Human Primary Cell Based BioMAP® Systems
Modelling the Host-Tumour Stromal and Vascular Microenvironments
to Support Compound Screening and Therapeutic Development

ELRIG Meeting, March 11th 2014
Telford UK
In 2012 there were estimated 14.1 million cancer cases worldwide

- Lung cancer was the most common cancer worldwide with 13% of the total number of new cases diagnosed in 2012
- Breast cancer (women only) was the second with nearly 1.7 million new cases
- Colorectal cancer was the third with nearly 1.4 million new cases

In 2013, more than 1.6 million Americans were newly diagnosed with some form of cancer

- In 2013, about 580,350 are expected to die of cancer, almost 1,600 people per day in the US
- However, 13.7 million are currently living with their disease (US)

5-year relative survival rate for all cancers diagnosed between 2002 and 2008 is 68%, up from 49% in 1975-1977

- Earlier diagnosis
- Better treatment strategies
Largest fraction of approvals in Oncology with 8 anti-cancer drugs approved in 2013 (30%)

- **Kinase inhibitors**: Trametinib (MEK inhibitor; GSK1120212; GSK), Dabrafenib (BRAF inhibitor, GSK2118436; GSK), Ibrutinib (BTK inhibitor; Pharmacyclics/Janssen), Afatinib (RTKi; Boehringer Ingelheim)
- **Biologics**: Obinutuzumab (anti-CD20, Roche-Genentech) and Ado-trastuzumab emtansine (Herceptin-microtubule disrupter ADC; Roche-Genentech)
Cancer and Associated Microenvironment

What to Target?

Goal: To develop more effective anti-cancer therapies

Molecularly targeted therapies
- Target-selective molecules – “Chisel”
- Safer non-selective agents - “Hammer”
- Combination therapies – “Strategic”

Target Validation
- Specificity/selectivity
- Potency

Assess impact on host-tumor biology
- Tumor cell proliferation
- Inflammation
- Angiogenesis
- Stromal invasion/metastasis

Anti-proliferative agents
- Cytotoxic agents
- Kinase inhibitors
- Epigenetic Agents
- Biologics
- Immunomodulators
Modeling Human Disease Biology for Drug Discovery

Primary Human Cell Systems

“Omics”

“Modeling”

Scale (M)
- $10^{-9}$ M
- $10^{-8}$ M
- $10^{-7}$ M
- $10^{-6}$ M

Time (sec)
- $10^6$ sec
- $10^2$ sec
- $10^4$ sec
- $10^5$ sec

molecules arrays pathways cells tissues humans
BioMAP® Systems Model Complex Disease Biology

*color indicates different disease-like environment

Human Primary Cells

- Endothelial cells
- PBMC
- Fibroblasts
- Monocytes
- Macrophages
- Bronchial Epithelium
Advantages of Human Primary Cells

• **Physiological Relevance**
  - Particularly valuable for unprecedented and challenging targets

• **Easier transition from screening to translational to clinic**
  - Biomarkers and patient classification can be addressed early

• **Fulfills FDA & regulatory agencies mandate for more human-based data for INDs**
  - “…The Critical Path Initiative (CPI), launched in March 2004, includes promoting *in vitro* screening trials on relevant *human cells* is important to evaluate NDE

• **Most first-in-class NMEs are still discovered using phenotypic assays**
Why Activate Multiple Pathways?

One Pathway Active

Activation of a single pathway provides limited information
Why Activate Multiple Pathways?

Multiple Pathways Active

Expression Level

Synergy

Feedback

IFN$\gamma$

IL-1$\beta$

VCAM

Feedback

Synergy

Why Activate Multiple Pathways?
Why Activate Multiple Pathways?

Multiple Pathways Active

Expression Level

IFNγ → TNF-α → IL-1β → VCAM

Expression Levels: Low, Low, High
Drug Effects on Biomarker Levels In a BioMAP System

Significant Increase in Biomarker Level

99% Significance Envelope

Significant Decrease in Biomarker Level

Biomarkers (Readout Parameters)

Log expression ratio (Drug/DMSO control)
Drug Effects on Biomarker Levels In a BioMAP System

Control (no drug)

99% Significance Envelope

Dose Response

Drug Effects on Biomarker Levels In a BioMAP System

Biomarkers (Readout Parameters)

Drug
- 10,000 uM
- 3,333 uM
- 1,111 uM
- 370.370 nM
- 123.457 nM

Log expression ratio (Drug/DMSO control)

- 3C

Drug
- 3C

Control (no drug)

99% Significance Envelope

Dose Response

Biomarkers (Readout Parameters)

- CCL2/MCP-1
- CD100/VCAM-1
- CD141/Tissue Factor
- CD142/Selectin
- CD87/Selectin
- CXCL8/IL-8
- CXCL5/IGF-2
- HLA-DR
- SIR

Drug
- 3C

Control (no drug)

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Biomarkers Provide Insight on Biological Processes

- MCP-1: Monocyte recruitment into inflammatory tissues
- Tissue Factor: Platelet activation and thrombosis
- HLA-DR: Antigen presentation and T cell activation
BioMAP Profile
Activity Profile of a Kinase Inhibitor

BioMAP Systems

Log expression ratio
(Drug/DMSO control)

Readout (Biomarkers)

Control (no drug)

Dose Response

3C  LPS  SAg  HDF3CGF  KF3CT  Mphg
Assays are Robust and Highly Reproducible

Repeats of BSK-714 (Multiple independent experiments)

12 experiments, each performed at different time, different donors, different lots of the same compound

Profile shape and EC50’s remains the same experiment-to-experiment

Pearson correlation routinely >0.9 (perfect match = 1)
BioMAP® Primary Human Cell Based Screening Platform

Reproducible Assays + Robust Data Management = In Vitro Human Data
Recently Approved Kinase Inhibitors

**BTK Inhibitors**

- **Ibrutinib**
  - PCI-32765
  - Orally available, irreversible inhibitor of BTK (IC50 0.46 nM)
    - Additional targets including Blk (0.5 nM), Bmm (0.8 nM), **EGFR** (5.6 nM), Itk (10.7 nM) and Jak3 (16.1 nM) detected using KINOMEScan
  - FDA approved as a treatment for patients with mantle cell lymphoma (MCL) in November 2013 and for chronic lymphocytic leukemia (CLL) in Feb 2014
    - Annual sales forecasts of $4.5 billion by 2019

*Spot size ➔ binding affinity as % of control*

*KINOMEScan® TREEspot™ analysis by D Treiber, DRX*
BioMAP Profile of Ibrutinib

**Efficacy at Lower Doses**

- At lower, more clinically relevant doses, Ibrutinib strongly inhibits B cell activation and proliferation (370 → 4.8nM dose range)
  - No cytotoxicity is detected
  - Inhibition of B cell activation responses >>> T cell responses (SAg and BT systems)

- **Effects on BTK non-expressing epithelial, endothelial and fibroblasts cell types**
  - also detected at these doses
    - Not consistent with primary target expression – indicative of secondary targets
- **Ibrutinib** top database match using non-immune cell based BioMAP systems only $\rightarrow$ Gefitinib ($r = 0.868$)
- Gefitinib (Iressa, AZ/Teva) an EGFR tyrosine kinase inhibitor in clinic for breast, lung and other cancers
- Similar to Erlotinib (Tarceva, OSI, Roche)
Skin Rash Predicts Efficacy in Oncology

Not suitable for RA development

- “The presence and severity of skin rash is associated with improved clinical efficacy in patients receiving EGFR inhibitors”  PMID 19452131.

- Skin rash AE reported in CLL patients in clinical trials for Ibrutinib
  - The toxicity of ibrutinib appears modest with the major adverse effects being nausea (26%), respiratory tract infection (29%) and rash (26%). ASCO 2012. Abstract No: 6515, CLL trial

Newer Kinase Targets

MEK Inhibitors

Trametinib
GSK1120212

- Trametinib (GSK1120212), is a MEK1 and MEK2 inhibitor approved by FDA for the treatment of late-stage metastatic melanoma in 2013

  - Disease recurrence on single-agent trametinib occurs within 6 to 7 months so Trametinib now approved for use in combination with combined with a BRAF inhibitor for the treatment of patients with BRAF V600E/K-mutant metastatic melanoma.
• Trametinib (GSK-1120212) is highly active, but not cytotoxic, in all BioMAP Diversity Plus systems at concentrations similar to clinical exposure levels (reported Cmax of 48nM)

• Activities detected at two or more concentrations include effects on cell proliferation, cytokine and chemokine production, immune cell recruitment and inflammation markers and activities impacting vascular and matrix remodeling related biology
Recently Approved Kinase Inhibitors

B-Raf Inhibitors

**Vemurafenib**
**PLX4032**

- **Vemurafenib** is a B-Raf enzyme inhibitor approved by FDA for the treatment of late-stage metastatic melanoma in 2011
  - First drug designed using fragment-based lead discovery to gain regulatory approval
  - Highly selective killing of tumor cells than carry BRAF V600E mutation
  - Emerging resistance becoming an issue (40% cases)
• Vemurafenib is selectively active, but not cytotoxic, at concentrations similar to clinical exposure levels (reported Cmax of $62 \pm 17 \mu g/mL = 126.6 \pm 34.7 \text{nM}$)

• Activities detected at two or more concentrations are annotated and include
  - Inflammation related impact: decreased CD40, M-CSF and sIgG; increased V-CAM
  - Matrix-modulation/tissue remodeling effects: decreased Col-III and PAI-I
  - These are effects unrelated to primary target – B-Raf$^{V600E}$ since cancer cells are not present in Diversity Plus BioMAP systems
How can we “model the tumor” in vitro?

• Host components
  • Activated fibroblasts, endothelial cells
  • Angiogenesis, ECM deposition and remodeling

• Immune components
  • Activated peripheral blood mononuclear cells (PBMC)

• Tumor component
  • Patient versus cell line?
    – Cell lines are genetically well-characterized
    – Patient pool is diverse and unknown
New BioMAP® Oncology Systems
Modeling Tumor-Host Microenvironments

Human Primary Stromal Fibroblasts ("Stro")

Human Primary Endothelial cells ("Vasc")

Primary PBMC (Peripheral blood mononuclear cells)

Cancer Cell Line (e.g. Colon Adenocarcinoma cells (HT-29)

Adapted From Burton ER et al., J. Biol. (2009)
Modeling the Human Tumor Microenvironment

Primary Human Cell Types

- Dermal fibroblasts or HUVEC
  “Host stromal/vascular cells”
- Human PBMC
  “Host immune cells”
- Colon adenocarcinoma (HT29) or Non-small cell lung cancer (NCI-H1299)
  “Tumor epithelium “

Tumor-stromal microenvironment

- Optimized Co-culture of Host and Tumor cells

Activation conditions

- Optimized TCR activation conditions

Host-Tumor Stromal or Vascular In Vitro models

- Drug Testing
  Measurement of select stromal-, vascular-, immune- and oncology-based protein readouts
## Development of BioMAP® Oncology Systems

**StroHT29 and VascHT29**

<table>
<thead>
<tr>
<th>System</th>
<th>Primary Human Cell Types</th>
<th>Disease Relevance</th>
<th>Readout Parameters</th>
<th>System Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>StroHT29</td>
<td>HT-29 colon Adenocarcinoma cell line + Primary Human Fibroblasts + PBMC</td>
<td>Oncology: Host Tumor-Stromal Microenvironment</td>
<td>VCAM-1, uPAR, Collagen I, Collagen III, IP-10, MMP-8, PAI-1, PBMC Cytotoxicity, sGranulysin B, sIFNγ, sIL-10, sIL-17A, sIL-17F, sIL-2, sIL-4, sIL-6, SRB, sTNFα, sVEGF, TIMP2, TPA, uPA</td>
<td>The StroHT29 system consists of primary human fibroblasts co-cultured with an adenocarcinoma cell line, HT-29 plus human PBMC stimulated via the T cell receptor (SAg) for 48 hours. These conditions model the host-tumor stromal microenvironment by capturing the complex interactions between tumor cells and the host stromal network and infiltrating immune cells recruited into the tumor mass.</td>
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<td>HT-29 colon Adenocarcinoma cell line + Primary Human Endothelial cells + PBMC</td>
<td>Oncology: Host Tumor-Vascular Microenvironment</td>
<td>MCP-1, VCAM-1, CD40, CD69, uPAR, Collagen IV, IP-10, MIG, PBMC Cytotoxicity, sGranulysin B, sIFNγ, sIL-10, sIL-17A, sIL-2, sIL-4, sIL-6, SRB, sTNFα</td>
<td>The VascHT29 system consists of primary human vascular endothelial cells co-cultured with an adenocarcinoma cell line, HT-29 plus human PBMC stimulated via the T cell receptor (SAg) for 48 hours. These conditions model host-tumor vascular microenvironment by capturing the complex interactions between tumor cells and the host vascular network and infiltrating immune cells associated with angiogenesis.</td>
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**BioMAP Models Tumor-associated Immune Regulation**

**HT-29 Cells Inhibit sIFNγ in the StroHT29 System**

- **IFNγ levels are decreased only tumor cell HDF/PBMC co-cultures (StroHT29)**
  - Consistent with suppression of immune responses by tumors (PMIDs: 16972901 and 18759925)
- **Production of sGranzyme B is also decreased only in StroHT29 (not shown)**
- IL6 levels are decreased only tumor cell HuVEC/PBMC co-cultures (VascHT29)
- VEGF highly induced only in the presence of the Tumor cells and is elevated independently of TCR stimulation
- Epithelial-derived cancer cells produce a variety of growth factors that promote angiogenesis. These growth factor RTKs are the targets of agents such as Iressa, Tarceva, etc. (PMID: 11350918)
• Paclitaxel is a first line treatment for breast and lung cancers by regulating cell division
• Reported to have immune-maintaining features (Zitvogel et al, 2008)
• Dose-dependent stromal, angiogenic, and immunomodulatory reductions
Synergistic reduction in sGranzyme B, sIL-2, sIL-4 and sIL-6 upon combination treatment with MEK (GSK-1120212) and mTOR (Everolimus) inhibitors
Several critical challenges remain to be addressed in order to develop safer and more effective anti-cancer drugs.

- Improved screening strategies using complex models of cancer cells plus the host microenvironments are needed.
- Combination therapies need to be identified and optimized early in development phase to accelerate clinical approval of both agents.
- New strategies showing great promise include immunotherapeutics and Antibody Drug Conjugates (ADC) require human screening models to assess the human tumor-targeting antibody as well as the chemotherapeutic payload.

Screening tools such as BioMAP® predictive models for efficacy and safety will significantly support anti-cancer compound discovery, lead optimization and pre-clinical development.
BioSeek Team,
DiscoverX, San Francisco

GM/CSO
Ellen Berg

Biology
Alison O’Mahony
Hans Layman
Jennifer Melrose
Sylvie Privat

Assay Operations
Mark Polokoff
Dat Nguyen
Liisa Alajoki
Lilly Litrus
Tomeo Minami
Charleen Rayl
Elijah Johnston
Naomi Brown
Mary Plavec
William Tang

Bioinformatics
Jian Yang
Antal Berenyi
Hannah Wang

Project Management
Carmen Bryant
Stephanie Fong
Monique Duval