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</th>
<th>Success</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>64</td>
<td>6%</td>
</tr>
<tr>
<td>II</td>
<td>67</td>
<td>6%</td>
</tr>
<tr>
<td>III</td>
<td>17</td>
<td>6%</td>
</tr>
<tr>
<td>IV</td>
<td>5</td>
<td>6%</td>
</tr>
</tbody>
</table>

Efficacy!
95% attrition

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

The best model of a cat is a cat or, better, the cat itself.

Norbert Wiener (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

- Project dependent

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

Hanahan D and Coussens LM, Cancer Cell 2012

Orthotopic approaches should be preferred whenever possible

Imaging is required to follow orthotopic tumours

In vivo imaging has long been indispensable in clinical practice
From whole body to subcellular scale

Regulations & Ethics
- Home office
  - Certificate holder
  - Certificate of designation
- Ethical Review Committees
- Health and safety
  - Zoonoses / allergies
  - Chemical risk (anaesthetics, drugs)
  - Ozone
  - Laser safety
  - Radio safety

Longitudinal studies to improve the use of animal models... and the 3Rs!
- Post mortem endpoints
  - 12 mice
  - 4 time points
- Imaging
  - 12 mice
  - Adaptable few points
  - 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
- Residual disease
- Relapse
- Combination therapy

Disease progression correlates inversely with animal welfare

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
  - Scanner
  - Probes
  - Contrast 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

www.london-research-institute.org.uk

First Charity dedicated to cancer

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

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

Example 2: Non Small Cell Lung Cancer [GEMMA]
- Micro-CT
- Response to treatment
- Metagene

The haematopoietic hierarchy

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

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

Labelling cells with luciferase (gene transfer)
- Transgenic animals
  - Ubiquitous or pathway specific
  - Constitutive or inducible

Dynamic/longitudinal tracking & (semi-)quantification
- 48, 48, 33 (9 cell divisions)
- 96 for animal cells
- Carolien Woolthuis & F. Lassailly
  - Metabolic Imaging Group
  - UCL
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)

Fluorescent source
Excitation λ

Whole body Fluorescence Imaging (VIS / NIR)

Adapted from Weissleder R, Nat. Biotech 2001

GFP
Applications: tracking fluorescently labelled leukemic cells

Fluorescent / fluorogenic probes (compatible microscopy)

Imaging glucose metabolism

Could NR-2-deoxyglucose (2DG) be used to monitor human leukemia development non-invasively?
**Study design**

- Luciferase (+)
- Human AML cell lines: U937, HL60, KG1
- NOD/SCID-γcnull (control)

**Cells injection**

- Day 0
- Day 7
- Day 14
- Day 21
- Day 28
- End

**Bioluminescence imaging (BLI)**

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

**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

Hongguang Liu et al. PloSOne 2010

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

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

Tony Gee

30MBq Ctrl
Combined NIR confocal and multiphoton microscopy

- Laser lines:
  - 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

- Benefits:
  - Limited background signal
  - Deeper penetration
  - Low photo-bleaching
  - Low tissue photolysis
  - Possible to image in diffusing environment
Multiparametric analysis of intact bone marrow microenvironment

Intervital 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*


### 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 tumours & metastasis
  - Bone biology
In vivo X-Ray Micro-computed tomography (micro-CT)

In vivo X-ray micro computed tomography provides high resolution images of small structures, allowing for detailed analysis of internal features. The resolution is achieved through the use of very precise X-ray sources and detectors. This technique is particularly useful in medical applications, where the ability to visualize internal structures without invasive procedures is critical. The spatial resolution can be as high as 10 to 20 micrometers, making it possible to study small tumors, lesions, and other microstructures.

Physiological monitoring

Physiological monitoring during the imaging process allows for real-time assessment of the subject's condition. This can be crucial in applications where the subject's response to the imaging process needs to be monitored, such as in the case of live animals or human subjects.

35 µm / 18 µm / 9 µm

At 180° / 360°

Physiological monitoring

30 / 40 acquisitions

Imaging autochthonous lung cancers (GEMMs)

GEMMs (Genetically Engineered Mouse Models) are used to study the development and progression of diseases, including lung cancer. These models allow researchers to study the effects of specific genetic alterations on cancer development, providing insights into the mechanisms underlying cancer progression.


Mouse models of autochthonous lung cancer


Conditioned media of lung cancer cells induces lung adenomas in transplanted fetal mouse lungs


Lung adenocarcinomas induced in mice by mutated Kras can spontaneously regress or progress to alveolar adenocarcinomas

Johnson L et al., Nature. 2001

Somatic activation of K-ras oncogene causes early onset lung cancer in mice


Adenoviral or lentiviral delivery of Cre recombinase

Polia K et al., Genes Dev. 2006

Lung adenocarcinomas induced in mice by mutated EGFR receptors found in human lung cancers respond to a tyrosine kinase inhibitor or to down-regulation of the receptors.

Lung tumours: Conventional analysis

Counting external nodule

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

Ex vivo

Snapshot (stain!)

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

Scan 1
Scan 4

Clare Sheridan, Francois Lassailly
Signal Transduction Lab
Dynamic monitoring of response to intervention

Kumar MS et al. Cell 2012

Castellano E & Sheridan C – Cancer Cell 2013

Dynamic monitoring of response to intervention

CondiKonal EGFR-L858R + dox 4 month

Selecting resistance to Erlotinib in an inducible model of h-EGFR-L858R

Elza de Bruin Downard's Lab

Assessing combination therapies to overcome Erlotinib resistance
Thoracic CT versus micro-CT

Contrast enhanced micro-CT (blood pool agent)

Automating detection, segmentation and quantification

Computer Aided Detection (CAD)
Skeleton analysis

Whole skeleton phenotyping

Bone morphometry

Collaboration with Prof. Paul Gissen – GOSH/ICH-UCL

Immunobiology Lab

Hematopoietic Stem Cells Lab

DNA Damage Response Lab

Ultrasound Imaging Facility

Bioluminescence Fluorescence Intravital microscopy (confocal/multiphoton)

X-ray microCT

Ultrasound

LRI In Vivo Imaging Facility

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Ultrasound
Further developments for the Francis Crick Institute

Photoacoustic imaging

Transient thermoelastic expansion => US waves
LIVIm
Lifelong Imaging Imaging

- Platform to foster exchanges in the field of In Vivo Imaging
- In Vivo Imaging: imaging of live tissues
  - 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!
Together we will beat cancer

Biological Resource Unit
Experimental Histopathology
Flow cytometry
Computing Department
Luis Pizzaro
Kheng Swee Andreas Bruckbauer
Facundo Baksta
Crick – BRF work package group
Kathleen Mathers
Gary Childs

Tumour Modelling Core
Paul Mackin

Division Imaging Sciences
Tony Gee

Signal Transduction Lab
Dominique Bonnet
Haematopoietic Stem Cell Lab
Ilaria Malanchi
Tumour Host Interaction
Erik Sahai
Tumour Cell Biology
Simon Boulton
DNA Damage Lab
Caetano Reis e Sousa
Immunobiology Lab
Axel Behrens
Mammalian gene expression Lab
Charles Swanton
Translational Cancer Therapeutics Lab
Centre for Advanced Biomedical Imaging
Mark Lythgoe
Bernard Siow
Adam Badar
Tammy Kalber