Non-invasive in vivo imaging for basic and translational research

From whole body to subcellular scale in health and disease

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Cancer Research UK, London Institute
Research Associate, Centre for Advanced Biomedical Imaging (CABI), UCL

Animal models are needed
For basic and translational research

- Study development & homeostasis of normal tissues
- Physiopathology of human and animal diseases
- Validate new targets
- Test new therapeutic strategies
  - PK
  - PD
  - Toxicity

... but predictive ones

Rates are for ten large pharmaceutical companies in the USA and Europe for the period 1991-2000

<table>
<thead>
<tr>
<th>Phase</th>
<th>Number of Drugs Entering Phase</th>
<th>Number of Drugs Completing Phase</th>
<th>Success rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>60</td>
<td>61</td>
<td>10%</td>
</tr>
<tr>
<td>II</td>
<td>18</td>
<td>17</td>
<td>10%</td>
</tr>
<tr>
<td>III</td>
<td>4</td>
<td>3</td>
<td>75%</td>
</tr>
<tr>
<td>IV</td>
<td>3</td>
<td>2</td>
<td>67%</td>
</tr>
<tr>
<td>V</td>
<td>5</td>
<td>3</td>
<td>60%</td>
</tr>
</tbody>
</table>

Efficacy !

95% attrition

Sharpless NE et al, Nat. Rev. Drug Disc. 2006
Conventional preclinical model in oncology

The best model of a cat is a cat or, better, the cat itself
Norbert Weiner (1894-1964), Mathematician

Essentially, all models are wrong, but some are useful
George E. P. Box (1919-2013), Statistician

What can we do?

1) Improve the (intrinsic) quality of animal models
2) Optimise the way we use animal models (extrinsic)
3) Develop best practice to maximise robustness and reproducibility
4) Report all studies appropriately
Select the best available model in oncology

<table>
<thead>
<tr>
<th>Mouse cancer cells</th>
<th>Human cancer cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syngeneic transplantation models</td>
<td>Xenograft of human cancer cell lines</td>
</tr>
<tr>
<td>Subcut, IP, IV, orthotopic</td>
<td>Subcut, IP, IV, orthotopic</td>
</tr>
<tr>
<td>Genetically engineered mouse models</td>
<td>Patient-derived xenografts</td>
</tr>
<tr>
<td>(GEMMA)</td>
<td>Subcut, orthotopic</td>
</tr>
</tbody>
</table>

Orthotopic approaches should be preferred whenever possible

Hanahan D and Coussens LM, Cancer Cell 2012

In vivo imaging has long been indispensable in clinical practice

01/12/2014
From whole body to subcellular scale

Regulations & Ethics

Health and safety
- Zoonoses / allergies
- Chemical risk (anaesthetics, drugs)
- Oxygen
- Laser safety
- Radio safety

Immunocompromised animals

Ethical Review Committee

Longitudinal studies to improve the use of animal models... and the 3Rs!

Post-mortem endpoints & rilmen

Imaging

12 mice
Adapted from grants
Paired data
Longitudinal studies to improve the use of animal models... and the 3Rs!

- Minimise inter-individual variability
  - Improved sensitivity to detect small but biologically meaningful effects

- "Staging" for subsequent randomisation before intervention

- Enabling approach for exploring complex scenarios
  - Disease progression correlates inversely with animal welfare
  - Imaging can be used to assess and 3Rs requirements

Dramatically reduce the need for survival studies

A strategic role for in vivo imaging facilities

Support:

- Study design
- Choice of animal models (including reporters etc)
- Choice of imaging technologies
  - Scanners
  - Probes
  - Contracting agents
  - Reporter systems
- Image acquisition
- Image analysis (segmentation/quantification)
- Data interpretation and preparation for publication
- Data storage and data sharing

- SOPs
- Blinded analysis
- Randomisation
- Reproducibility
- Relevance
- Best practice

First charity dedicated to cancer

42 Research groups
15 Core Technology platforms
**In Vivo Imaging Facility**

- Bioluminescence
- Near Infrared Fluorescence
- Intravital microscopy

**Some applications**

- Example 1: Haematopoietic stem cells and acute myeloid leukaemia
  - Manipulation
  - Near infrared fluorescence
  - Intravital microscopy

Example 2: Non-Small Cell Lung Cancer (GEMM)
- Micro-CT
- Response to treatment
- Analysis

**The haematopoietic hierarchy**

D. Bonnet and J Dick, Nat Med, 1997
Simplified model for the stem cell niche

Hypoxia, Bone, Haematopoiesis, Stem Cells, Osteoblasts, Osteoclasts, Endosteum, Endothelial Cells, Sinusoids, Megakaryocytes, Vascular Niche, Regular Cells, SDF:1 (rich), Pericytes, Astrocytes, MSC, Nes.n:1, SNC, Osteomacs

Orthotopic AML patient-derived xenografts

Human haematopoietic stem cells

Orthotopic AML patient-derived xenografts

Human leukaemia stem cell (AML pat.)

Mouse AML microenvironment is able to support and maintain the functionality of normal HSC and LSC over long period of times

Recapitulating the human disease from the patient leukaemia stem cells
**Optical whole body imaging**

![Image](image1.png)

**Whole body Bioluminescence imaging**

![Image](image2.png)

**Dynamic/longitudinal tracking & (semi-)quantification**

![Image](image3.png)
SensiKvity

Tracking small numbers of stem/progenitor cells

Flow cytometry (engraftment)

Proliferon (BLI)

Scramble shRNA β-catenin

Bioluminescence imaging

Sensitivity and specificity

β-catenin loss of function in orthotopic AML
Lassailly F. et al, Leukemia 2013 (Bonnet’s Lab)

PTEN loss of function in prostate cancer cells
Ros S. et al, Cancer Discovery 2012 (Schulze’s Lab)

Hematopoietic stem cell tracking & BM reconstitution
Lassailly F. et al, Leukemia 2013 (Bonnet’s Lab)

T cell based immunotherapy in orthotopic AML
Lassailly F. et al – Leukemia 2014 (Bonnet’s Lab)

Ongoing

Experimental lung metastases
Drug efficacy
Bioluminescence imaging

Sensitivity and specificity

- Low resolution
- Quantification affected by depth of the signal

Need for gene transfer

⇒ Not suitable for routine tracking of primary AML or solid tumours from human origin (PDX)

Optical whole body imaging

- Bioluminescence & Fluorescence
- Spectral unmixing
- Epifluorescence & Transillumination
- 2D, 3D
- 1 to 5 animals

Whole body Fluorescence Imaging (VIS / NIR)

Adapted from Weissleder R, Nat. Biotech 2001
Applications: tracking fluorescently labelled leukemic cells

![Image of fluorescent cells](image)

Staining with lipophilic NIR dye

DiO, DiI, DiD, DiR, PKH26 etc...

Lassailly et al., Blood 2010

**Fluorescent / Fluorigenic probes [compatible microscopy]**

<table>
<thead>
<tr>
<th>Target</th>
<th>Application</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood pool agents</td>
<td>Angiogenesis / BVD</td>
<td>Non-specific</td>
</tr>
<tr>
<td>Proteases</td>
<td>Hypoxia / metastasis</td>
<td>Non-specific</td>
</tr>
<tr>
<td>Integrins (αvβ3)</td>
<td>Neoangiogenesis, tumours</td>
<td>Targeted</td>
</tr>
<tr>
<td>CA IX</td>
<td>Hypoxia</td>
<td>Targeted</td>
</tr>
<tr>
<td>CA XII</td>
<td>Hypoxia</td>
<td>Targeted</td>
</tr>
<tr>
<td>UCP2, UCP3, NFO</td>
<td>Hypoxia / metabolism</td>
<td>Targeted</td>
</tr>
<tr>
<td>Integrins</td>
<td></td>
<td>Targeted</td>
</tr>
<tr>
<td>CA IX</td>
<td></td>
<td>Targeted</td>
</tr>
<tr>
<td>VEGF-R</td>
<td>Angiogenesis</td>
<td>Targeted</td>
</tr>
<tr>
<td>Glucose Transporter</td>
<td>Metabolism / hypoxia</td>
<td>Targeted</td>
</tr>
<tr>
<td>Annexin V</td>
<td>Apoptosis</td>
<td>Targeted</td>
</tr>
<tr>
<td>Caspases</td>
<td>Apoptosis</td>
<td>Targeted / activatable</td>
</tr>
<tr>
<td>MMP-2, -3, -9, -13</td>
<td>Inflammation, angiogenesis, metastasis</td>
<td>Activatable</td>
</tr>
<tr>
<td>Cathepsin B, L, S</td>
<td>Inflammation, angiogenesis, metastasis</td>
<td>Activatable</td>
</tr>
<tr>
<td>Cathepsin K</td>
<td>Osteoclasts</td>
<td>Activatable</td>
</tr>
<tr>
<td>Hydroxy Apatite</td>
<td>Bone remodelling</td>
<td>Targeted</td>
</tr>
</tbody>
</table>

How about your own probe(s)?

Lassailly F. et al. Blood 2013

**Imaging glucose metabolism**

Could [18F]fluoro-2-deoxy-D-glucose ([18F]FDG) be used to monitor human leukemia development non-invasively?

Positron Emission Tomography (PET)

![Image of PET scan](image)

[18F]fluoro-2-deoxy-D-glucose ([18F]FDG)

Lassailly F. et al. Blood 2013
Luciferase (+) Human AML cell lines U937 HL60 KG1 NOD-SCID-γc null Control NOD-SCID-γc null

Cells injection
Day 0 Day 7 Day 14 Day 21 Day 28 End

Bioluminescence imaging (BLI)

May Zaw
Thin Collaboration with Haematopoietic Stem Cells Lab
Dominique Bonnet
Carolien Woolthuis
Linda Ariza-McNaughton
Alessandro Di Tullio

Near-infrared (non-radioactive) 2-Deoxy-glucose (2DG) imaging

ALI

Near-infrared (non-radioactive) 2-Deoxy-glucose (2DG) imaging

Fluorescence tomography

New developments: Cerenkov Luminescence Imaging

Cerenkov light:
Electromagnetic radiation emitted when a charged particle (such as an electron) passes through a dielectric medium at a speed greater than the phase velocity of light in that medium.
New developments: Cerenkov Luminescence Imaging

Common radionuclides for molecular imaging

<table>
<thead>
<tr>
<th>Nuclide</th>
<th>Half-life</th>
<th>Energy (MeV)</th>
<th>Photo (MeV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18F</td>
<td>109.8 min</td>
<td>0.87</td>
<td>×</td>
</tr>
<tr>
<td>123I</td>
<td>13.7 h</td>
<td>2.9 MeV</td>
<td>×</td>
</tr>
<tr>
<td>64Cu</td>
<td>12.79 h</td>
<td>0.806 MeV</td>
<td>×</td>
</tr>
<tr>
<td>111In</td>
<td>2.8 h</td>
<td>0.8 MeV</td>
<td>×</td>
</tr>
<tr>
<td>89Zr</td>
<td>3.7 days</td>
<td>0.81 MeV</td>
<td>×</td>
</tr>
<tr>
<td>151Eu</td>
<td>6.2 h</td>
<td>0.92 MeV</td>
<td>×</td>
</tr>
<tr>
<td>99Mo</td>
<td>66.8 h</td>
<td>0.92 MeV</td>
<td>×</td>
</tr>
</tbody>
</table>

Hongguang Liu et al. PLoSOne 2010

Preliminary test with (18)F-Fluoride (bone)

Tony Gee

Preliminary test with (18)F-Fluoride (bone)
Combined NIR confocal and multiphoton microscopy

Laser Lines:
- Ar Argon 458 / 488 / 514 nm
- Diode 561 nm
- HeNe 633 nm
- Pulsed multiphoton (Mai Tai Deep-See)

Detectors:
- 34 detectors (live spectral unmixing)
- 5 Non Descanned Detectors (NDDs)

Two (multi)-photon excitation
Pulsed laser (femto-second) to excite a single point in space

- Limited background signal
- Deeper penetration
- Low photobleaching
- Low tissue phototoxicity
- Possible to image suffusing environment
Multiparametric analysis of intact bone marrow microenvironment

Intravital microscopy of human leukemic cells in intact bones

Non-invasive time-lapse of human hematopoietic stem cells
Microscopic imaging of deep tissues

Intestinal crypt homeostasis revealed at single-stem-cell level by in vivo live imaging

Ritsma et al. Nature 2014

Some applications

- Example 1: Haematopoietic stem cells and acute myeloid leukaemia
  - Bioluminescence
  - Near Infrared Fluorescence
  - Intravital microscopy

- Example 2: Non Small Cell Lung Cancer (NSCLC)
  - Micro-CT
  - Response to treatment
  - Relapse

X-ray micro-CT

- SkyScan 1176 (SkyScan/Bruker microCT)
  - Lung treatment & evaluation
  - Bone biology
  - Vascular biology
In vivo X-ray Micro-computed tomography (micro-CT)

35µm / 18µm / 9µm
180° / 360°
Physiological monitoring
30 / 40 acquisitions

Imaging autochthonous lung cancers (GEMMs)

  Native kras is expressed in a late-onset lung cancer model
  Mutant kras induces spontaneous autochthonous tumour mouse
  - Lung-specific inducible expression of a non-differentiating insertional Kras allele

  Lentiviral delivery of Cre recombinase
  - Retroviral vector
  - Cre recombinase

- Poli4 K et al, Genes Dev. 2006
  Lung adenocarcinomas induced in mice for mutant EGFR receptors
  - Lentiviral delivery

Lung tumours: Conventional analysis

Counting external nodule

Histology
- nodules diameter, surface
- % surface tumors / lung

Ex vivo
Snapshot [static!]

Imaging a moving "sample" at 35 µm resolution: need for "gating"

Retrospective gated imaging: "physiologic monitoring"

Retrospective gated imaging: "image based sorting"
Longitudinal tracking of individual tumours over weeks

35µm isotropic, retrospective gating

Longitudinal quantification of single nodule volume
Dynamic monitoring of response to intervention

Kumar MS et al. Cell 2012
The CAS9 Transient Knockdown Network is Regulatable for P53 Oncogene-Driven Non-Small Cell Lung Cancer

Cannistra J & F. Lassailly – Cancer Cell 2014
Engagement of transmembrane P75NTR with RAS in Lung Tumor Initiation

Kras LA2-G12D/+; Rosa26 CreERT2/+; Gata2 +/+, Flox/+, Flox/Flox


Kumar MS et al. Cell 2012
CondiKonal EGFR-L858R + dox 4month Selecting resistance to Erlotinib in an inducible model of h-EGFR-L858R

Seq 4-weeks InhIBition

ErloKnib 2 weeks
No treatment

1 2

Tumour 1: 4.4 mm
Tumour 2: 40.8 mm

ErloKnib 2 weeks

1 2

Tumour 1: 2.9 mm
Tumour 2: 23.5 mm

Elza De Bruin Downward’s Lab Tumour 1: 2.8 mm
Tumour 2: 25.1 mm

ErloKnib 2 weeks

1 2

Tumour 1: 3.2 mm
Tumour 2: 26.7 mm


Assessing combination therapies to overcome Erlotinib resistance

Reduced NR11 expression confers resistance to EGFR inhibition in lung cancer

Seq 4-weeks InhIBition

ErloKnib 2 weeks

1 2

Tumour 1: 2.9 mm
Tumour 2: 23.5 mm

ErloKnib 2 weeks

1 2

Tumour 1: 3.2 mm
Tumour 2: 26.7 mm


20
Thoracic CT versus micro-CT

Contrast enhanced micro-CT (blood pool agent)

Automating detection, segmentation and quantification

Computer Aided Detection (CAD)
Further developments for the Francis Crick Institute

Develop Novel Technologies

Photoacoustic imaging

Rolling wave propagation
LIVIm

- Platform to foster exchanges in the field of In Vivo Imaging
  - Any aspect of in vivo imaging
  - Animals (Human)
  - Any imaging technology
  - Any type of application

Registration is free — but required!
livim@cancer.org.uk

MANY THANKS!

NC3R

- Biological Resource Unit
- Experimental Histopathology
- Flow cytometry
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- Luis Pizzaro
- Kheng Swee Lian
- Andreas Bruckbauer
- Facundo Baksta
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- Division Imaging Sciences
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- Ilaria Malanchi
- Tumor Host Interaction
- Erik Sahai
- Tumor Cell Biology
- Simon Boulton
- DNA Damage Lab
- Caetano Reis e Sousa
- Immunobiology Lab
- Axel Behrens
- Mammalian gene
cs Lab
- Charles Swanton
- Translational Cancer Therapeutics Lab
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- Bernard Siow
- Adam Badar
- Tammy Kalber

May Zaw Thin

In Vivo Imaging Facility