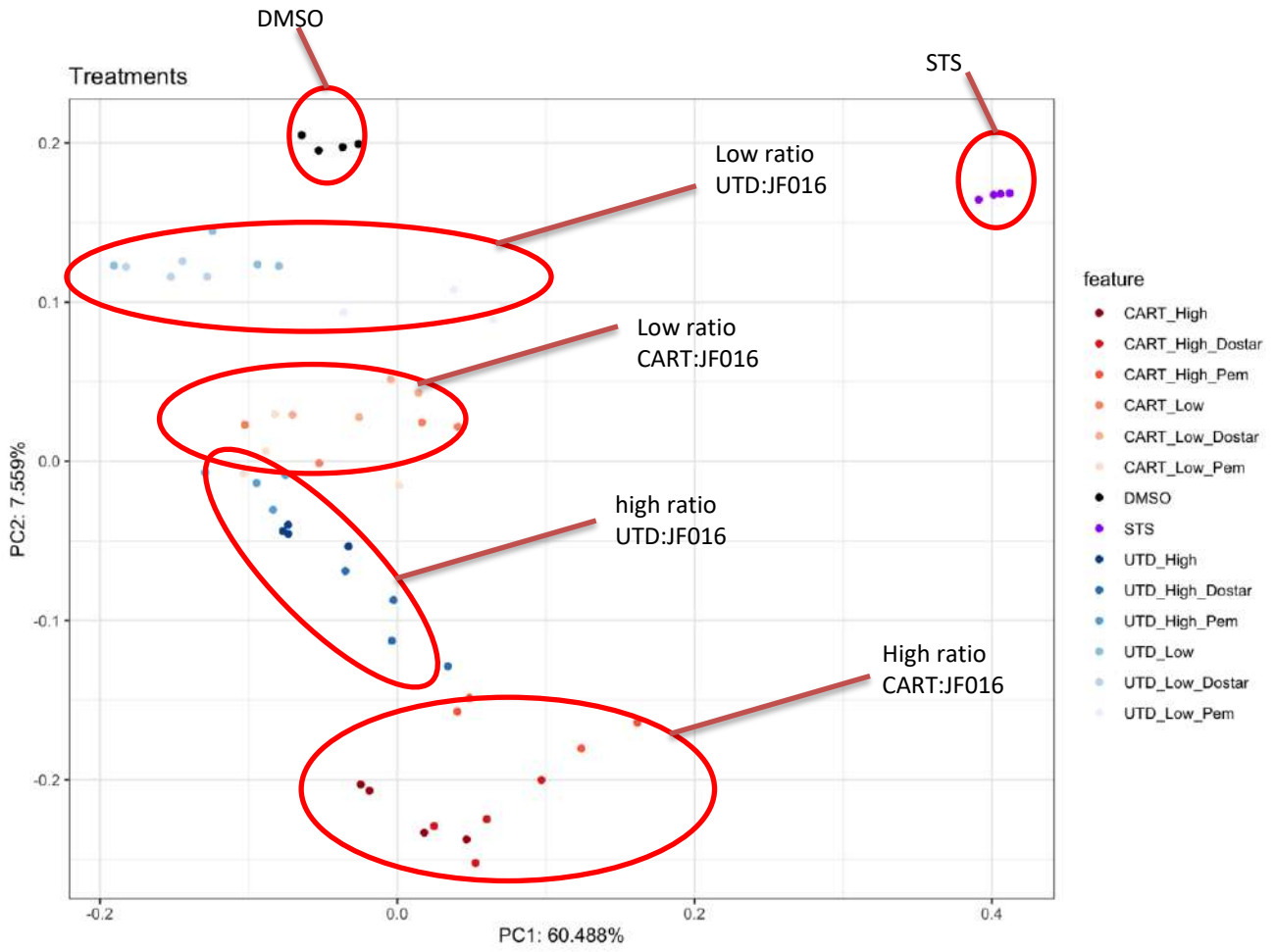
CRC with
- CAR-T
- Untransduced T cells



Xin Liu, Mark Li

We can discriminate the RNA profiles of each treatment condition

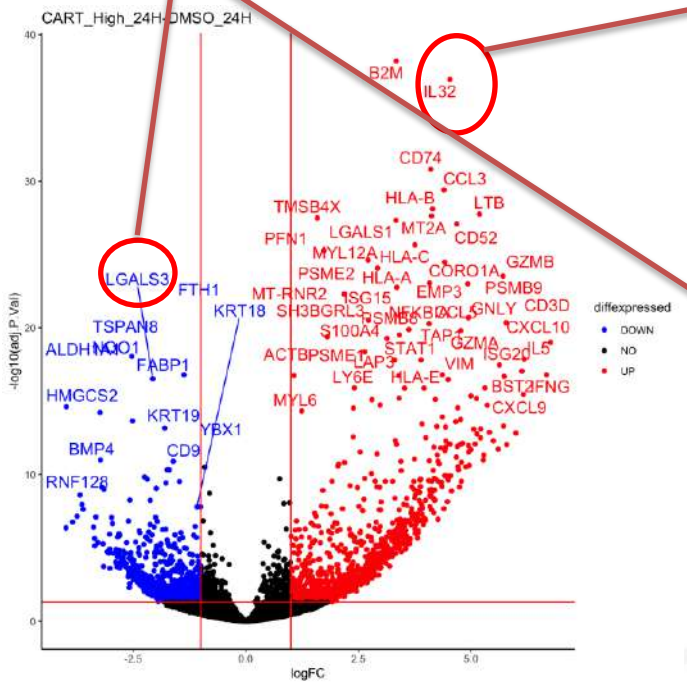
Each dot is the transcriptome of that treatment well.

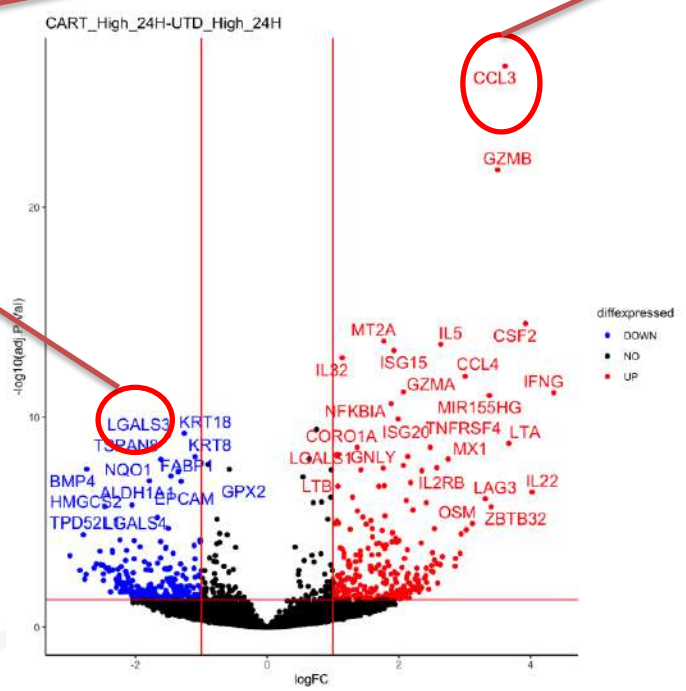


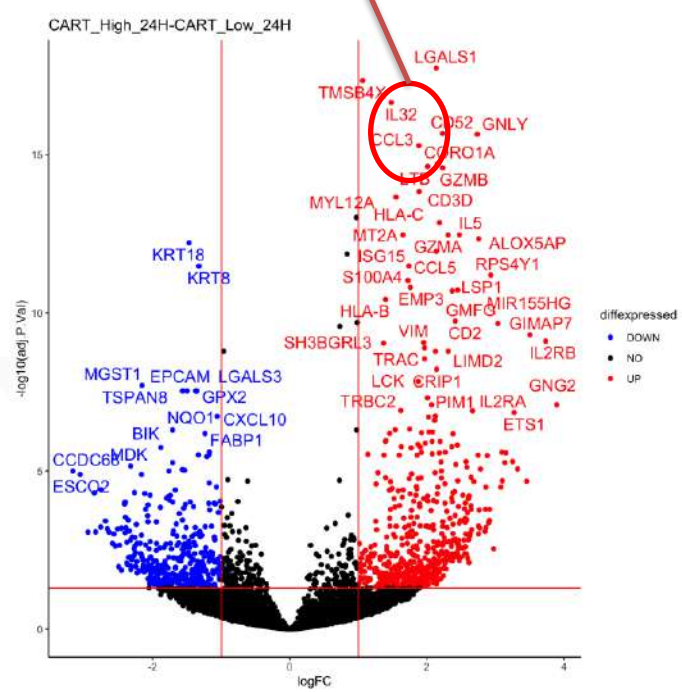
Differential expression of co-culture conditions

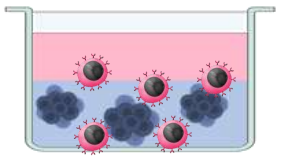
Required for efficient T cell killing

Galectin-3 promotes secretion of proteases that decrease epithelium integrity in human colon cancer cells



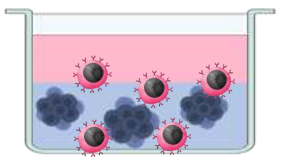




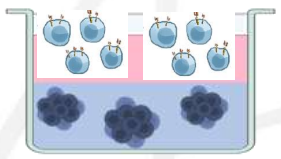


CAR-T

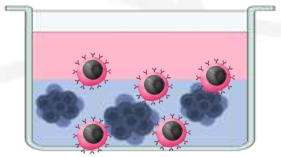
CRC alone



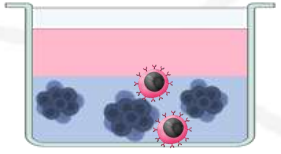
CAR-T



Untransduced T cells

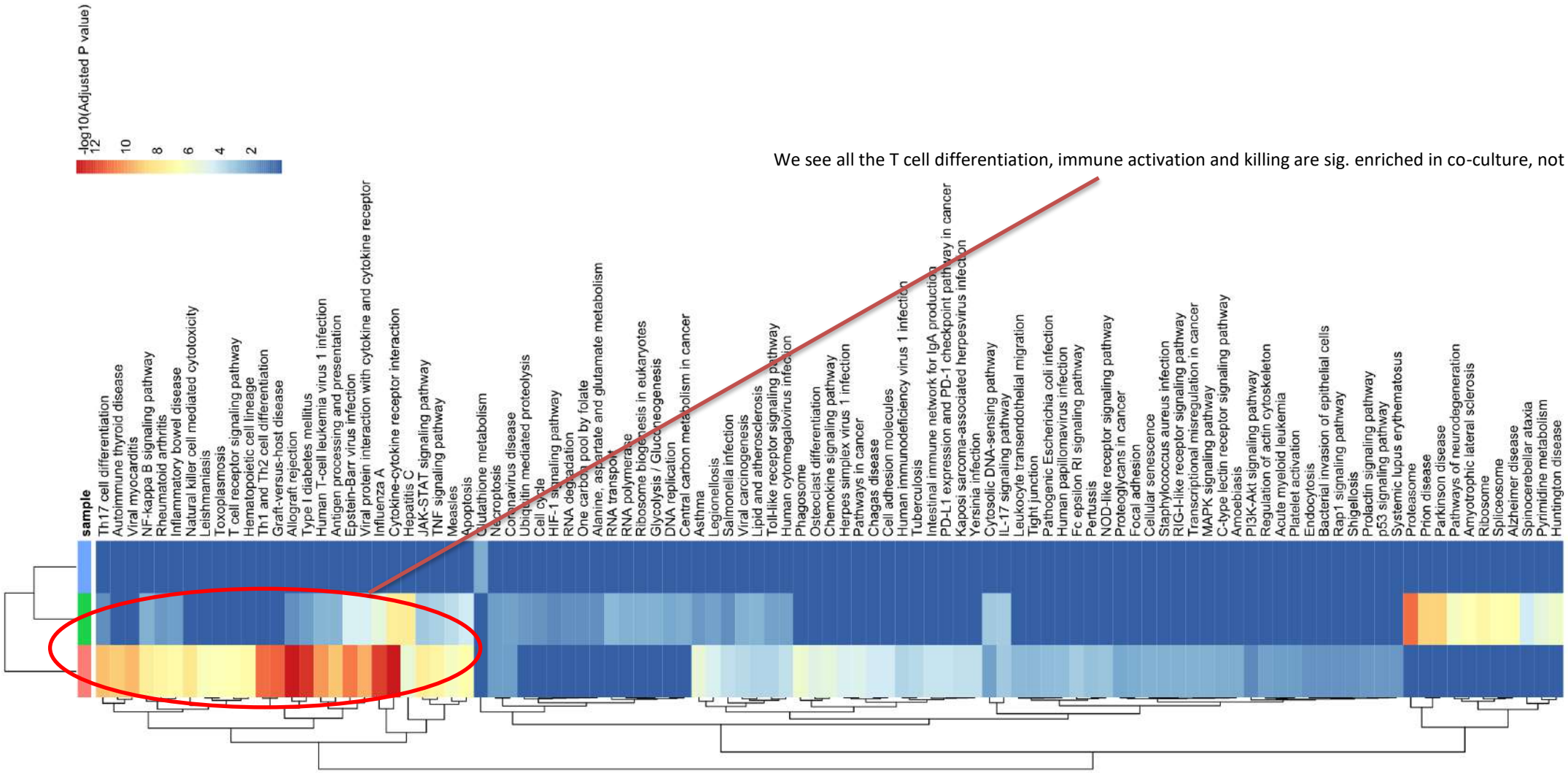


CAR-T high



CAR-T low

Co-culture identifies different expression signature



We see all the T cell differentiation, immune activation and killing are sig. enriched in co-culture, not much in CART alone.

Multi-OMICS up next..

Currently working on more immunology focussed assays, drugs and CRISPR

Develop the high throughput flow applications (Novocyte with Hudson plate crane), particularly liquid handling multiplexed staining

Developing assays with Metabolomics Australia (Bio21) to quantify metabolite signalling

Working with Mass Spectrometry and Proteomics (Bio21) to miniaturise

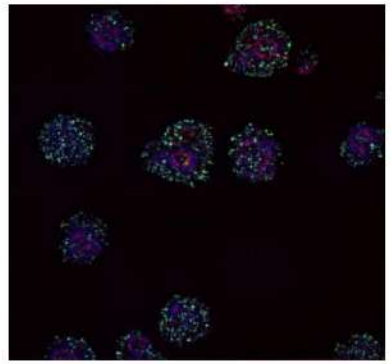
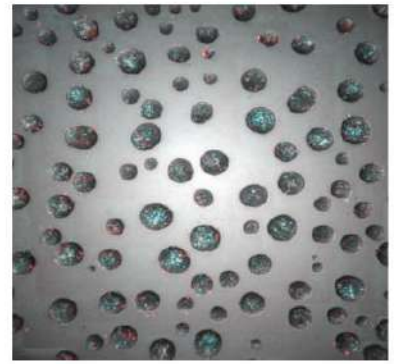
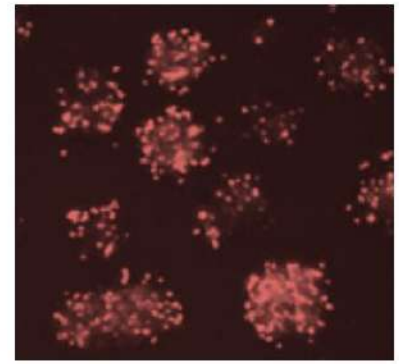
Data integration pipelines

VCFG team – an innovative and collaborative partnership

- Prof Kaylene Simpson – Head, project management, grant support, strategy
- Dr Susanne Ramm – 2IC, R&D lead, compound screening, imaging and analysis
- Dr Mark Li – 3D screen support, fee for service and analysis, tech development
- Karla Cowley – 2D data analyst, high content microscopy, IT/server liaison
- Dr AnnRann Wong – Data analysis, screen support, fee for service projects
- Xin Liu – MAC-seq, RNA-seq, spatial transcriptomics, joint with VCFG, MGC and Bioinformatics
- Hasan Quraishi – Integrating MAC-seq with high content screening, Masters of Data Analytics (Monash)
- Jennii Luu – Lab manager, automation specialist, screening method development
- Robert Vary – Equipment training, screen support, fee for service, CRISPR
- Dr Ada Koo – Assay development, high throughput metabolomics (Bio21), iLAB management
- Kavya Pamulapati – Equipment training, maintenance, screen support
- Dr Twishi Gulati – PA National service Coordinator (NCRIS), CRISPR, business development
- Louise Scerri - administration

Organoid Nexus 2024: High Throughput Innovation Meeting

When: November 7, 2024
Time: 8:45am arrival for 9am start, concludes at 5:15pm
Where: Bio21 Institute, 30 Flemington Rd, Parkville VIC
Attendance: In-person only
Cost: $27.50 per person (incl. GST)
Presentation EoI deadline: October 16, 2024
Registration deadline: October 30, 2024
Interstate travel awards available

Functional High Throughput Technologies AUSTRALIA