Advances in High Content Analysis: Phenotypic Drug Discovery

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ELRIG Drug Discovery 2012, Manchester
Outline

• High Content Analysis

• Evolution of HCA

• Technology and Platforms

• Applications
  • Target identification and validation
  • Phenotypic Screening & Toxicity Prediction
  • Physiologically relevant models for MoA studies
  • Tissue-based & automated immunohistopathology

• Observations & Trends
High-Content Analysis

“An automated cell biology method drawing on optics, chemistry, biology and image analysis to permit rapid, highly parallel biological research”

Access to complex phenotypes intractable by non-HCS methods

Cellular movement e.g. speed of migration

Tissue organisation
Subpopulations
Heterogeneity

Spatial events e.g. translocation

Morphological alterations e.g. cytoskeletal

Cell differentiation

Represents targets in their native environment

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Evolution of HCA

High-content approaches underpin significant assay development and technology delivered to projects at AstraZeneca. Can be used to embrace complexity of biology.
High Content Biology Facility

- Latest HCA platform technology is enabling the application of assays with increased physiological relevance earlier in drug discovery.
Application of HCA to Drug Discovery

Frontloading Toxicty Prediction

MoA & prioritization & reprofilng of compounds for clinical testing in Physiological Models of Disease

Discovery

Development

<table>
<thead>
<tr>
<th>Target selection</th>
<th>Lead Generation</th>
<th>Lead Optimisation</th>
<th>Pre clinical</th>
<th>POM</th>
<th>POP</th>
<th>POC</th>
<th>Phase III / Launch</th>
<th>Product maint.</th>
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Validation of Targets & Pathways in Physiological Models of Disease

Phenotypic Screening Paradigms

Mechanistic understanding of compound action using Automated Histopathology
Physiologically Relevant Models

- Human primary tissue key to implementation of HCA in drug discovery

**Primary Human Myoblasts/ Satellite Cells**

Quadriceps muscle (*vastus lateralis*) dissected *post mortem*

- Yield > 5 x 10⁵ cells/g
- Myf5+ Satellite cells/ myoblasts
- Muscle myotubes stained for myosin-heavy chain

**Human Adipose Derived Stem Cells (ADSCs)**

- Subcutaneous adipose tissue
- Isolate subcutaneous adipose tissue
- Aspirate and wash fat
- Digest with collagenase
- Isolate stromal-vascular fraction
- ADSCs
- Adipogenic differentiation

- Human primary tissue key to implementation of HCA in drug discovery
Example: Organotypic model of Angiogenesis

- Formation of new vessels from pre-existing vasculature
- Multistage process involves endothelial cell proliferation, migration & differentiation; challenging to model *in vitro*
- Role in many pathological processes including tumour formation, macular degeneration, psoriasis

- Assessment of vessel density & morphology in a human primary HDF:HUVEC co-culture organotypic model

Isherwood B. (2011) International Drug Discovery
Higher throughput screening

- Laser Scanning Cytometer: Acumen Explorer (TTP LabTech Ltd)

- Identify modulators without detailed MoA characterization
  - low resolution
  - whole-well scanning
  - fast (~9 min/plate)
  - analysis on-the-fly

Dose-response: Mycophenolic Acid

% Inhibition Total Tube Area

IC$_{50}$ = 396 nM
Multiparametric Profiling

• High-content imaging instruments

• Detailed profiling of MoA of vascular modulating compounds upon tubule structure & other cellular processes (i.e. cell cycle):
Automation of co-culture models

- Hepa-filtered enclosed Agilent Biocel system for automated screening:

<table>
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<tr>
<th>Cell plating</th>
<th>Assay ready plate transfer</th>
<th>Reagent addition</th>
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<td>Analyte capture</td>
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TGFBR Inhibitor (A83-01)

Univariate analysis of Z’ & pIC$_{50}$

Manual
- pIC$_{50}$ 6.23

Automated
- pIC$_{50}$ 6.35

3 nM
Custom developed automated Image Analysis

- Enables contextual & relational analysis of image objects
- Objects can be custom defined to include biological knowledge
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1 – Pre-processing
Custom developed automated Image Analysis

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2 – Finding Vessels

Tubule Area Parameters Quantified from Vessel Mask
Custom developed automated Image Analysis

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3 – Skeletonization

Tubule Length & Branchpoint Parameters quantified from Skeletonization
Custom developed automated Image Analysis

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4 – Nuclei Segmentation

Nuclei Count & Morphology Parameters quantified from segmentation
Custom developed automated Image Analysis

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4 – Classification

Nuclei classified by Cell type

- Nuclei
- Endothelial
- Fibroblast
Highly flexible workflow - automated data pipeline

- 90 compound screen monitoring 2 λ & 16 sites/well = 86,400 images
- Multiple parameters (~15) = 1,296,000 data points...

- Combines & manipulates data from different sources using pipeline tools:

**Input**
- Compound Information
- Cell Plate Information
- Analysis Results

**Dataset**
- Tools
  - Normalization
  - QC
  - Stats; PCA

**Output**
- Spotfire
- AZ Screening Dbase
- Pipeline Pilot Viewer
Highly flexible workflow - interactive QC

Dose-responses for Total Tube area of 90 molecule VM screen:
Highly flexible workflow- integrated statistics

1. Survival
   - TKI
   - mTOR/PI3K/Akt
   - Microtubule Effects

2. Migration
   - p38MAPK
   - Src
   - Rho Kinase

3. Activation/ Differentiation
   - Raf/ERK Signalling
   - TGFBR Signalling
   - VEGF Signalling

Data Normalization
Outlier Removal (Controls)
Dimension Reduction
Active Compounds Identified
Clustering

- 85% data explained PC1-3 (96% PC1-6)
- 68 compounds identified as active at, at least a single dose (by Mahalanobis Distances, thresholded at 0.1% significance level)
Phenotypic Assays for Drug Discovery

- HC phenotypic analysis of cell-based assays to complement traditional target-centric Lead Identification programs

Cell Cycle | Morphology | Apoptosis

Phenotypic fingerprint (morphology assay)

MCF7 cells & Aphidicolin

Temporal Effects in Cell Based Assays

- Increased dimensionality of kinetic profiling enables analysis of temporal effects and optimization of endpoint assays

Isherwood B. et. al. (2011) Pharmaceutics. 3 p141-170
Combinations Profiling

- Increased dimensionality of kinetic profiling enables analysis of temporal effects. In addition to measurement of phenotypic responses, additional data can derive further understanding of compound mode of action:

- **DEVD-NucView™ 488 caspase substrate** to monitor onset of apoptosis in live cell assay (no wash steps)

![Image of DEVD-NucView™ substrate](image1)

![Image of kinetic imaging](image2)

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**Staurosporine (1 μM) & DMSO Control (0.1%)**

**Caspase Positive [Feature 1]**

**All Wells Mean vs Time**

**Kinetic Imaging**
- Phase Contrast
- Fluorescence
- Mask

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| B Isherwood | 06 September 2012 | Innovative Medicines | Discovery Sciences | 23 |
### Kinetic Profiling: Apoptosis

**Example Combination Output:**

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#### Summary statistics used to obtain measure of synergistic effects - allows comparison across cells from different patient backgrounds

![Graph](image_url)
Imaging cells in 3D

- 3D Tumour Cell Transwell Invasion Assay (2-photon confocal)

![Diagram of HT1080 cells in 3D environment with media and fibrillar collagen/Matrigel.]

Hoechst: Cell number
Calcein/Sytox: Cell Viability
Tagged Proteins: Functional Biosensor

% No. cells invading beyond 60μm
• Automated Z-stack capture & Image Analysis

HT1080 cell invasion through collagen

Number of kits available

Imaging cells in 3D

- Air-Liquid Interface culture model of airway epithelium
- Monitor effects of smoke on epithelial breakdown and progenitor division post fixation & ICC:

  - Classify cell phenotypes & spatial relationships using definiens
  - Smoke drives epithelial cell proliferation, causes mixing of immature and mature cells, decreases tight junction proteins
Tissue HCA

- Beta-cell proliferation a key endpoint in diabetes research
- Gold-standard process utilises isolated rodent islets. Requires process of isolation and dispersion. Numerous washes and poor adhesive properties of dispersed islets reduces cell number

Reduced timelines (months > weeks) and islet (hence animal) number required

Isolated pancreatic islets

Adhesive slide-based assay

Dedicated Slide Scanner
Automated Immunohistopathology

• Pancreatic beta-cell mass in type 2 diabetes

Low resolution scanning can report beta cell mass

Traditional methods involve cell counting by hand on DAB stained sections.
Automated Immunohistopathology

• Dedicated slide imaging coupled with advances in automated image analysis. Example of pancreatic and beta-cell analysis:

  - **Time saving:** From 1.5 h to 25 min per animal
  - **Improved Data Quality:** From 10 images per animal to whole sample (>100)
  - **Detailed cellular resolution** (e.g. stain intensity, morphology-islet area and shape) as well as tissue area & cell count
Key Observations

- **Range of complementary imaging systems**, fast and multimodal enables platform selection to meet a breadth of applications.

- **Application is dependent upon integration with other specialties** - advanced cell biology, liquid handling & automation, informatics and data processing, image analysis techniques.

- **Prosecution of Phenotypic functional endpoints** to complement traditional target-centric approaches. Powerful technique to allow quantification from physiologically relevant models of disease.
Future Challenges & Prospects

• Increasing use of organotypic systems: primary tissue & stem cell biology- robust analysis & calibration at scale

• Development and adoption of novel modalities & biosensors to monitor e.g. small molecule location, additional functional endpoints and multiplexed options

Talbot C et.al. (2008) J. Biophotonics. 1 p514-521
Future Challenges & Prospects

• Linkage of phenotype with pathway & disease through integration of data from diverse sources e.g. genomics, proteomics & systems pathology

• Full exploitation of information rich data using machine vision technology or single cell analysis developed using bioimage benchmark collections

Isherwood B. et. al. (2011) Pharmaceutics. 3 p141-170
Acknowledgements

High Content Biology
James Pilling
Sam Peel
Sandeep Daya
Anesh Sitaram
Lauren Drowley
Mark Roberts
Wendy Vernon
Tracy Gorman
Claire Priest
Elizabeth Mouchet
Anne Camm
Linda MacCallum
Gillian Barrett
Dave Nicholls
Shaun Hawley
Neil Carragher
Ed Ainscow
Peter Caie
Rob Eagle
Jo Francis
Rebecca Walls
Tom Houslay
Michael Sullivan
Patrick O’Shea

Discovery Statistics
Mike Dymond
Susan Lovick

Oncology iMED
Sandra Brave
Hannah Greenwood
Claire Barnes
Kirk Schroeder
Vince Groppi
Dyke McEwen
Beth Leslie
Brad Neagle
Imperial College London
Paul French
Clifford Talbot
Sunil Kumar
Grant Zimmermann
Jeb Ledell

R&D Information
Jeremy Campbell

Kings Mill Hospital
David Walsh
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