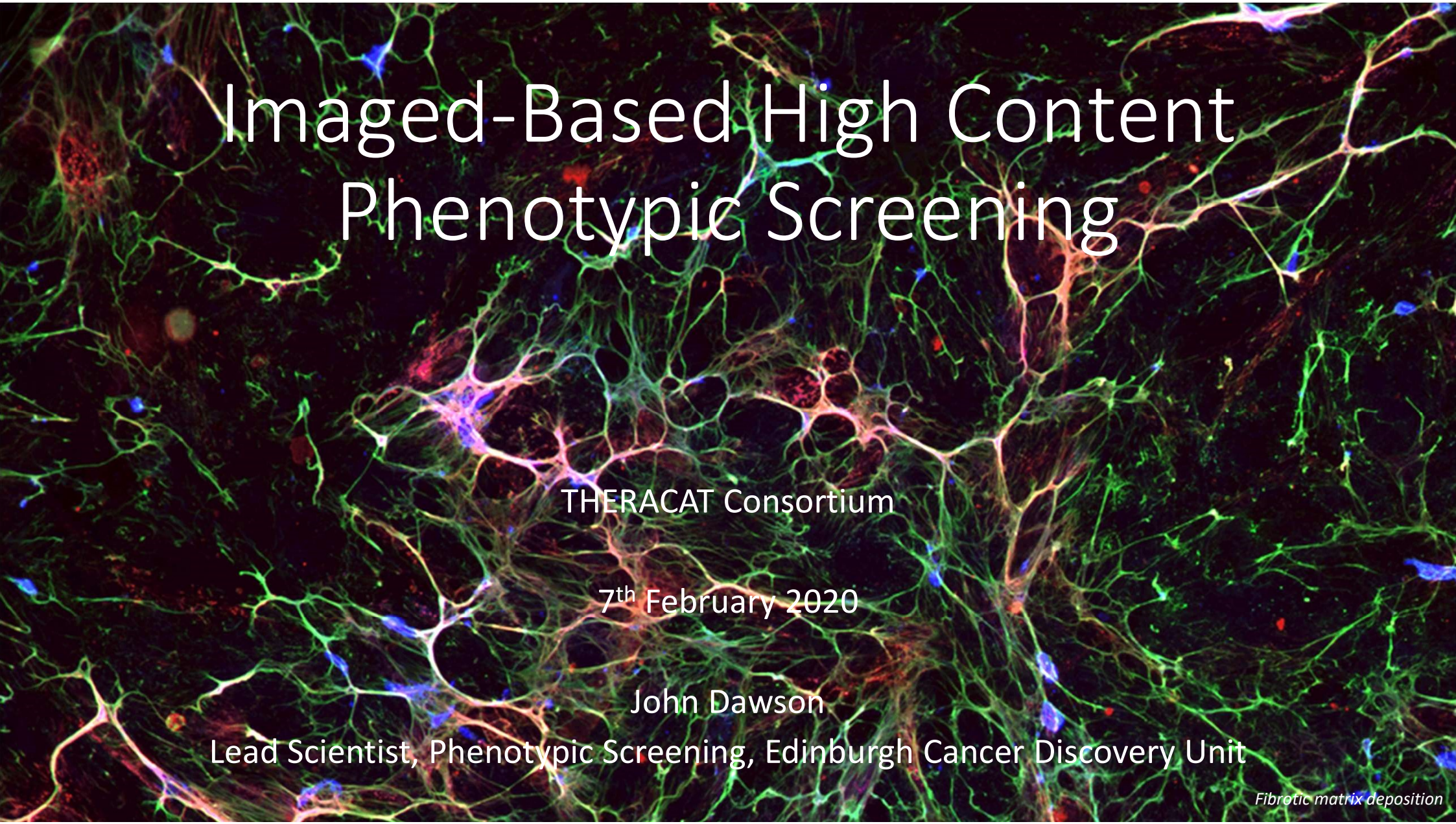
Imaged-Based High Content Phenotypic Screening



THERACAT Consortium

7th February 2020

John Dawson

Lead Scientist, Phenotypic Screening, Edinburgh Cancer Discovery Unit

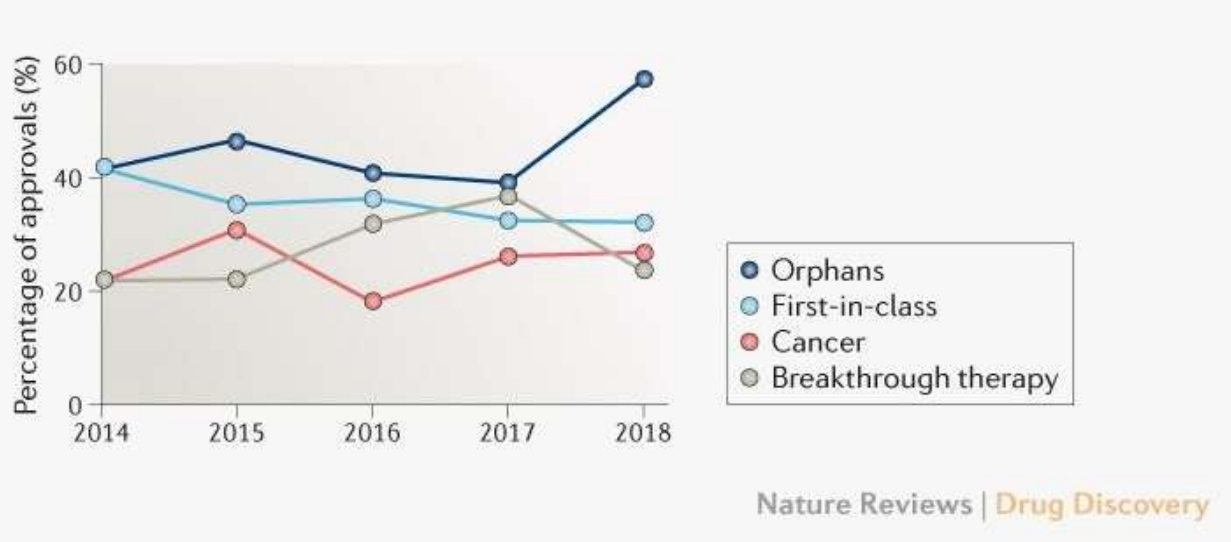
Fibrotic matrix deposition

Overview

1. Drug discovery challenges and why we use phenotypic assays

2. Imaging technologies advancing high-content analysis (HCA) and developments in model assays

3. Examples of phenotypic HCA assays.

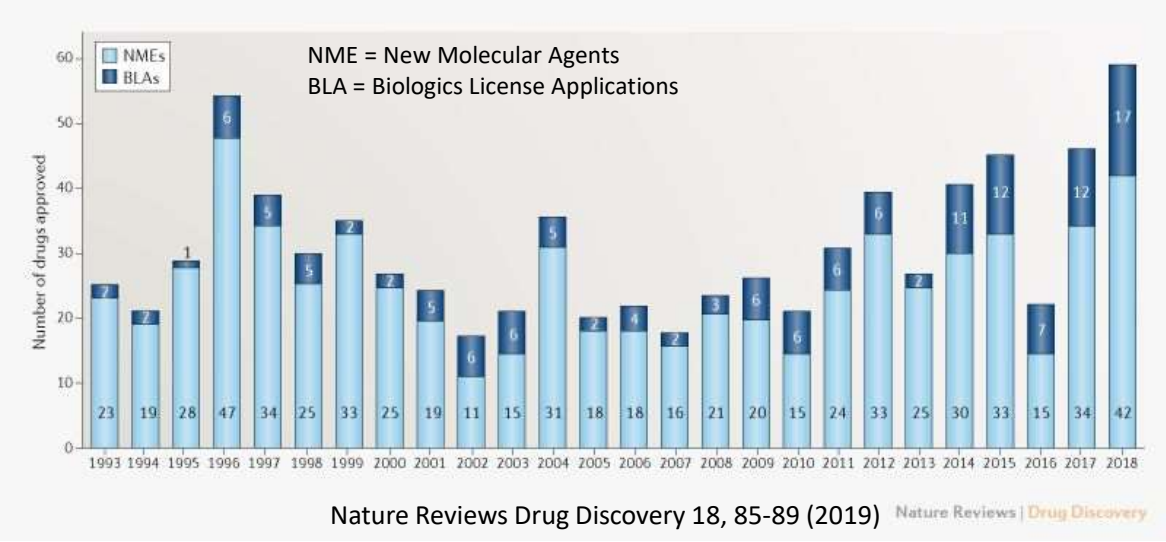


Nature Reviews Drug Discovery 18, 85-89 (2019)

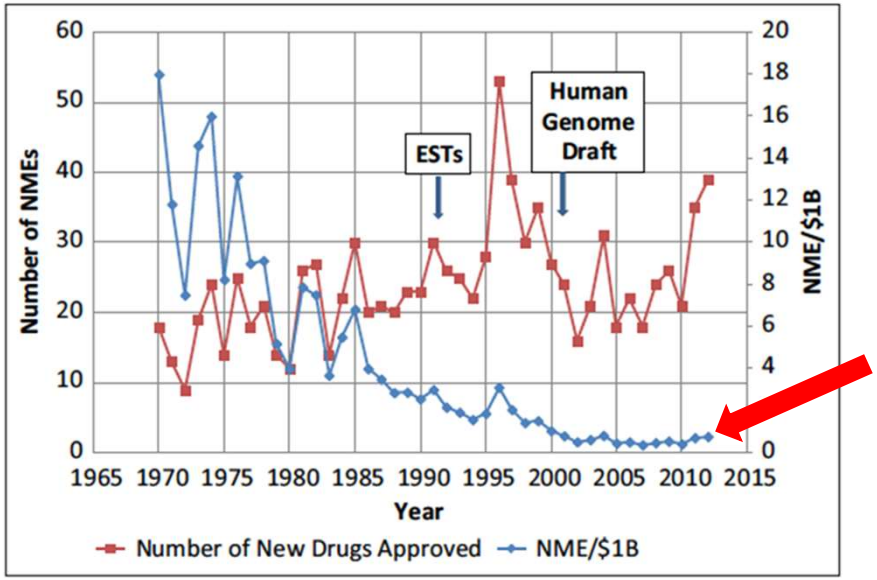
The rising cost of drug discovery

In 2016 a study calculated that it costs $2.558 billion to produce a new drug (*Journal of Health Economics Volume 47, May 2016, Pages 20-33)*

In 2018 a retrospective study of clinical trials between 2000-2015 of 21,143 compounds revealed that the highest three success rates were 32.6% for clinical studies of ophthalmology drug candidates, 25.5% for cardiovascular drug candidates, and 25.2% for infectious disease products. The lowest percentage came from oncology trials, at just 3.4%. *(Biostatistics, Volume 20, Issue 2, April 2019, Pages 273–286)*

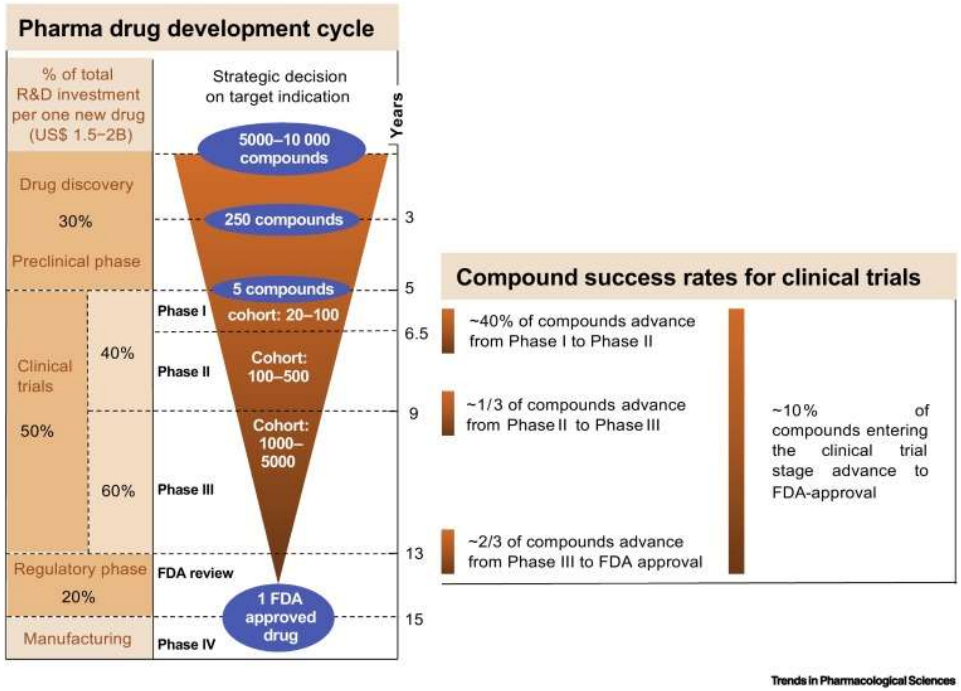


Nature Reviews Drug Discovery 18, 85-89 (2019)

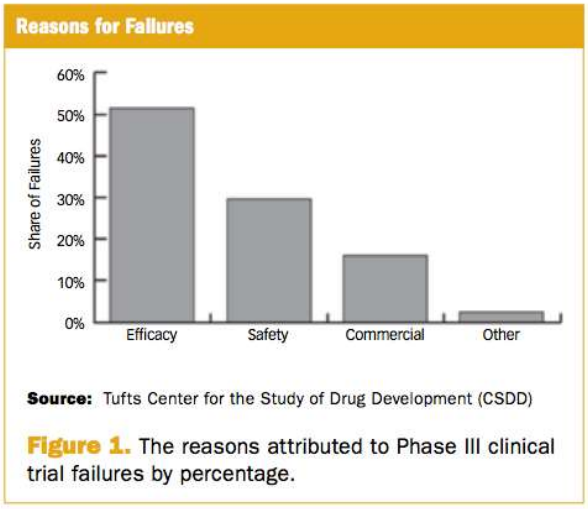


Adapted from: J Biomol Screen. 2013 18(10) 1143-1155

Reasons for clinical trial failure



Volume 40, ISSUE 8, P577-591, August 01, 2019



http://www.appliedclinicaltrialsonline.com/phase-iii-trial-failures-costly-preventable

It takes a long time to fail!

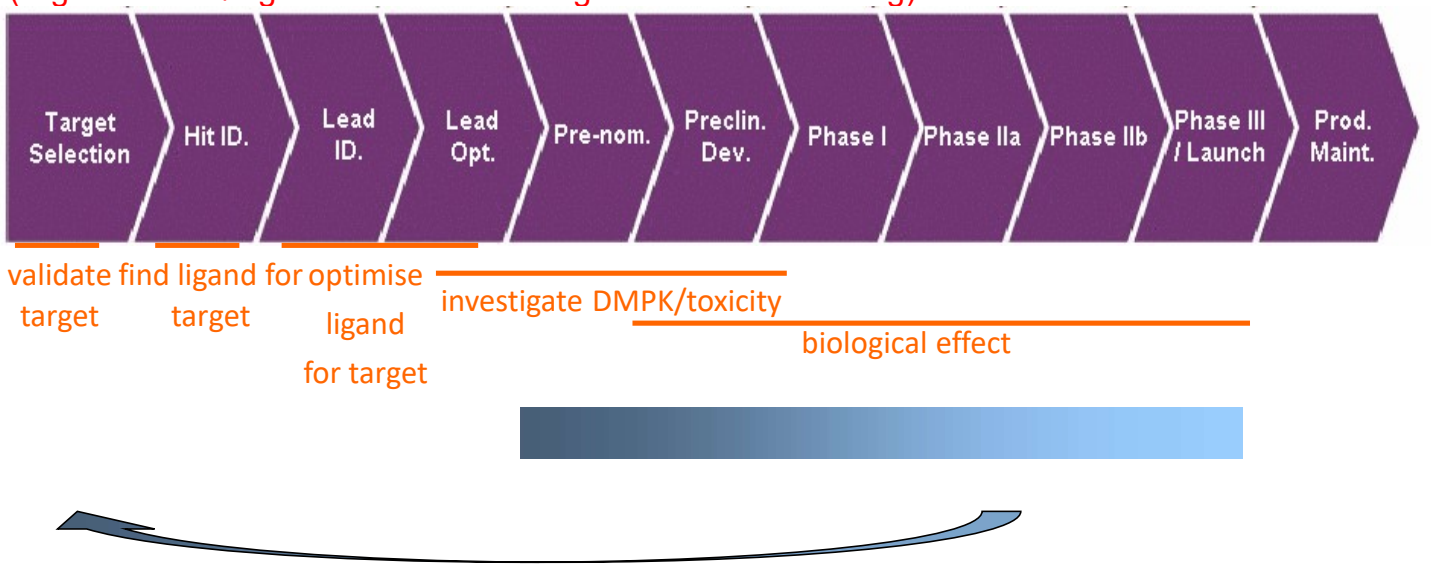
DPOM: Target-directed Drug Discovery (TDD): High Throughput Screening in target based drug discovery

It takes a long time to bring a candidate drug to preclinical testing

Phenotypic Drug screening: (drug discovery in reverse)
-just like the old days!

Problem: Current preclinical models of disease do not fully recapitulate clinical disease and therefore do not predict clinical efficacy.

Standard DPOM: Drug Project Operating Model
(High numbers/high attrition – most drugs fail in clinical testing)



Answer: We need earlier testing of compounds in biologically relevant assays.

This can be achieved by frontloading the biology by screening for candidate compounds using a phenotypic approach.

What is a phenotype?

"a set of observable characteristics of an individual resulting from the interaction of its genotype with the environment."

Why use phenotypes?

- Target agnostic; in contrast to target-based discovery.
  - *Useful for when diseases are poorly understood (no targets), are very heterogeneous (too many targets!) or targets are hard/complicated to drug.*
- Phenotypic screening has been successfully used for a long time and it can be better at finding "first-in-class" drugs (i.e. novel targets).
- Phenotypic screening is compatible with complicated, disease recapitulating models.
- Phenotypic screening can also work in tandem (and does in some companies) with current models of drug discovery.

Historical Drug Discovery Strategies:
Serendipitous Phenotypic Drug Discovery

(screening natural or synthetic chemical libraries in physiological based assays)

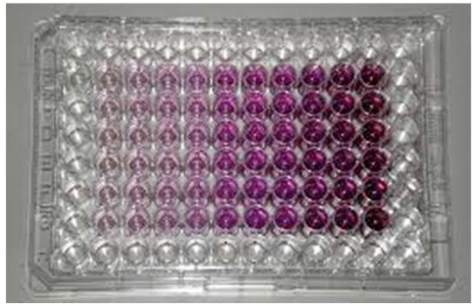
Animal models

Lianne and Bill Russell - Oak Ridge National Labs

https://www.ornl.gov/news/mammalian-genetics-pioneer-liane-russell-writes-mouse-house-history

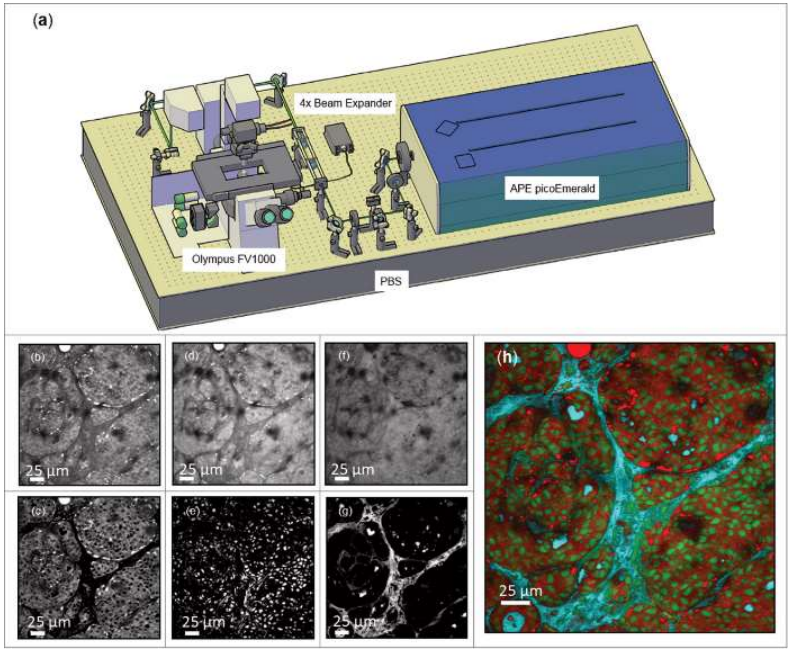
Eggs

Cell Viability



Why Use Phenotypes? – Biology is complicated

In vivo imaging of squamous cell carcinoma cells (green), collagen (blue) and red blood cells (red).

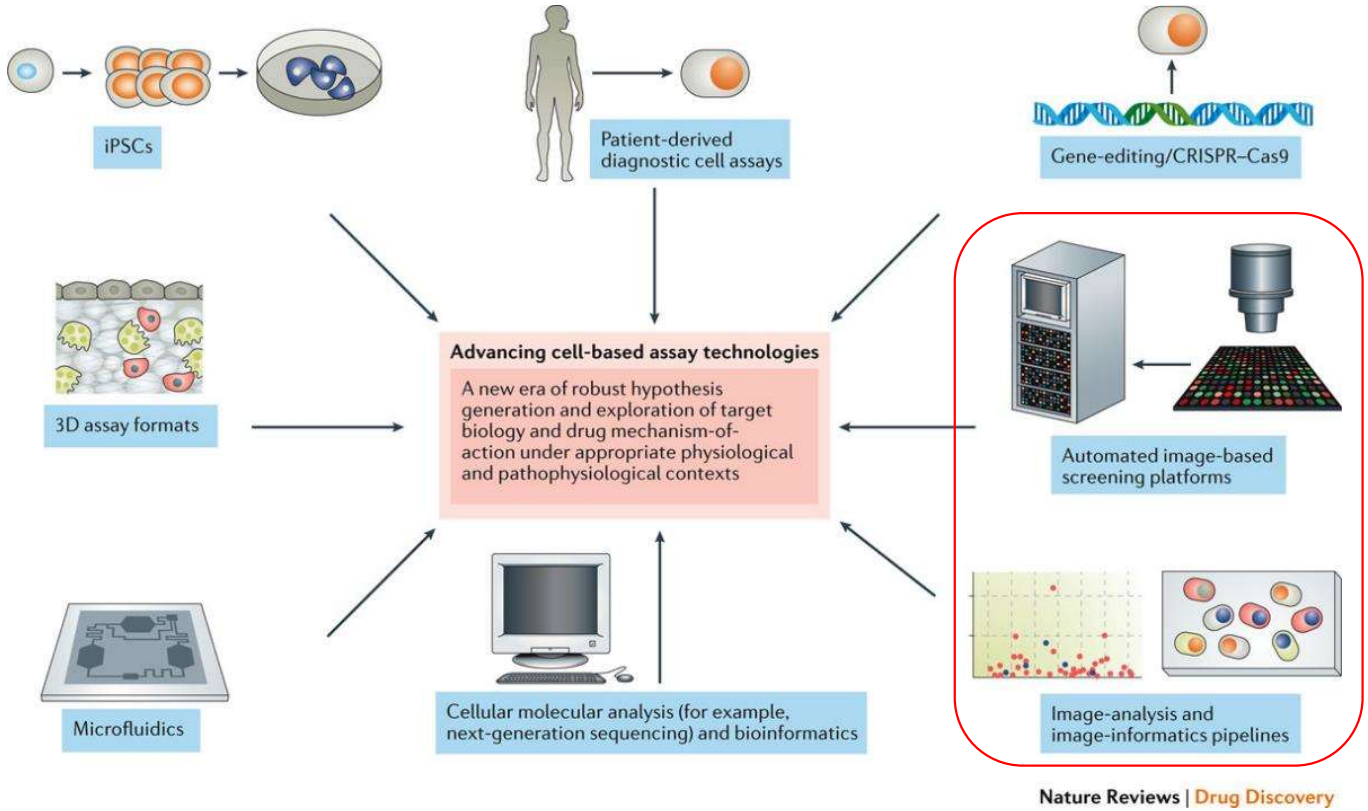


Multimodal imaging of cancer *in vivo*.

Lee et al., IntraVital Volume 4, 2015 - Issue 1

Are our disease models good enough?

- Recent advances in patient-derived primary cell models

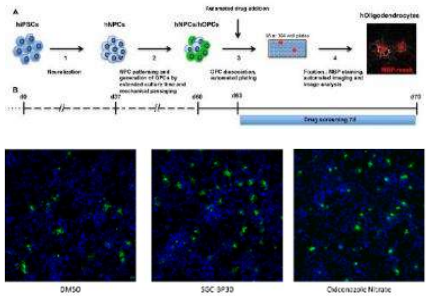


Horvath, P., Aulner, N., Bickle, M. et al. Screening out irrelevant cell-based models of disease. Nat Rev Drug Discov 15, 751–769 (2016) doi:10.1038/nrd.2016.175

Why Use Phenotypes? – Biology is complicated

Neurological

Screening for compounds promoting Oligodendrocyte differentiation using hiPSC cultures

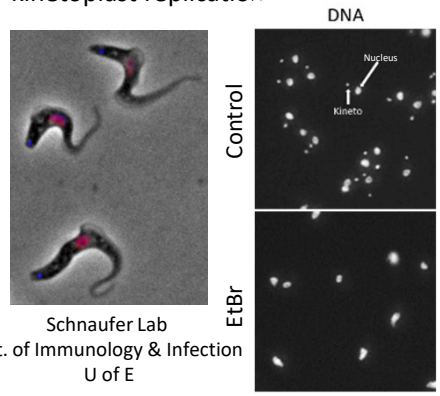


Dario Magnani/ Siddharthan Chandran
MRC Centre for Regen. Med. U of E

Outputs: $O4^+/MBP^+$ OLs, OL morphology

Infectious Disease

Inhibiting *Trypanosoma brucei* kinetoplast replication

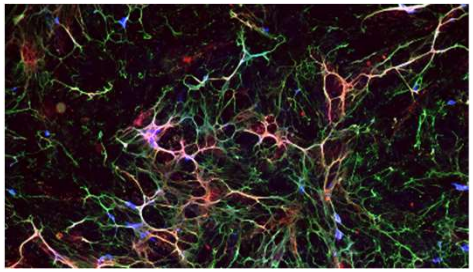


Schnaufer Lab
Inst. of Immunology & Infection
U of E

Outputs: Tryp viability, cell cycle stage and kinetoplast number

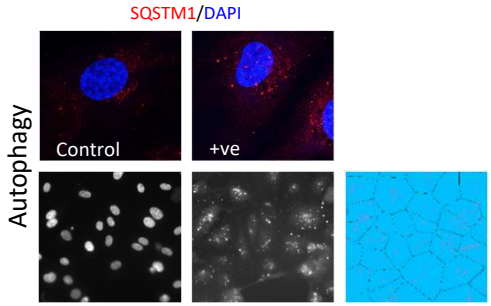
Respiratory

Fibrosis ECM deposition assay (Collaboration with UCB Pharma)



Outputs: cell viability, fibronectin, collagen I, III and IV

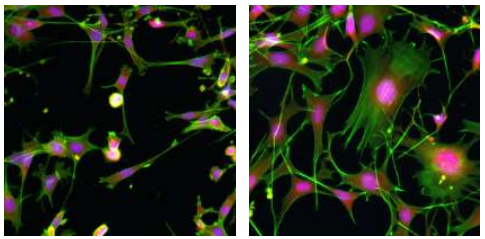
Cellular Processes

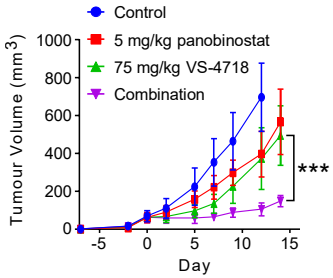


Andrea Martello Centre for Cardiovascular Science U of E

Outputs: Cell counts, autophagy puncta and localisation

Oncology

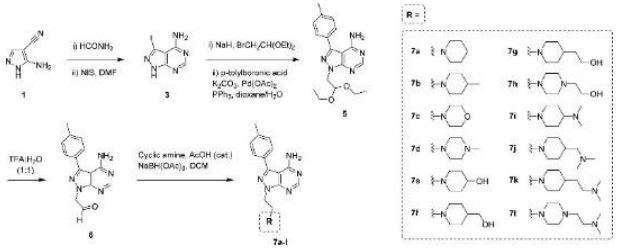


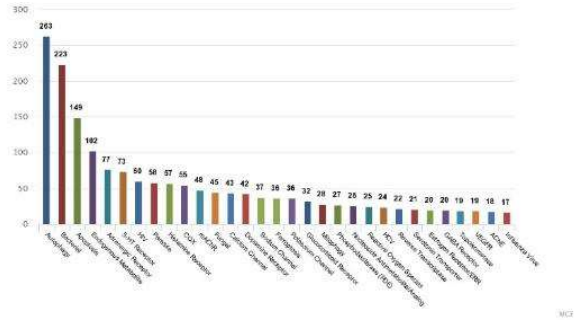


Outputs: Cell number, cell cycle and morphology

Edinburgh Cancer Discovery Unit Collaborations

What can be screened?



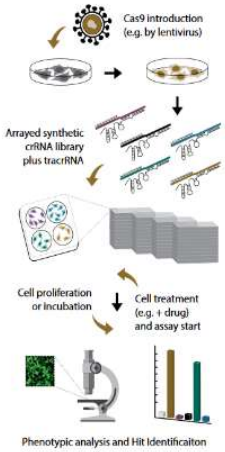


Small targeted chemical libraries (10s of compounds)

- Target can be known but not required for phenotypic screening.
- Can use very complicated phenotypes requiring difficult biological conditions
- Low throughput
- Very focused chemistry

Annotated compound libraries (10-1000s)

For example established compounds for repurposing or focused chemical probes sets



Large diverse chemical libraries (1000s, 10k+ compounds)

- Target can be known but not required for phenotypic screening.
- Harder to use very complicated biological conditions
- High-throughput
- Diverse chemistry

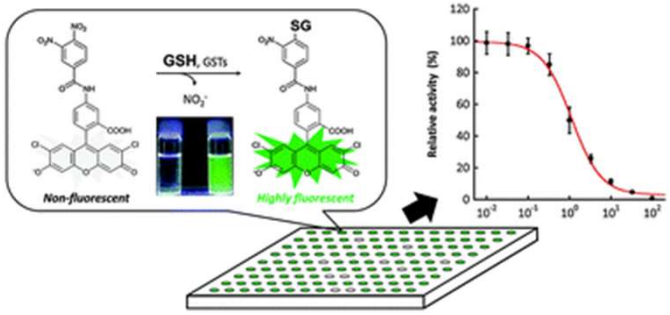
Genetic screens; CRISPR or RNAi (up to whole genome screens)

- No chemistry!
- Can search for novel targets
- Knockouts or knockdowns of genes is not equivalent to chemical inhibition.
- Can be difficult to introduce vectors into cells.

What do we mean by high-content phenotypic screening?

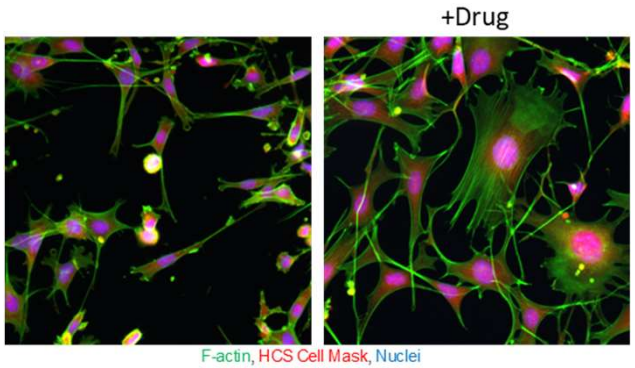
High-throughput screening

- Designed to assay thousands-millions of compounds rapidly in a (simple) **single** endpoint assay, e.g. inhibition of a target enzyme.



High-content screening

- Captures **multiple features** (high-content) per sample to quantify phenotypes.



Advances in technology enables - Edinburgh Phenomics Drug Discovery Capabilities

BIOCITY SCOTLAND

bio:ascent

(12k)120,000
FDA (1,280)
Annotated (>200)
Sub-libraries
(kinase/protease/Epigenetic/industry

Edinburgh Cancer Discovery Unit

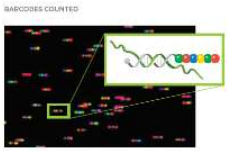
RPPA,Cytokine Arrays and NanoString
(antibody-based protein array and gene expression analysis)

RPPA and Cytokine arrays

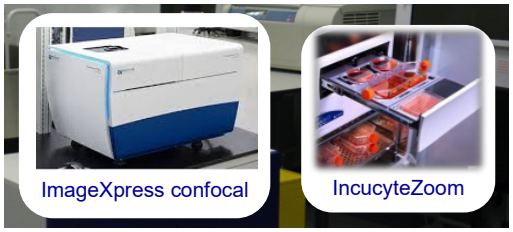
BioMek FX/NX

Nanoplotter 2.1E

nCounter Analysis System



High-content Image Acquisition and Analysis



ImageXpress-XL -PAA

IncucyteZoom

ImageXpress confocal

DEFINIENS
Understanding Images

CellProfiler
cell image analysis software

IGMM Image Database &
Local Compute Cluster: 1800 cores

THE UNIVERSITY of EDINBURGH
informatics

High-Content Screening Workflow

Plate Cells
[Genetic cell series]

Collagen I coated
384 well plates

Incubated
for 24 hours

Compound Addition

BioMek FX

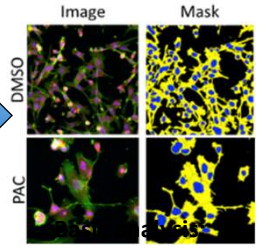
Incubated for
24 hours

Image Acquisition

ImageXpress

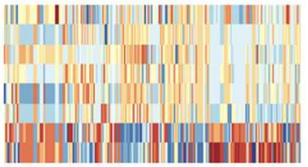
Cells fixed and labelled with
Hoechst, Phalloidin and HCS
Cell Mask

Image Analysis + Quantification



QC
Cell Number
Cell-Cycle Distribution

Data Processing
(multi-parametric
phenotypic analysis)



Clustering and classification of
similar / dissimilar compound
activity/cell phenotypes by
Principle Component Analysis

180 x 384 Well Plates:

16,896 Wells (1,216,512 images) – **large image sets (Tb's!)**

1,408 DMSO Wells

704 STS Wells

704 PAC Wells

144 Untreated Wells

Quality Control

Cell QC

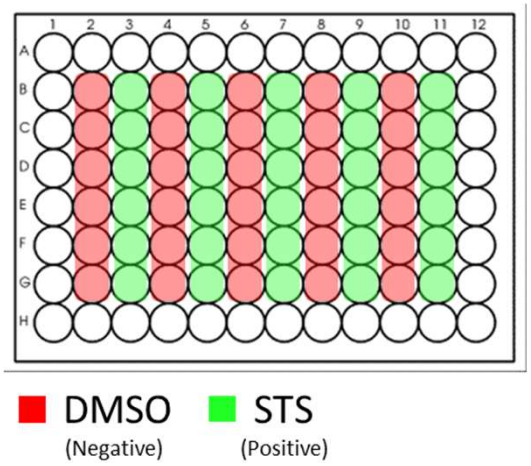
- Healthy cells free of mycoplasma
- Cells need to look and be growing normally
- Try to screen within a set number of passages

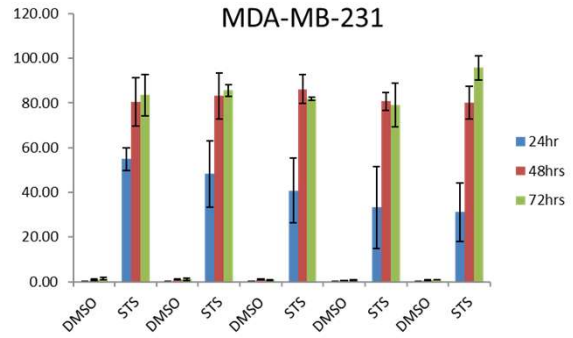
Image QC

- Identify any images that are out of focus or contain debris
- During acquisition correct for uneven illumination (can be done on the images as well)

Assay QC

Assay robustness is measured with positive and negative controls.



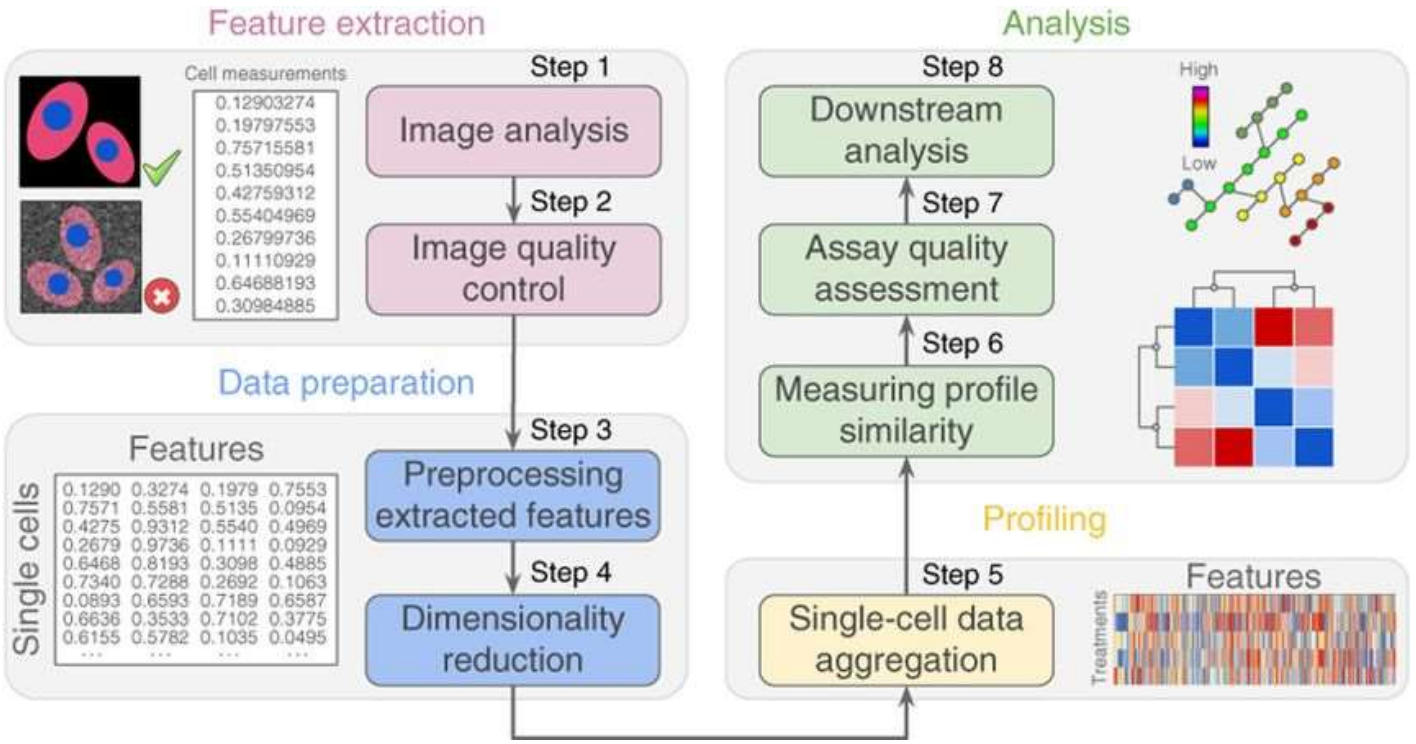


Z-Factor	24Hrs	48Hrs	72Hrs
MDA-MB-231	0.14	0.72	0.694253
HCC1569	-0.57	0.35	0.774934

Note that if σp = σn, 0.5 is equivalent to a separation of 12 standard deviations between μp and μn

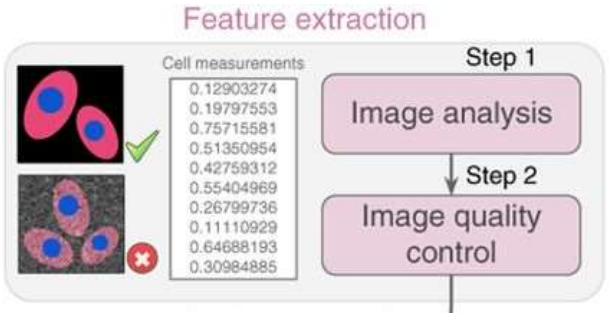
Z'-factor	Interpretation
1.0	Ideal. Z-factors can never exceed 1.
between 0.5 and 1.0	An excellent assay.
between 0 and 0.5	A marginal assay.
less than 0	There is too much overlap between the positive and negative controls for the assay to be useful.

Typical Analysis Work Flow – Data Analysis

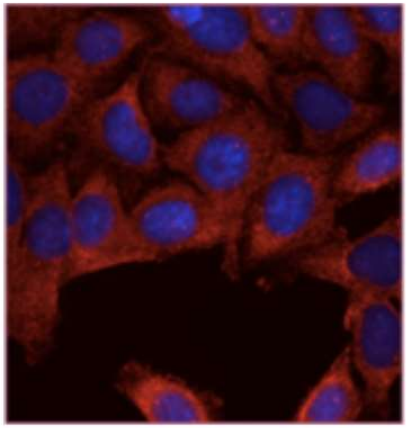


Nature Methods **volume 14**, pages 849–863 (2017)

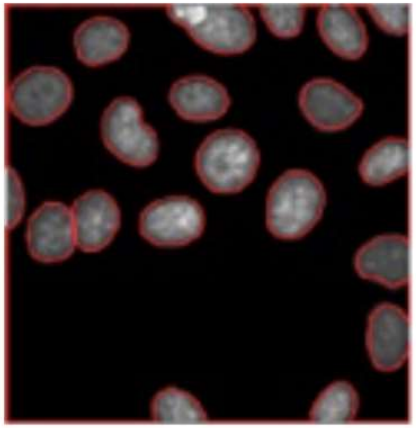
Describing the Phenotype – Image Analysis

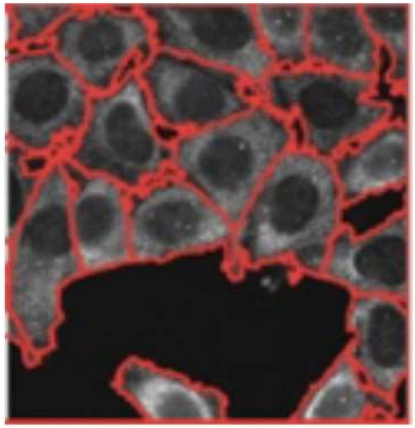


Original image:



Processed images:

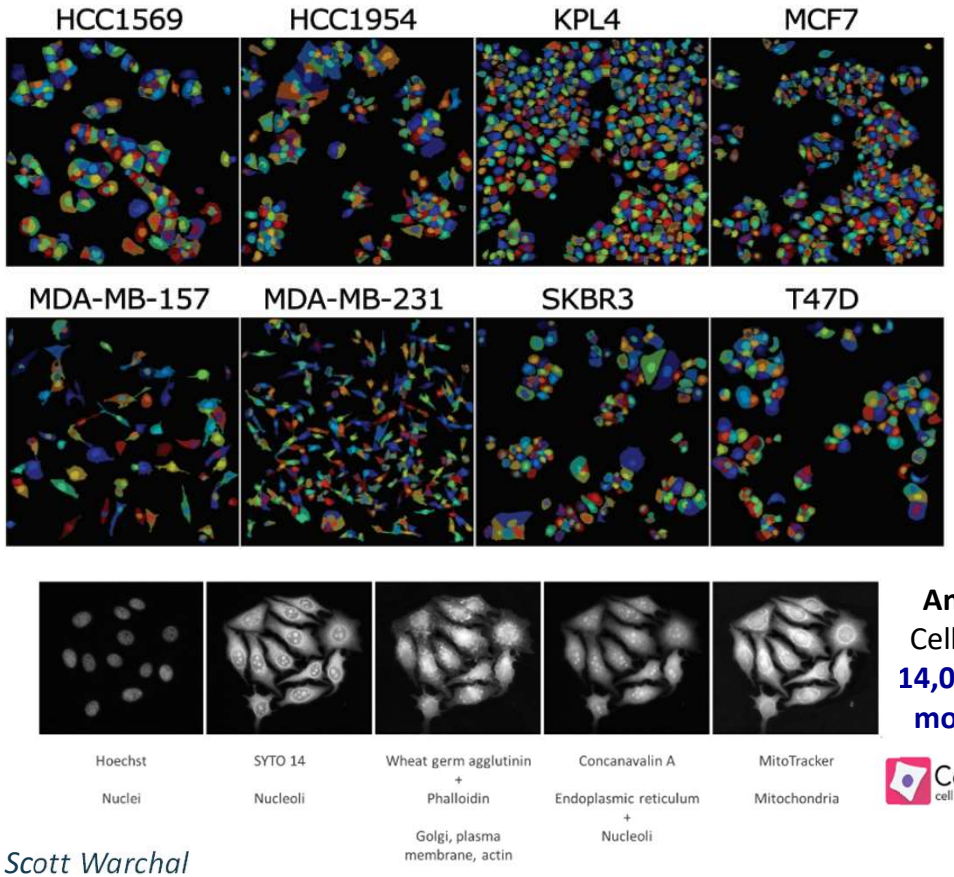




https://clue.io/connectopedia/cell_painting_features

Describing the Phenotype – Image Analysis

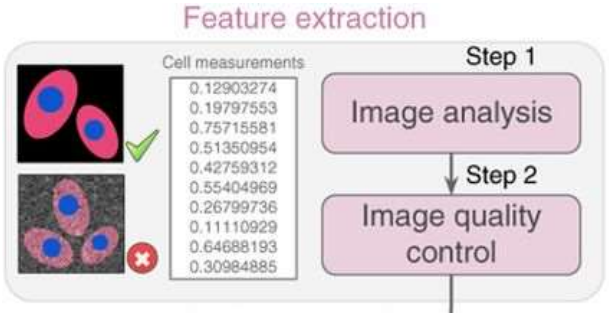
CellPainting - Unbiased phenotypic profiling



Analysis:
CellProfiler
14,000 small molecules

CellProfiler™ cell image analysis software

Scott Warchal



Multiparametric phenotypic profiling

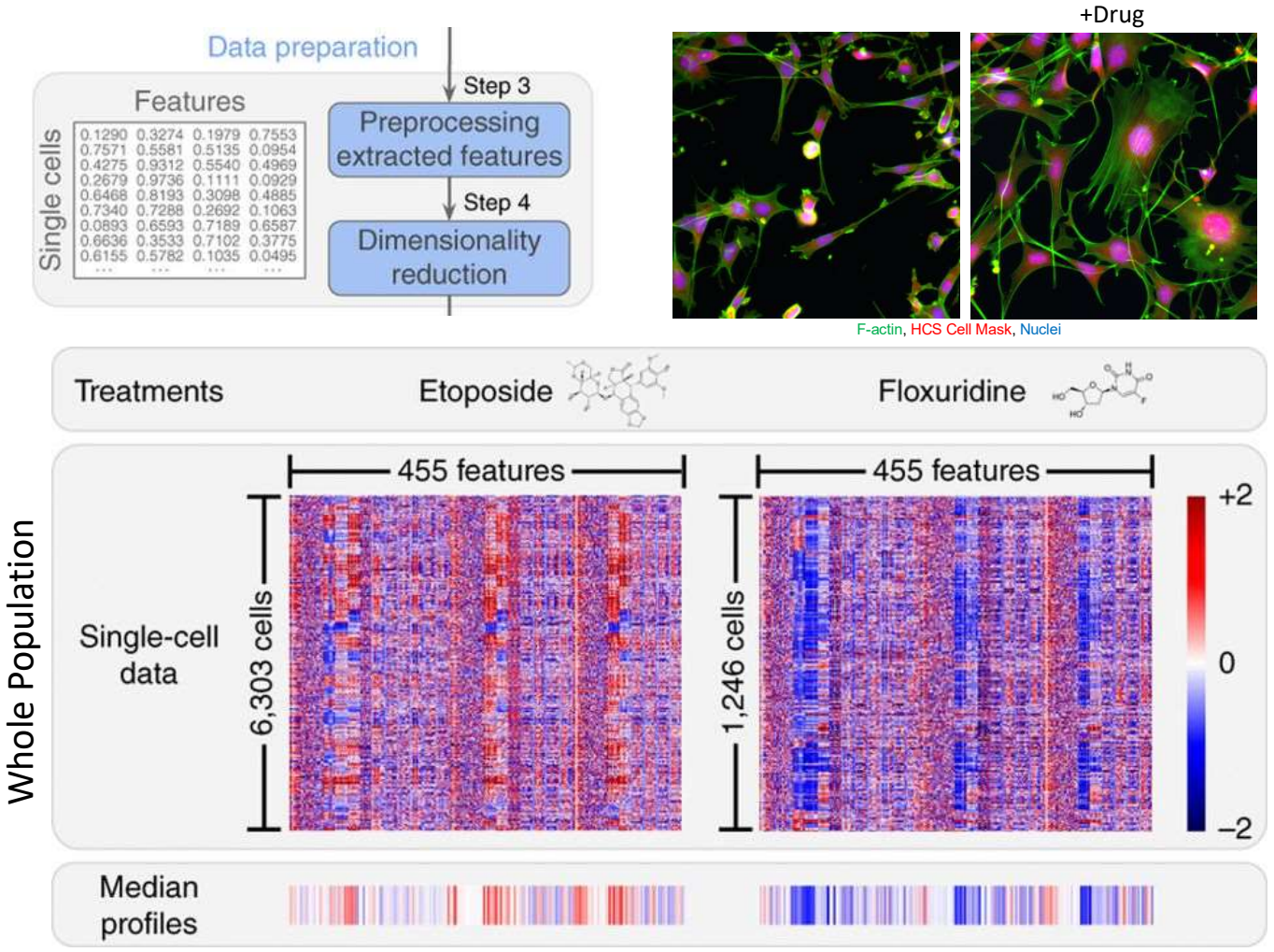
Channel	n
DNA	355
AGP	387
ER	387
Mito	387
RNA	387

Compartment	n
Cells	596
Cytoplasm	582
Nuclei	605

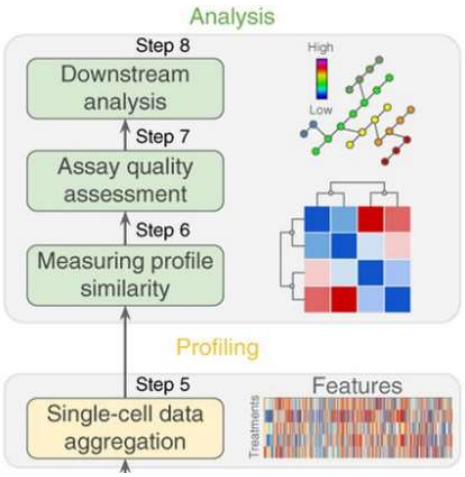
FeatureGroup	n
AreaShape	144
Correlation	300
Granularity	208
Intensity	225
Location	66
Neighbors	21
RadialDistribution	180
Texture	630

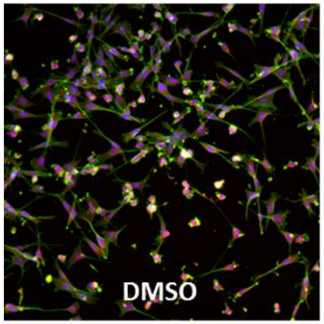
https://clue.io/connectopedia/cell_painting_features

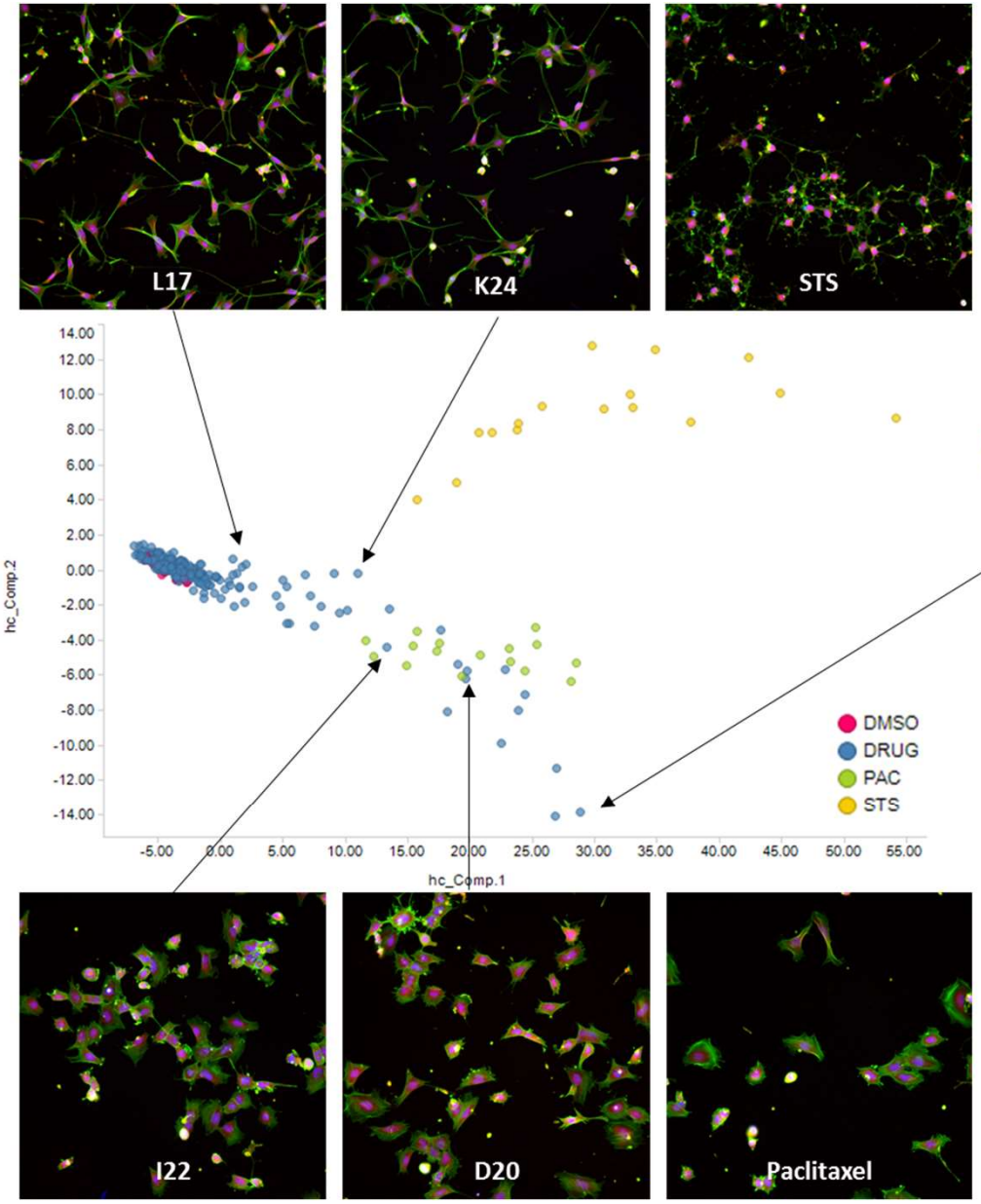
Describing the Phenotype – Data Processing



Describing the Phenotype – Data Profiling and Analysis

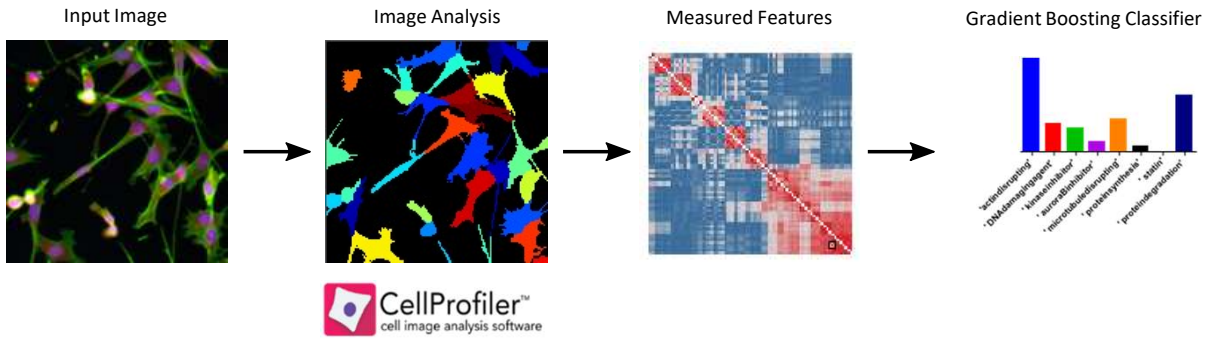




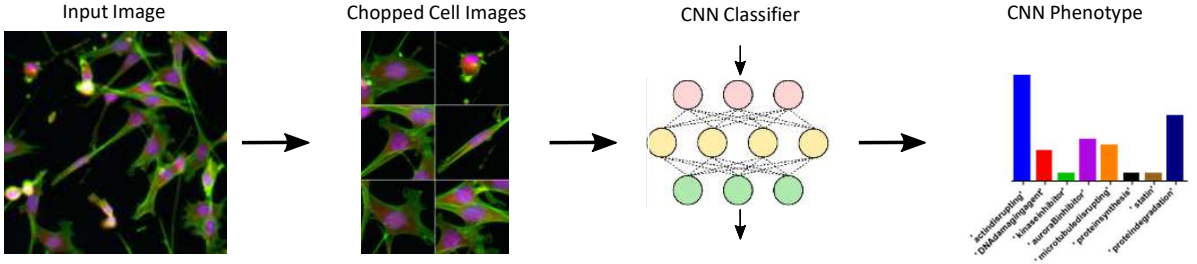


Describing the Phenotype – Data Analysis

i Image analysis algorithm segmentation and feature extraction; ensemble based tree classifier
User defined set of features to describe a phenotype.



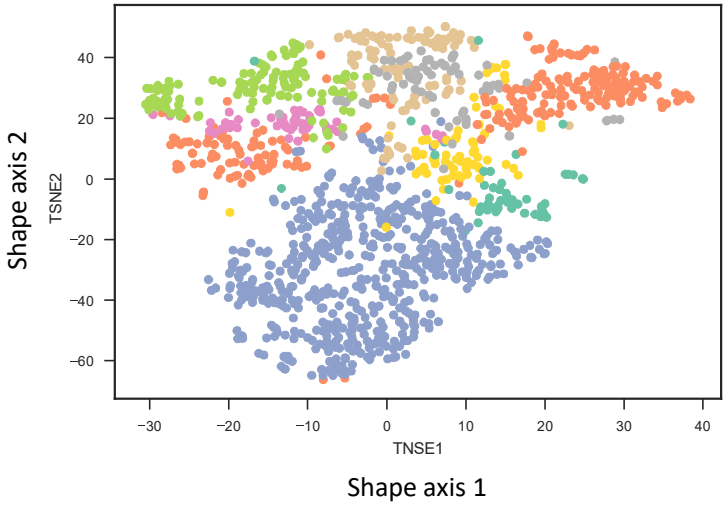
ii AI: Deep learning on raw image (300x 300 pixels) data; convolution neural network classifier (cNN)
AI learns features that describe phenotype.



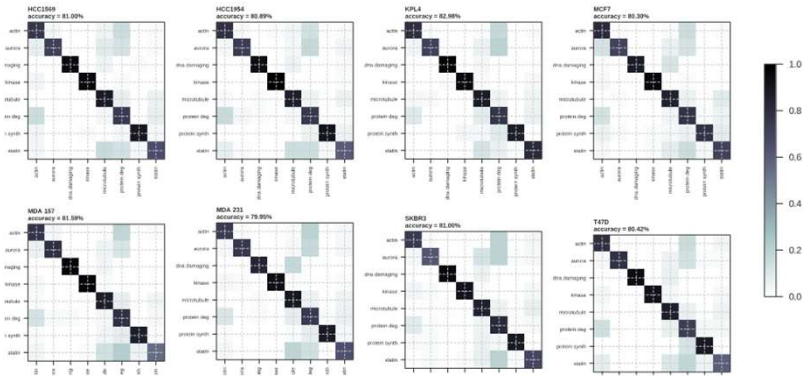
Warchal et al., 2019

Describing the Phenotype – Data Analysis

Compound Clustering



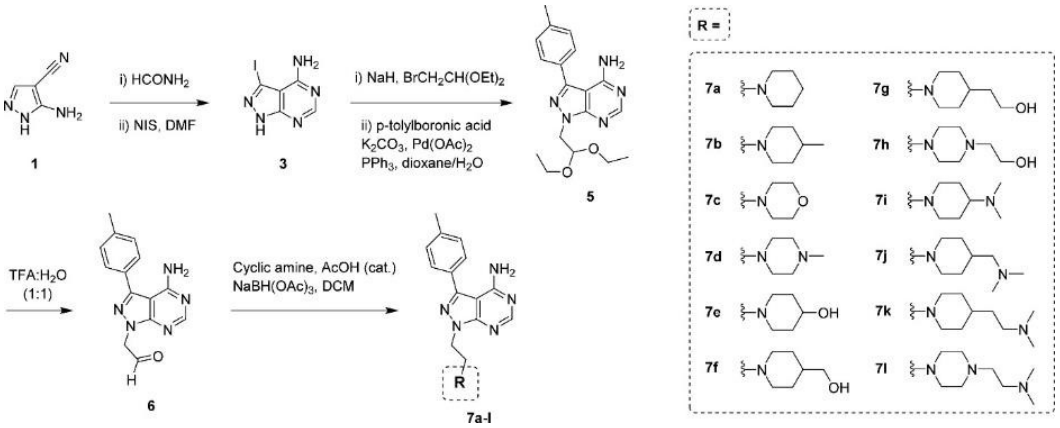
Compound MOA Prediction

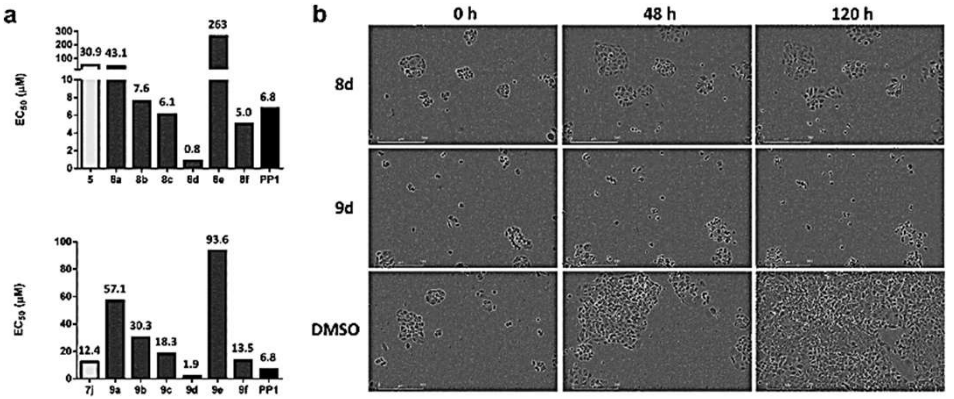


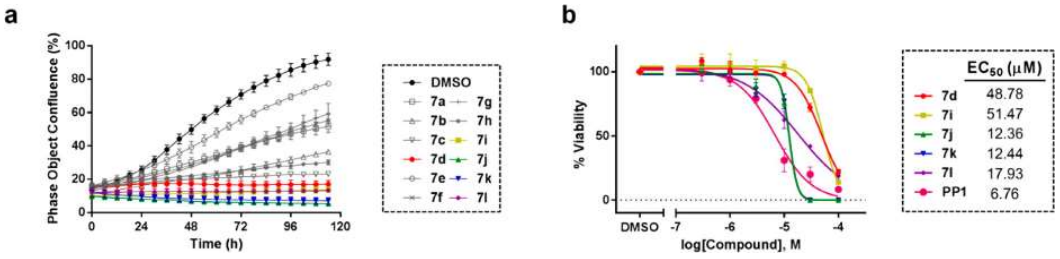
Take novel compounds and compare their fingerprints to those of known drugs

Warchal et al., 2019

1. Screening of small targeted-compound libraries – Src kinase inhibitors in breast cancer





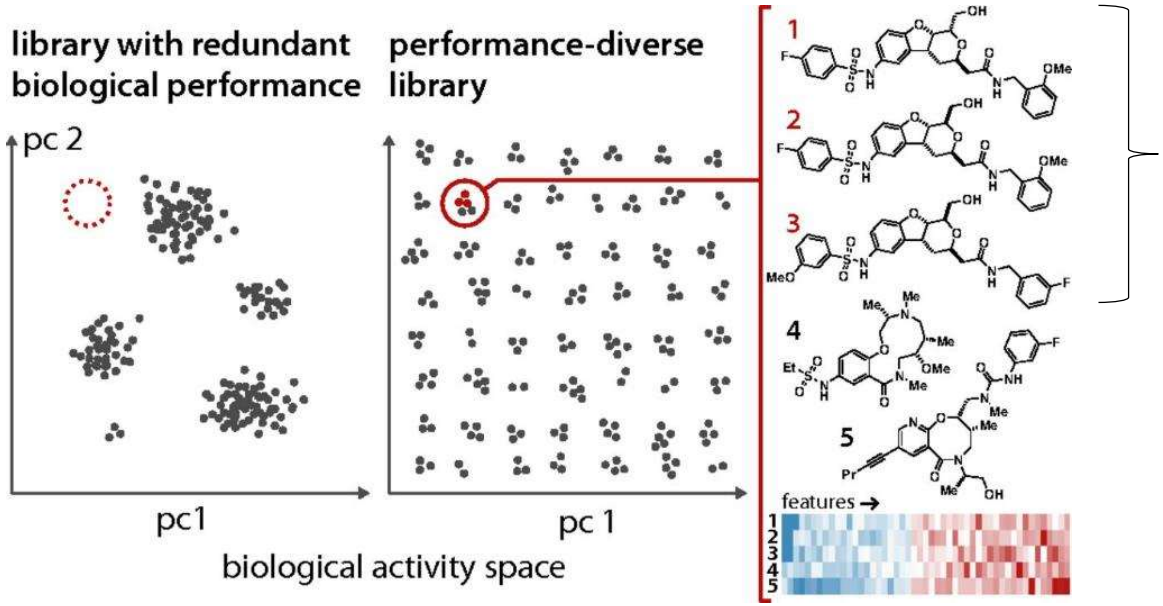


Simple phenotypic readouts to progress active compounds

- Kinetic growth (confluence)
- Cell Viability

Fraser et al., *J. Med. Chem.* **2016,** 59, 4697-4710.

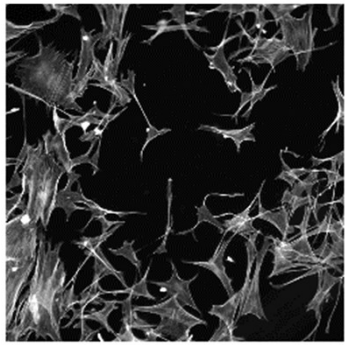
1. Using chemical diversity libraries to identify novel inhibitor starting points.

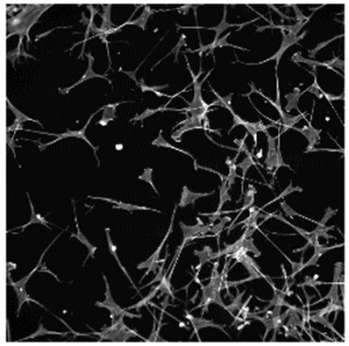


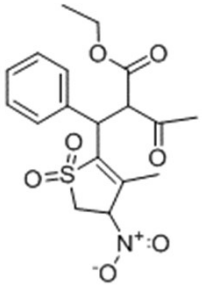
PNAS July 29, 2014 111 (30) 10911-10916

Chemical diversity libraries covers a wider range of chemistry and, potentially, more biology

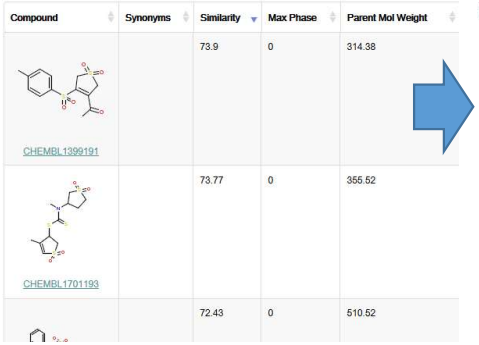
1. Chemical diversity library screening: Chemi-informatics

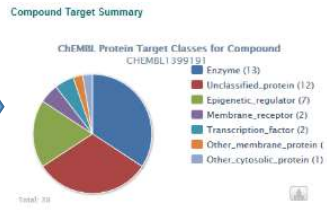




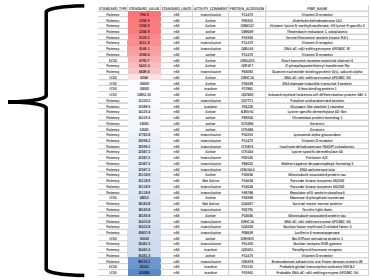


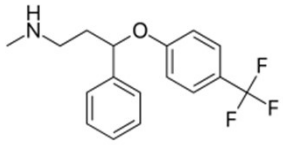
Structure similarity search on ChEMBL





Published Screening Data

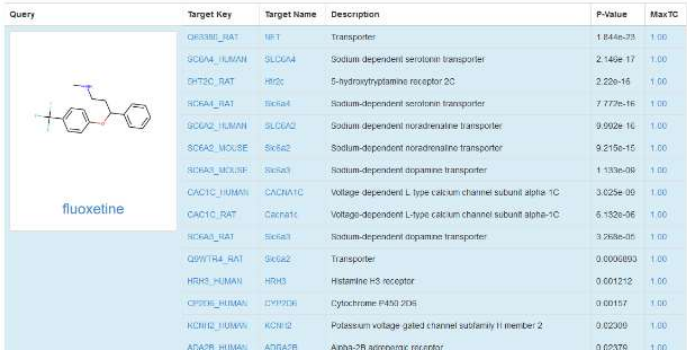




SEA

SEA relates proteins based on the set-wise chemical similarity among their ligands. It can be used to rapidly search large compound databases and to build cross-target similarity maps.

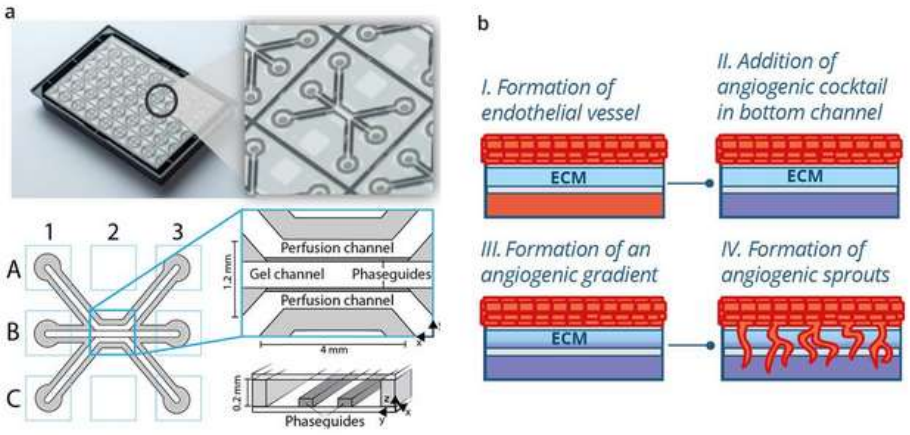
Predict targets

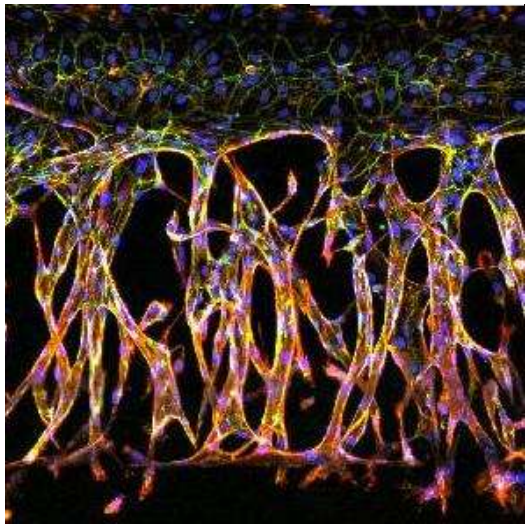


Keiser MJ, Roth BL, Armbruster BN, Ernsberger P, Irwin JJ, Shoichet BK. Relating protein pharmacology by ligand chemistry. Nat Biotech 25 (2), 197-206 (2007).

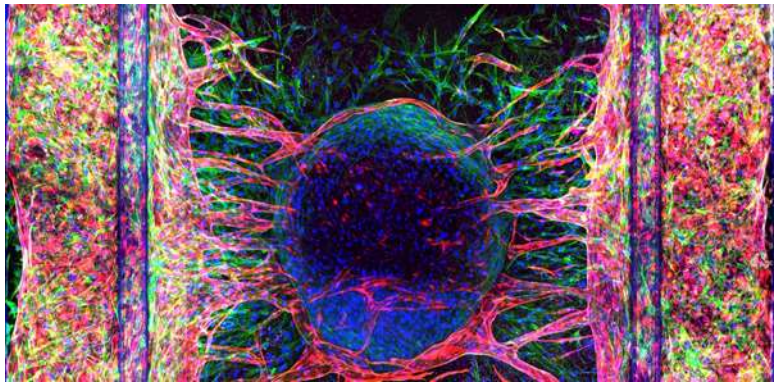
**other chemical similarity tools are available*

5. Miniaturised Complex 3D Assay Formats





Angiogenesis



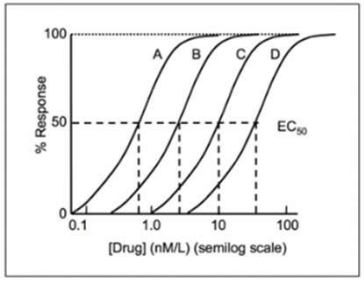
Vascularized tumour spheroids

MIMETAS
the organ-on-a-chip company

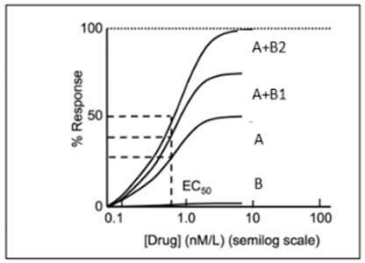
6. Combination drug screening

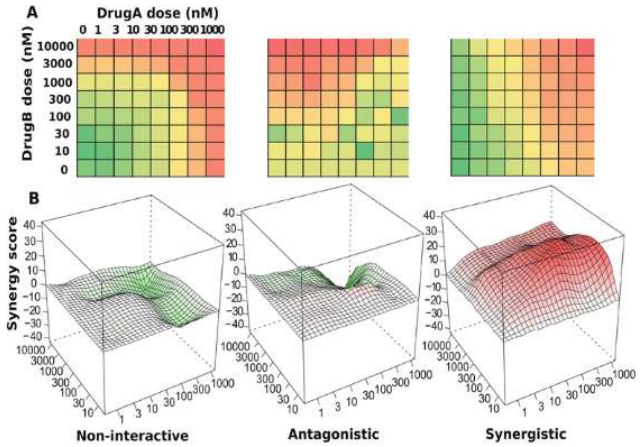
Chemical-chemical screening strategy

Potency

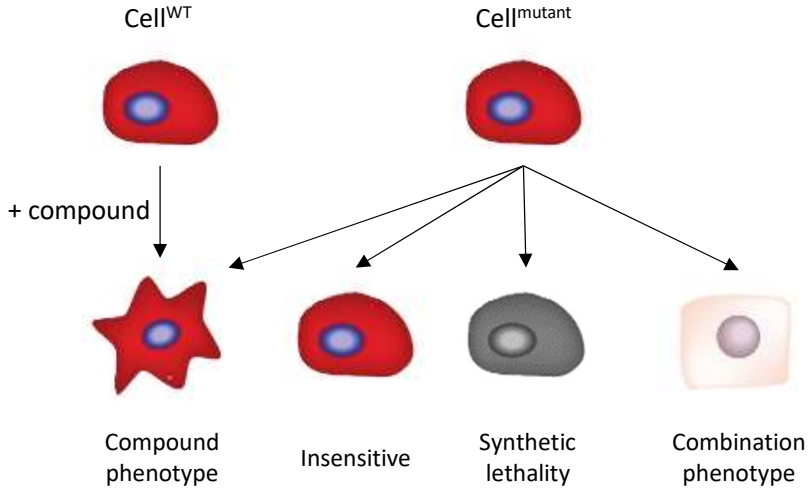


Potentiation



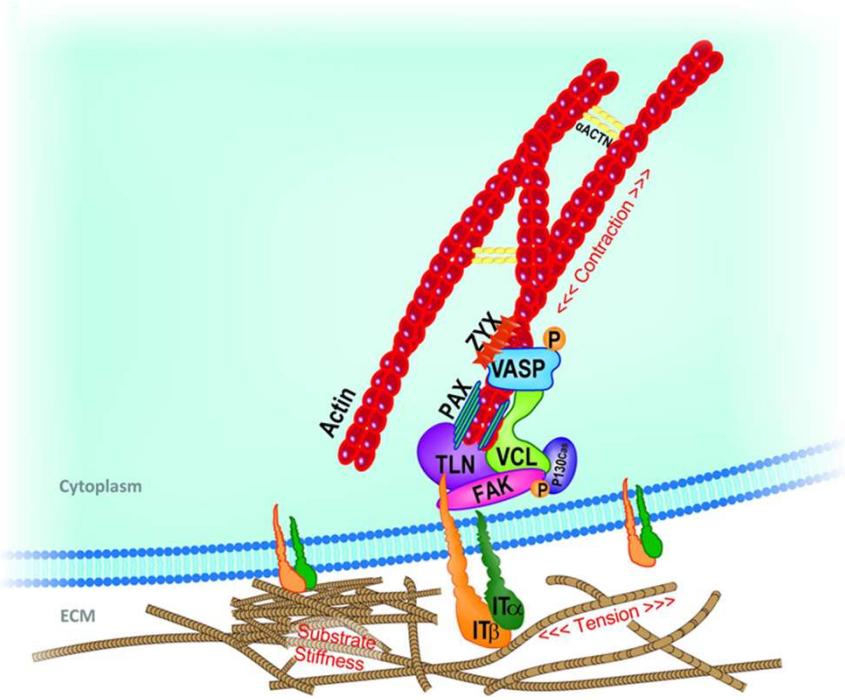


Chemical-genetic screening strategy



Multiple combinations becomes technically challenging especially with large dose matrix setups.

Focal Adhesion Kinase (FAK)



SCC FAK-Wild type

SCC FAK-G431A, F433A (CDC37 chaperone mutant)

SCC FAK-Kinase dead

SCC FAK-deleted

FAK Combination Phenotypic Screen Summary

Aim – To identify compounds that synergise with a FAK kinase inhibitor using a genetic kinase dead.

Plate Cells

SCC FAK-WT

SCC FAK-G431A, F433A

SCC FAK-KD

SCC FAK -/-

Incubated for 24 hours

Compound Addition

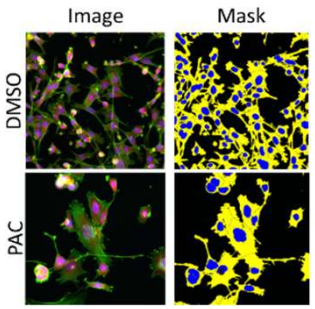
Incubated for 24 hours

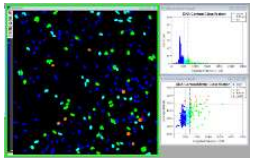
Image Acquisition

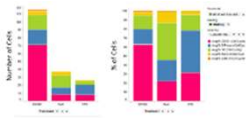
ImageXpress

Cells fixed and labelled with Hoechst, Phalloidin and HCS Cell Mask

Image Analysis & Quantification



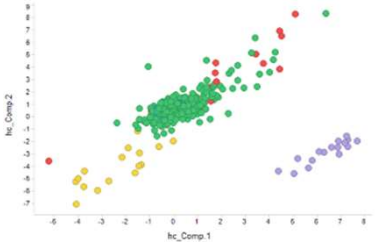




CellProfiler™ cell image analysis software

MOLECULAR DEVICES

Data Processing



- Median or Average Well Measurements
- QC
- Median normalised per plate.
- Z-score normalised.
- Feature reduction.
- Differential responses between FAK mutants.
- Data analysis & clustering.

13,936 Compounds Tested

Annotated Compounds

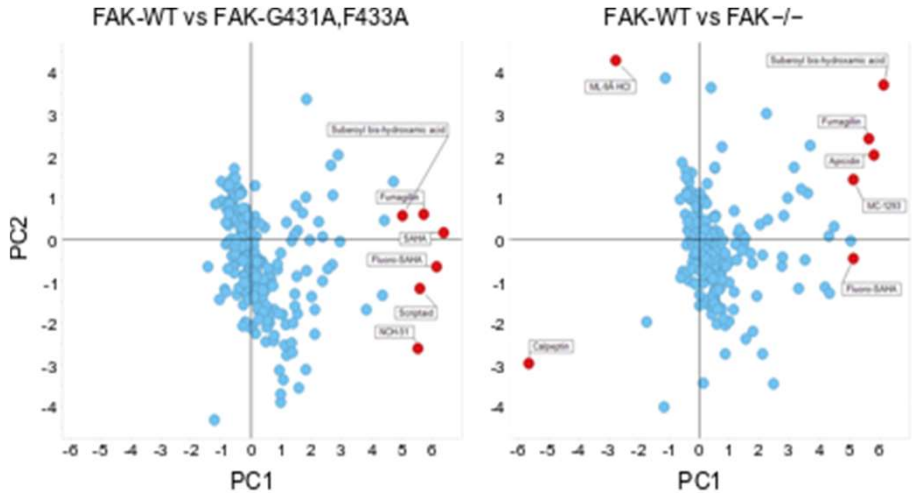
- 80 Kinase Inhib.
- 53 Protease Inhib.
- 43 Epigenetic Inhib.
- 1,280 FDA Approved Agents

→ Proof of principal combination example

Targets unknown

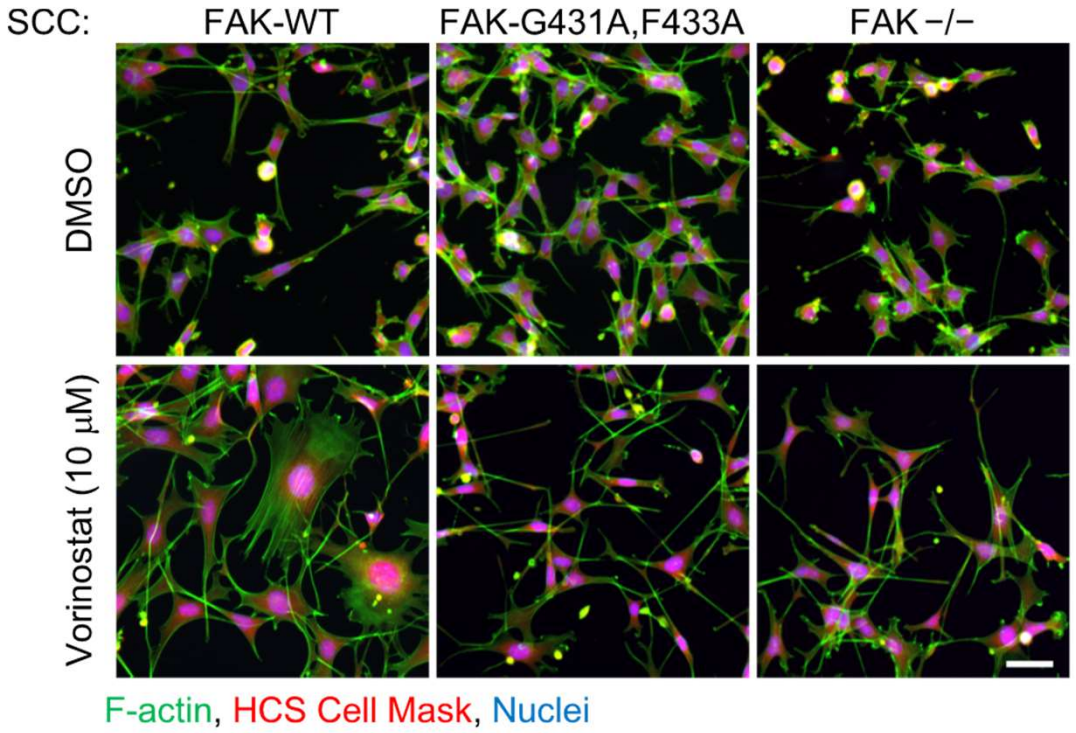
- 12K Bioascent Chemical Diversity Library (123 hit compounds)

Protease, Kinase and Epigenetic (PKE) annotated tool compound library (176 in total).

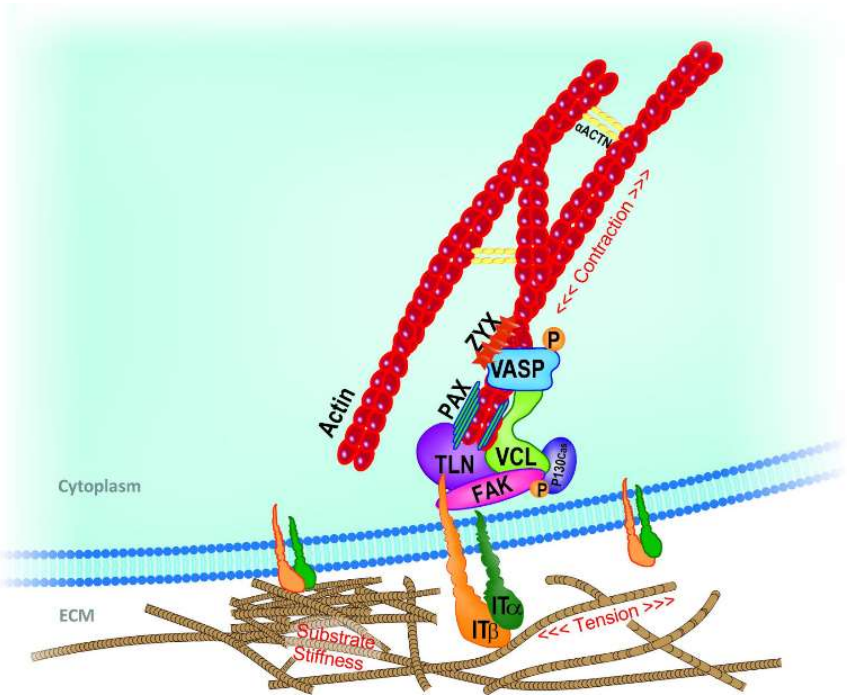


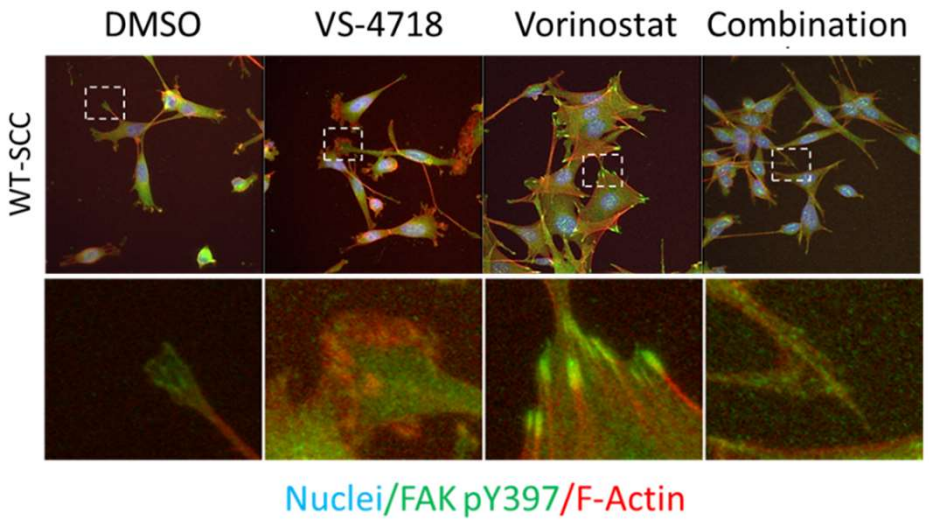
Chemical Name	Hit Score	Target	Cell Line
Suberoyl bis-hydroxamic acid	3.5	HDAC	−/−
Calpeptin	3.5	Calpain; Cathepsin L	−/−
SAHA	2.8	HDAC	G431A
Fumagillin	2.8	Met AP2	−/−
Apicidin	2.7	HDAC	−/−
ML-9A-HCl	2.7	MLCK	−/−
Fluoro-SAHA	2.7	HDAC	G431A
NCH-51	2.6	HDAC	G431A
Fumagillin	2.4	Met AP2	G431A
Scriptaid	2.4	HDAC	G431A
MC-1293	2.2	HDAC	−/−
Fluoro-SAHA	2.0	HDAC	−/−
Suberoyl bis-hydroxamic acid	2.0	HDAC	G431A

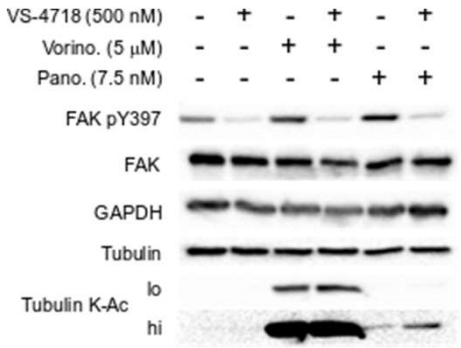
Proof of principle example – Histone deacetylase inhibitors

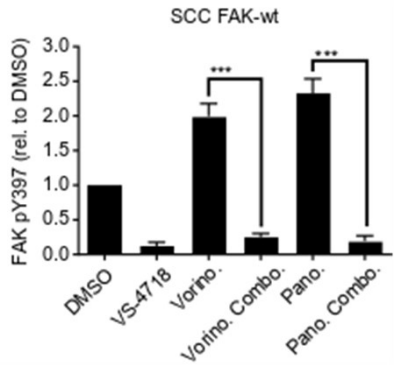


SCC model – HDAC combination with the FAK kinase inhibitor VS4718







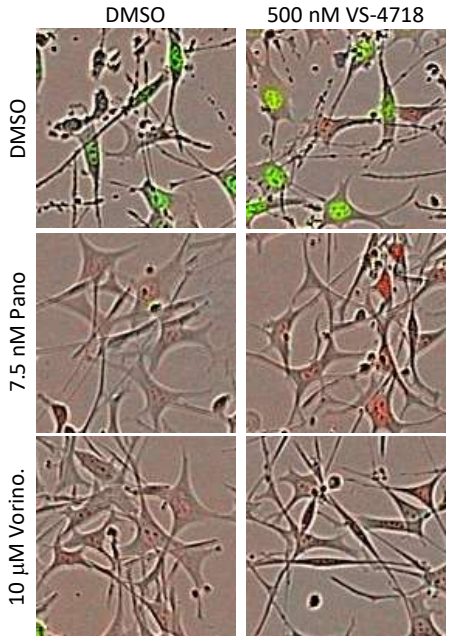


SCC-FAK model

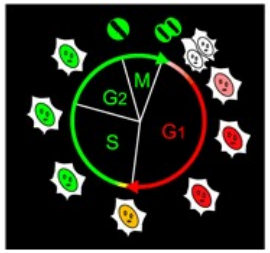
Vorinostat and **panobinostat** were selected to take forward for subsequent validation with a FAK kinase inhibitor (**VS-4718**).

Combination does not synergistically inhibit 2D proliferation of SCC cells.

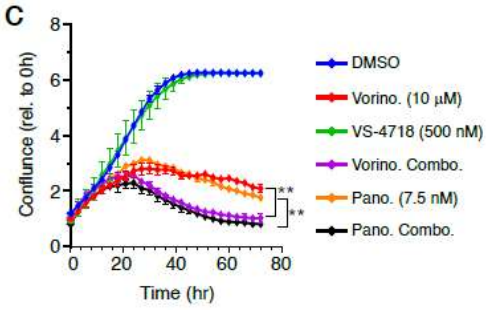
2D phenotype



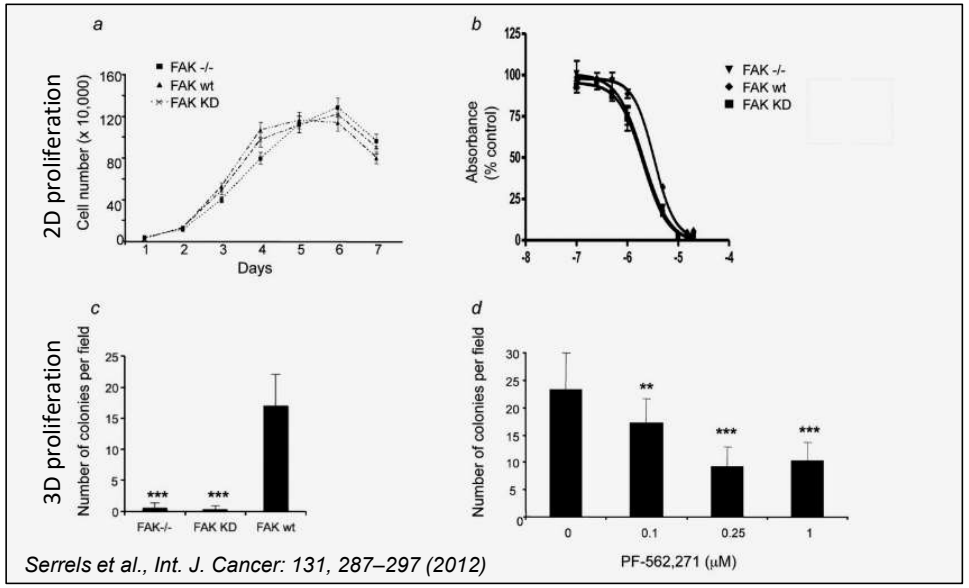
WT-SCC
24 Hours



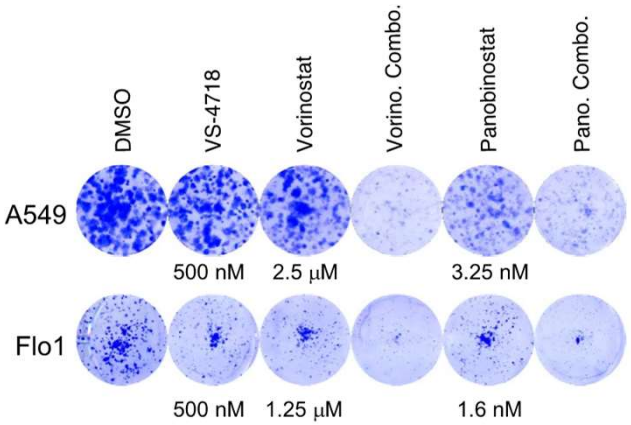
WT-FAK SCC FUCCI

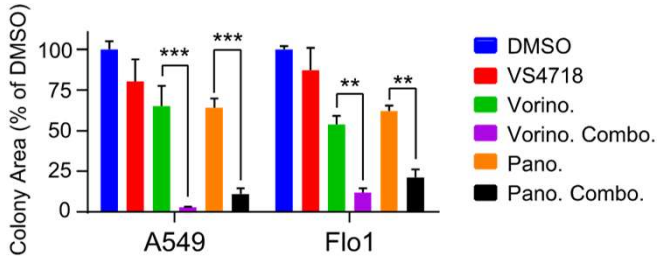


FAK is required for 3D, but not 2D proliferation



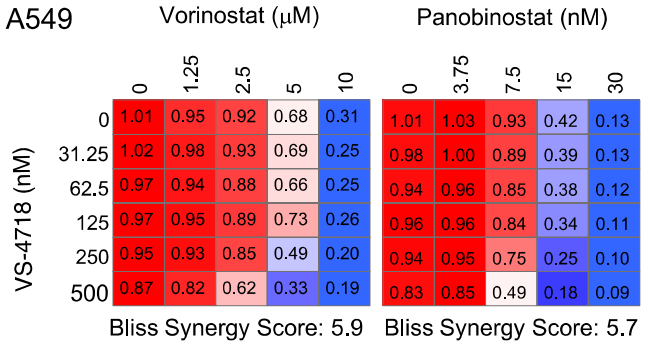
Combination of HDAC and FAK inhibitors blocks growth of A549 and Flo1 cell lines

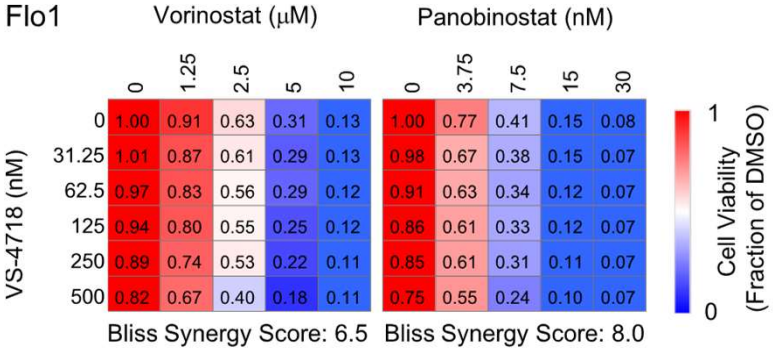




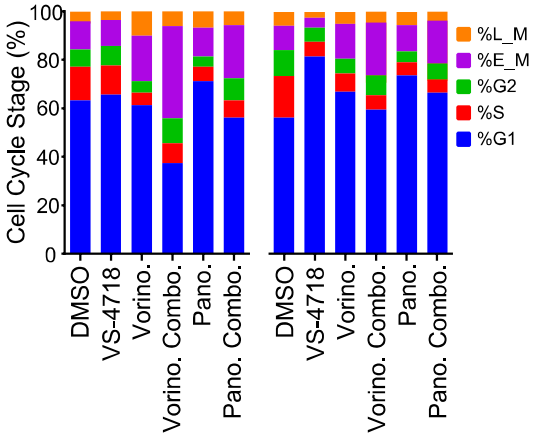
A549 cells are a lung adenocarcinoma

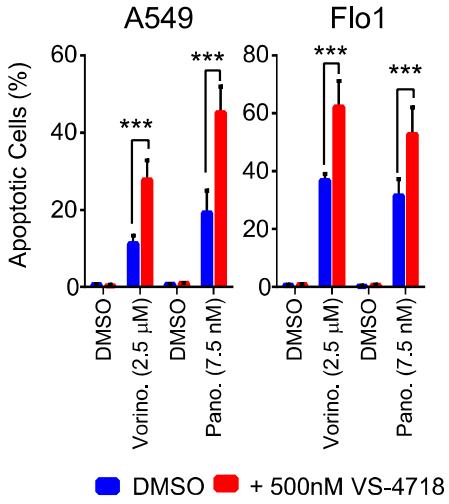
Flo1 cells are a oesophageal adenocarcinoma

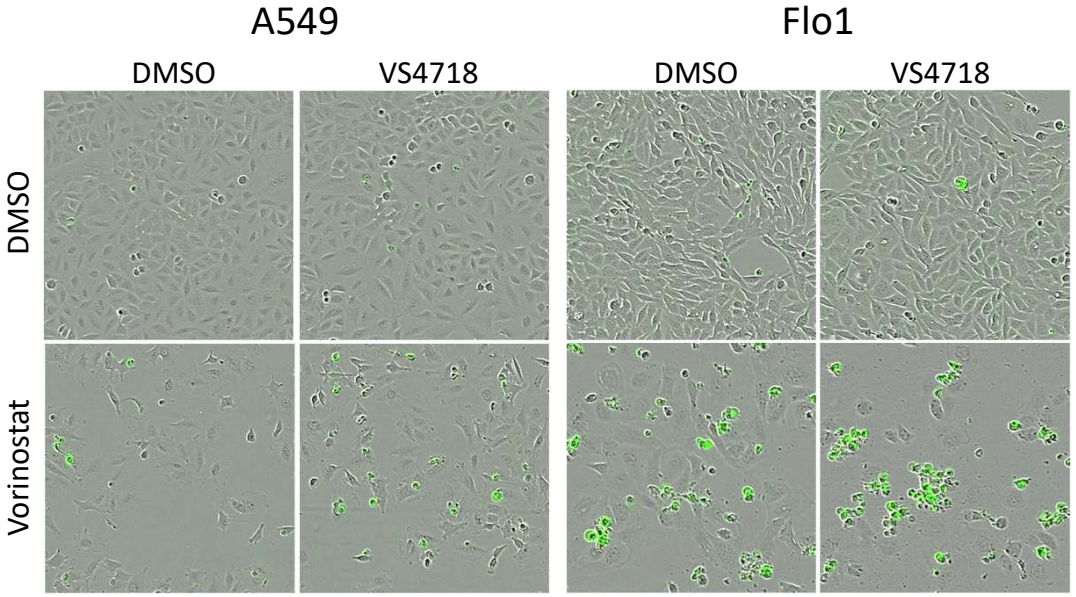




Combination of HDAC and FAK inhibitors induce cell cycle arrest and apoptosis.





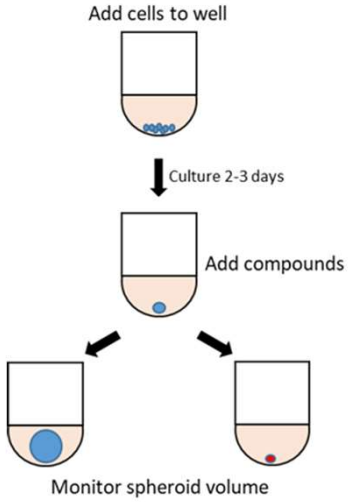


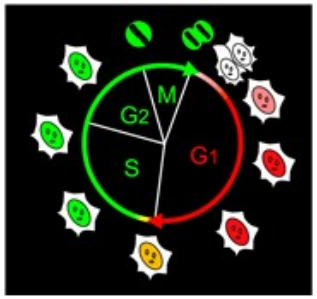
Apoptotic cells (NucView)

Phenotypic assays - 3D Spheroid Cultures

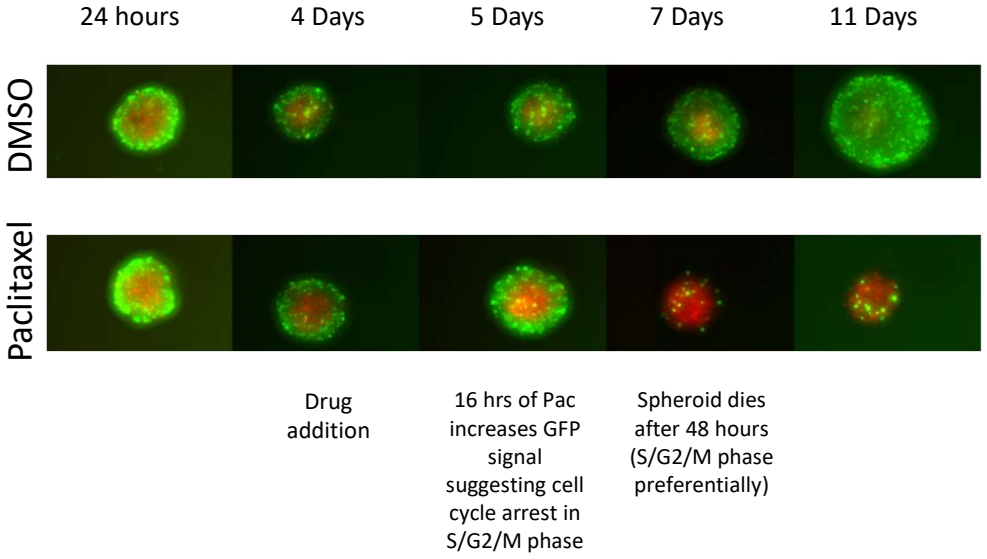
SCC cells expressing FUCCI (Fluorescent Ubiquitination-based Cell Cycle Indicator)

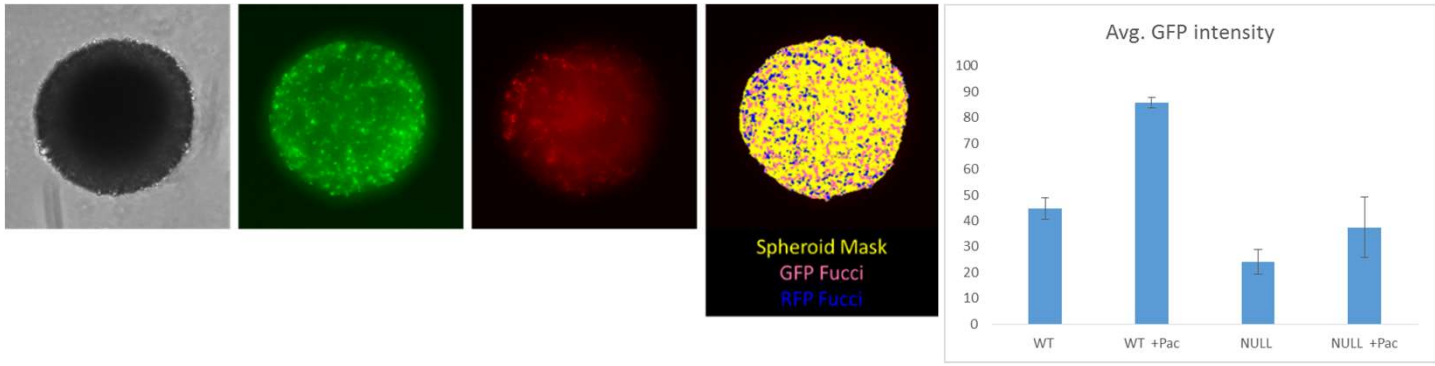
3D spheroid cell culture



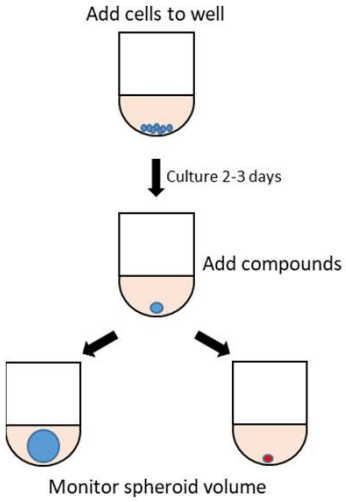


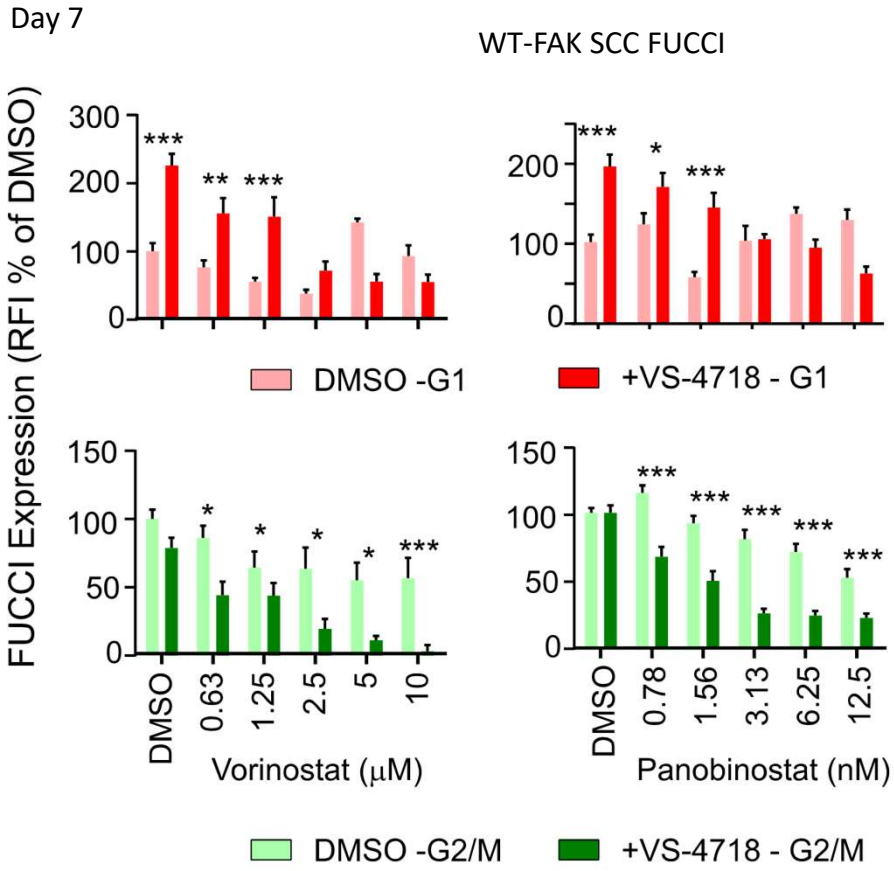
FUCCI Cell Cycle

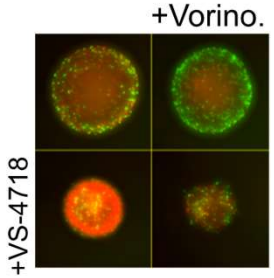


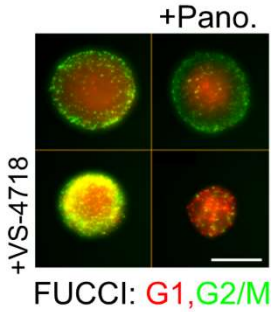


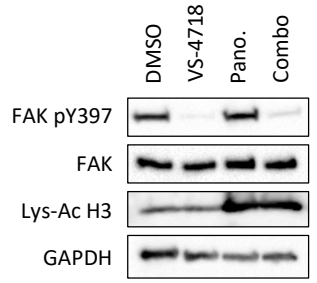
Combination of HDAC and FAK inhibitors blocks growth of SCC cells in a 3D spheroid model of growth.

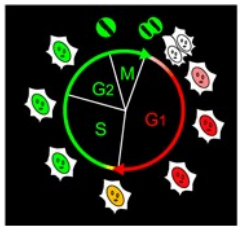




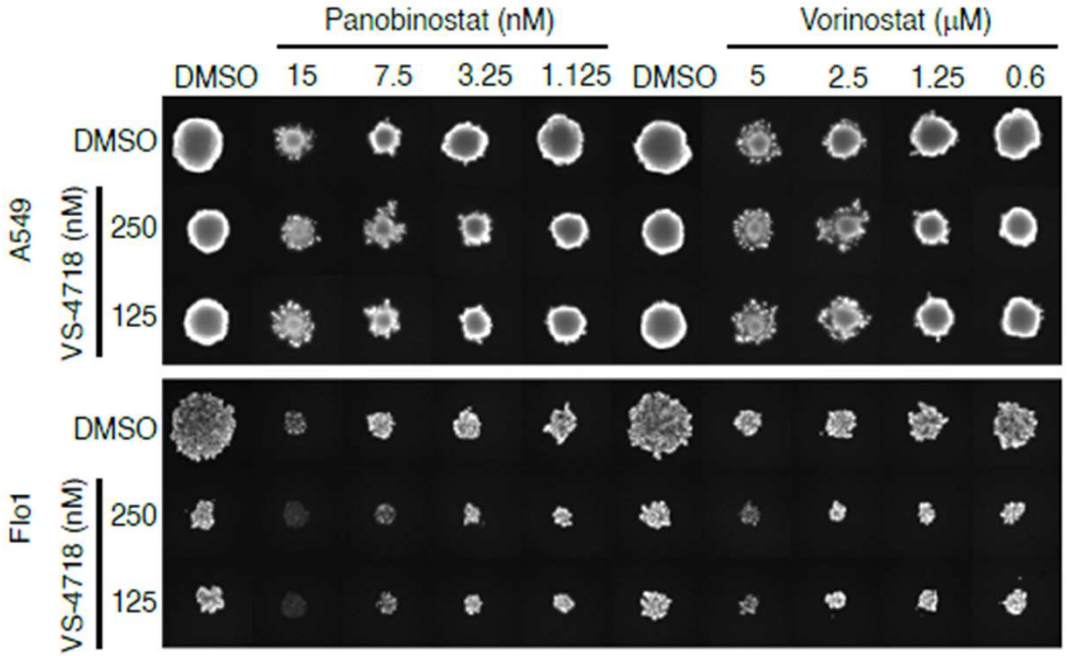






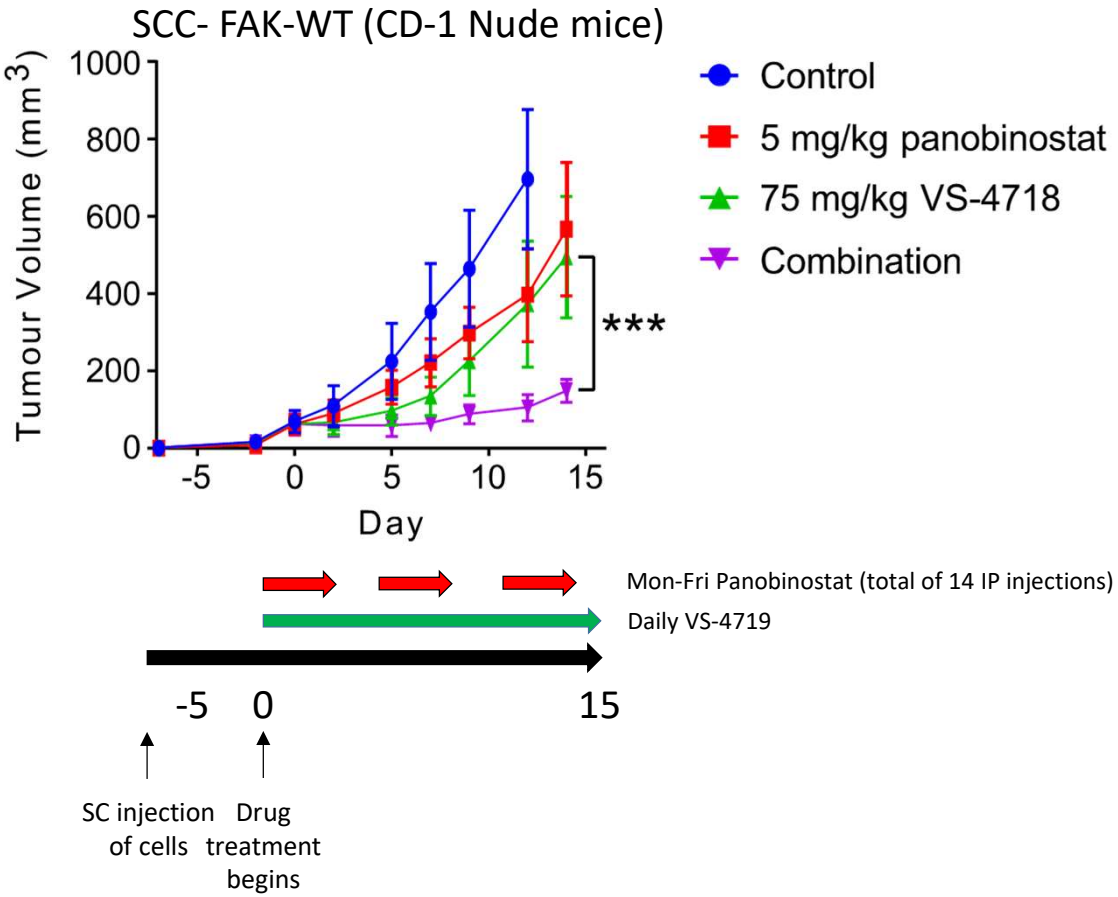


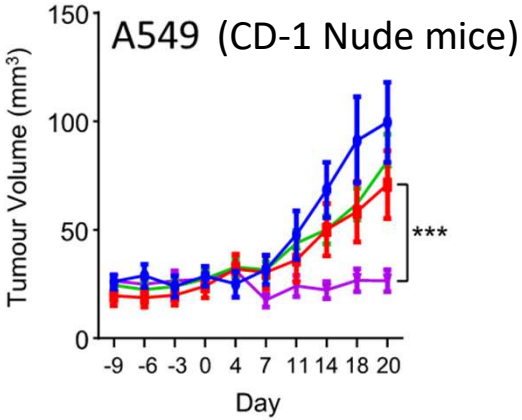
Combination of HDAC and FAK inhibitors blocks growth of A549 and Flo1 cells in a 3D spheroid model of growth.

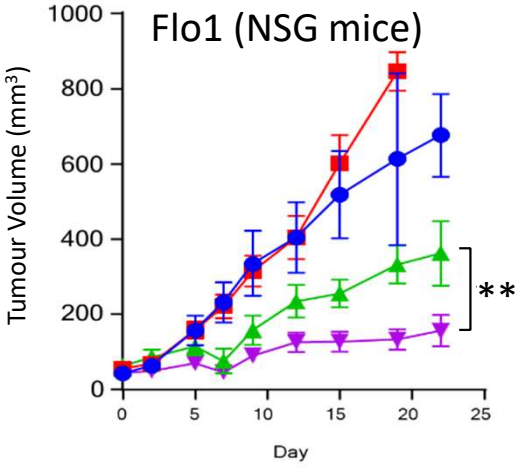


Spheroids labelled with Calcein AM viability dye on day 7

Combination of HDAC and FAK inhibitors blocks growth of tumours.

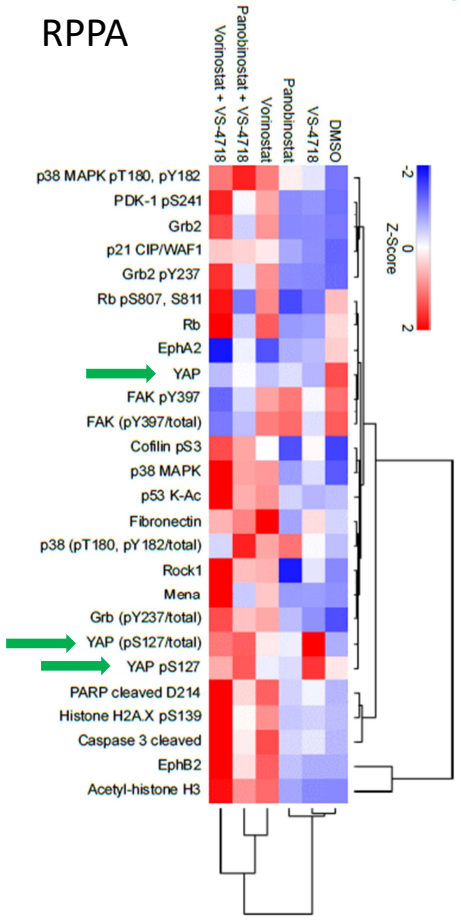






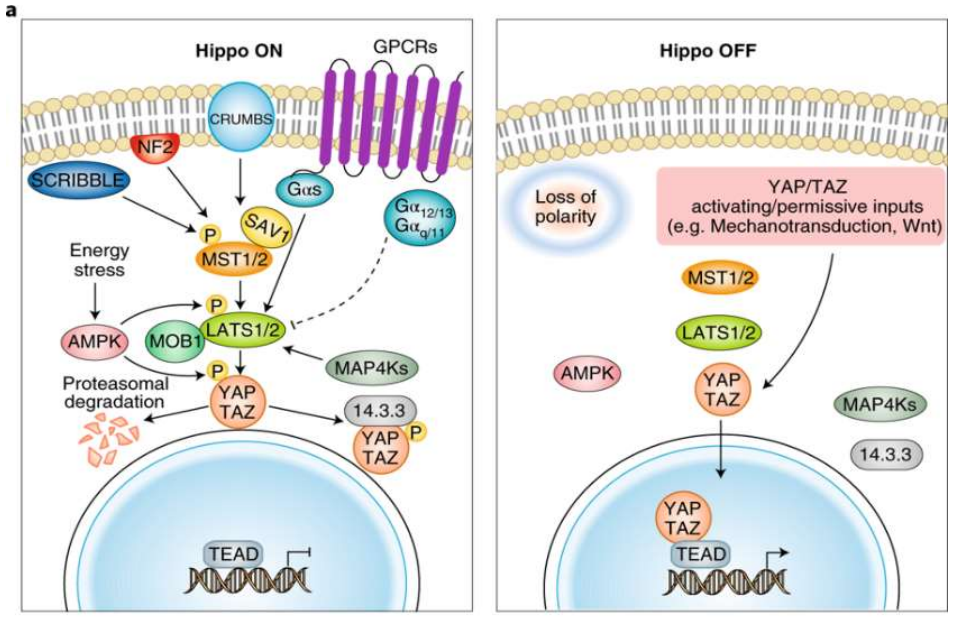
A FAK and HDAC inhibitor combination targets YAP signalling

RPPA



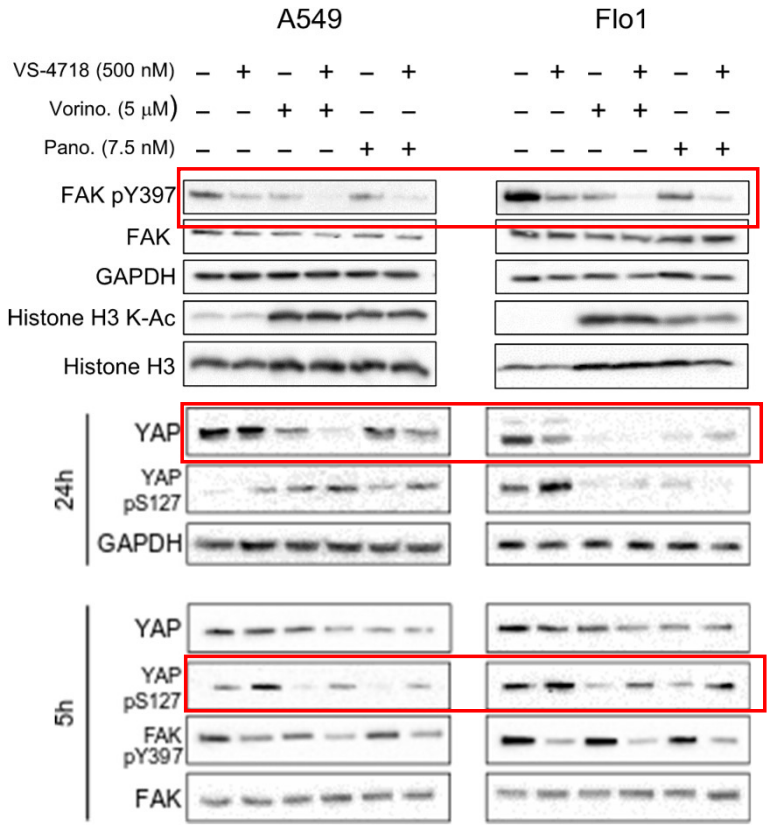
Profiled 120 antibodies covering a range of signalling pathways

Hippo signalling

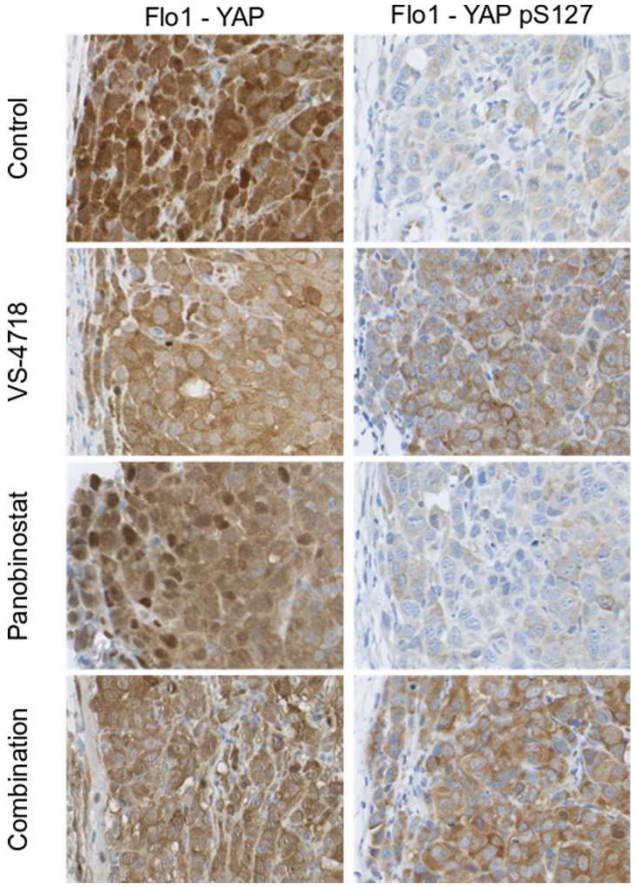


Combination of HDAC and FAK inhibitors abolishes FAK activity, and cooperatively inhibits YAP nuclear translocation and expression

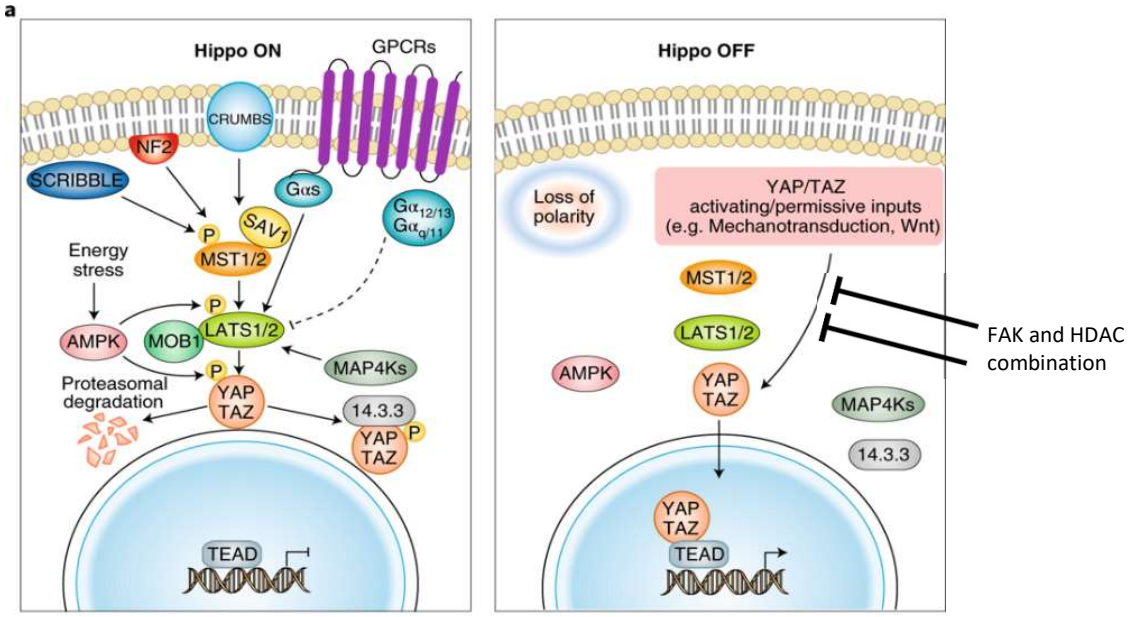
Validation



YAP expression in vivo



A FAK and HDAC inhibitor combination targets YAP signalling



Molecular Cancer Therapeutics

A Synergistic Anti-Cancer FAK and HDAC Inhibitor Combination Discovered by a Novel Chemical-Genetic High-Content Phenotypic Screen

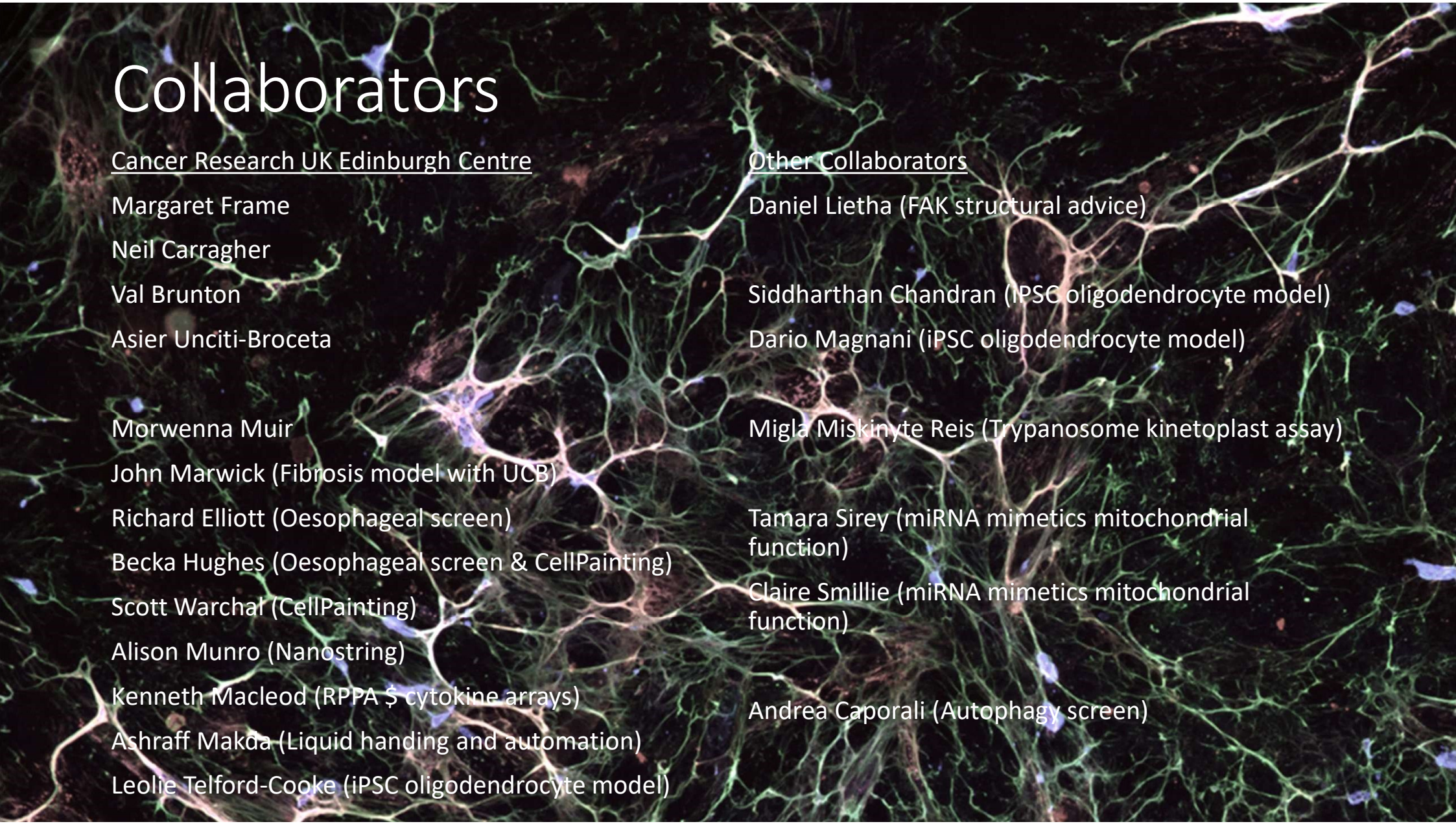
DOI: 10.1158/1535-7163.MCT-19-0330

Conclusions

1. Drug discovery challenges and why we use phenotypic assays

2. Imaging technologies and developments in model assays

3. Examples of phenotypic high-content analysis (HCA) assays



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