BIOMATH M263 Clinical Pharmacology Lecture

Studying tissue pharmacokinetics by Positron Emission Tomography (PET)

Imaging tumor responses to therapy

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Overview

1. What is PET and how does it work?
2. Using PET for imaging tumor responses to therapy
3. Studying tissue pharmacokinetics by PET
Classes of *in vivo* imaging approaches

- **functional**
  - cardiac function
  - kidney function
  - lung function, etc.
  - *ultrasound*
  - nuclear medicine
  - CT & MRI

- **structural**
  - anatomy lesions
  - X-rays, CT, MRI
Classes of \textit{in vivo} imaging approaches

**molecular**

- biology of disease
- Positron Emission Tomography (PET)

**functional**

- cardiac function
- kidney function
- lung function, etc.
- ultrasound
- nuclear medicine
- CT & MRI

**structural**

- anatomy lesions
- X-rays, CT, MRI
Hyperpolarized $^{13}$C MRI and PET: In Vivo Tumor Biochemistry

Ferdia A. Gallagher$^{1-3}$, Sarah E. Bohndiek$^{1,2}$, Mikko I. Kettunen$^{1,2}$, David Y. Lewis$^1$, Dmitry Soloviev$^1$, and Kevin M. Brindle$^{1,2}$

$^1$Cancer Research United Kingdom Cambridge Research Institute, Cambridge, United Kingdom; $^2$Department of Biochemistry, University of Cambridge, Cambridge, United Kingdom; and $^3$Department of Radiology, Addenbrooke’s Hospital, University of Cambridge, Cambridge, United Kingdom

PET/MRI: The Blended-Modality Choice of the Future?*

Norman E. Bolus$^1$, Remo George$^1$, Johnee’ Washington$^2$, and Bradley R. Newcomer$^1$

$^1$Nuclear Medicine Technology Program, Department of Clinical and Diagnostic Sciences, School of Health Professions, University of Alabama at Birmingham, Birmingham, Alabama; and $^2$Montgomery Cardiovascular Associates, Montgomery, Alabama
Question: What is PET?

Answer: A Molecular Camera
2-[F-18]Fluoro-2-Deoxy-D-Glucose (FDG)

- probes for metabolism, receptors, enzymes, DNA replication, gene expression, antibodies, hormones, drugs, etc.
- in nmole amounts
- labeled with F-18, C-11, O-15, I-124, Cu-64, etc.

How does PET work?

PET scanner
Classes of PET imaging probes

Small molecules
Typically drugs, drug & substrate analogs

Peptides

Antibodies

Aptamers

Nanoparticles

Minibody

Diabody
### Positron-emitting radioisotopes

<table>
<thead>
<tr>
<th>Isotope</th>
<th>Half-life</th>
<th>$\beta^+$ fraction</th>
<th>Max. Energy</th>
<th>Positron Range</th>
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<tbody>
<tr>
<td>C-11</td>
<td>20.4 min</td>
<td>0.99</td>
<td>0.96 MeV</td>
<td>0.4 mm</td>
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<tr>
<td>N-13</td>
<td>9.96 min</td>
<td>1.00</td>
<td>1.20 MeV</td>
<td>0.7 mm</td>
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<td>O-15</td>
<td>123 sec</td>
<td>1.00</td>
<td>1.74 MeV</td>
<td>1.1 mm</td>
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<tr>
<td>F-18</td>
<td>110 min</td>
<td>0.97</td>
<td>0.63 MeV</td>
<td>0.3 mm</td>
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<tr>
<td>Na-22</td>
<td>2.6 years</td>
<td>0.90</td>
<td>0.55 MeV</td>
<td>0.3 mm</td>
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<tr>
<td>Cu-62</td>
<td>9.74 min</td>
<td>0.98</td>
<td>2.93 MeV</td>
<td>2.7 mm</td>
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<tr>
<td>Cu-64</td>
<td>12.7 hours</td>
<td>0.19</td>
<td>0.65 MeV</td>
<td>0.3 mm</td>
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<tr>
<td>Ga-68</td>
<td>68.3 min</td>
<td>0.88</td>
<td>1.90 MeV</td>
<td>1.2 mm</td>
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<tr>
<td>Rb-82</td>
<td>78 sec</td>
<td>0.96</td>
<td>3.15 MeV</td>
<td>2.8 mm</td>
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<tr>
<td>I-124</td>
<td>4.18 days</td>
<td>0.22</td>
<td>3.16 MeV</td>
<td>2.8 mm</td>
</tr>
</tbody>
</table>

- The most widely used isotopes are F-18, C-11, O-15 and N-13
- These are produced in cyclotrons and used on-site or nearby due to the short half-life
- Longer lived isotopes, such as I-124, Cu-64 and Na-22, are produced in reactors
- Short lived isotopes like Rb-82 and Ga-68 are made using generators and must be used on site due to the very short half life
PET imaging probes/biomarkers

- Predict the presence of disease and/or characterize it
- Predict response to therapy

**General Markers:**
- Glycolysis - FDG
- Proliferation - FLT
- Lipids - FLC
- Apoptosis - Annexin V

**Specific Markers:**
- Neuroendocrine – Octreotide
- Parkinson's - Dopascan
- Alzheimer's - FDDNP
Use of PET in cancer management

- Initial diagnosis
- Staging
- Assessment of responses to therapy
- Detection of disease recurrence after treatment
FDG PET/CT monitoring of cancer treatment

SUV 4.8 5.4 4.2

non-responder

SUV 18.4 3.9 11.0

responder
NSCLC: an example of a partial response

PET

prior to therapy

CT

at three weeks
NSCLC: partial response

CT at 3 months confirms PET findings from 3 weeks
NSCLC: non-responder

prior to therapy

PET

at three weeks

CT
NSCLC: non-responder

CT at three months confirms PET findings from 3 weeks
Erlotinib responders vs. non-responders

PET/CT

baseline
follow-up

PET

SUV = 10.3
SUV = 4.6

percent survival

P=0.03

metabolic responders
metabolic non-responders

time (days)
FLT Imaging in glioblastoma: responses to Avastin + Irinotecan

Current approach

Standard Treatment
Staging with CT
Assess Responses with CT
Modify treatment based on CT

Future approach

PET based Treatment Selection
Assess Responses with PET/CT
Modify treatment
Expanding the use of PET in cancer

- Initial diagnosis
- Staging
- Treatment stratification
- Detection of disease recurrence after treatment
- Assessment of responses to therapy
A new PET probe for visualizing DNA metabolism

1-(2’ deoxy-2’,-\textsuperscript{18}Fluoro-Arabinofuranosyl) Cytosine
\textsuperscript{[18F]}FAC
Thymidine kinase 1 (TK1) and deoxycytidine kinase (dCK) are enzymes in the nucleoside salvage pathway.
$^{18}$F-FAC is a substrate for dCK

deoxycytidine kinase (dCK)

$^{18}$F-FAC-PO$_4$ trapped
Investigating probe specificity:
Lack of $^{18}$F-FAC uptake in dCK KO mice
\( ^{18} \text{F-FAC} \) resembles gemcitabine and related prodrugs

- If the activity of dCK in tumor lesions is low or absent, gemcitabine and the related prodrugs don't work!

- The problem is how to measure dCK activity?
$^{18}$F-FAC distinguishes dCK-positive and dCK-negative tumors and predicts responses to gemcitabine

Laing et al., PNAS 2009 Feb;106(8):2847-2852.
Many dCK dependent prodrugs are used in cancer and other diseases

<table>
<thead>
<tr>
<th>dCK-dependent prodrug</th>
<th>Structure</th>
<th>&gt;1,000 open clinical trials (ClinicalTrials.gov) and Indications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytarabine (Ara-C)</td>
<td><img src="image1" alt="Structure" /></td>
<td>&gt;150 studies in combination with a variety of drugs, in both solid and liquid tumors. Indicated in acute myeloid leukemia (AML) and lymphoma.</td>
</tr>
<tr>
<td>Fludarabine (Fludara) Bayer</td>
<td><img src="image2" alt="Structure" /></td>
<td>&gt;300 studies, many in B-cell chronic lymphocytic leukemia (CLL), some in bone marrow transplant conditioning. Indicated in CLL and non-Hodgkin's lymphoma.</td>
</tr>
<tr>
<td>Cladribine (Leustatin®) Ortho Biotech</td>
<td><img src="image3" alt="Structure" /></td>
<td>17 studies. Indicated in Hairy Cell leukemia, AML, a variety of lymphomas, Waldenstrom's Macroglobulinemia, CLL currently awaiting FDA approval for Multiple Sclerosis (developed by Merck/EMD Serono).</td>
</tr>
<tr>
<td>Gemcitabine (Gemzar) Lilly</td>
<td><img src="image4" alt="Structure" /></td>
<td>&gt;300 studies in a variety of cancers. Indicated in pancreatic, ovarian, breast and non-small cell lung cancers, and many others.</td>
</tr>
<tr>
<td>Clofarabine (Clolar) Genzyme</td>
<td><img src="image5" alt="Structure" /></td>
<td>64 studies in leukemia, NHL, AML, MDS. Indicated in pediatric ALL.</td>
</tr>
<tr>
<td>Decitabine (Dacogen®) Supergen</td>
<td><img src="image6" alt="Structure" /></td>
<td>32 studies in a variety of cancers. Indicated for myelodysplastic syndromes (MDS).</td>
</tr>
</tbody>
</table>
FDA approval process for PET probes

Investigational New Drug Application
Submit IND

Clinical Research

Phase I
Phase II
Phase III

New Drug Application
Submit NDA
NDA approval

Pre-Clinical Testing R&D

Imaging probe

Limited human studies can be done through:

- RDRC: Radioactive Drug Research Committee approval
- eIND: Exploratory Investigational New Drug Application

“Phase 0”
<table>
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<tr>
<th>Purpose</th>
<th>RDRC</th>
<th>eIND</th>
<th>IND</th>
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<tr>
<td>- Only for basic research</td>
<td>- For Micro-dose/limited dose and exposure studies</td>
<td>- For clinical investigation of radioactive probes</td>
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<tr>
<td>- Only for radioactive probes, where the non-radioactive form has been tested in humans</td>
<td>- Can be used to screen 2-5 probes simultaneously</td>
<td>- For therapeutic, diagnostic and preventative use</td>
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<tr>
<td>- Not intended for diagnostic/therapeutic</td>
<td>- Not intended for diagnostic/therapeutic</td>
<td>- For determining safety/efficacy</td>
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<table>
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<td>- Clinical protocol</td>
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<td>- Manufacturing under USP 823 guidelines</td>
<td>- Manufacturing under USP 823 guidelines</td>
<td>- Manufacturing under USP 823 guidelines</td>
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<tr>
<td>- Limited safety/tox studies in rats</td>
<td>- Tox studies in 1 specie</td>
<td>- Tox and safety pharm studies in 2 species</td>
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<tr>
<td></td>
<td>- No safety pharm studies</td>
<td>- Genotoxicity studies</td>
<td></td>
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<tr>
<td></td>
<td>- No genotoxicity studies</td>
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<tr>
<td>Up to 30</td>
<td>Up to 30</td>
<td>No limit</td>
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Imaging probe IND submission

1. Pre-clinical safety studies
2. Pre-IND meeting with FDA
3. IND submission
4. Initiate phase 1 clinical trials

### Toxicty Testing
- Duration: 14 days
- Analyze:
  - CBC
  - Blood Chemistry
  - Histopathology

### Safety Pharmacology
- Duration: 14 days
- Analyze:
  - EKG
  - Blood pressure
  - Heart rate
  - Weight
  - Pulse oximetry
  - Body temp

### Radiation Safety
- Dynamic analysis of radioactivity within major organs
- Absorbed dose calculations
Are INDs affordable for academic sites? YES!

The Department of Molecular and Medical Pharmacology at UCLA has established a streamlined, cost-effective path for obtaining INDs for imaging probes:

- **Pre-Clinical Safety/Tox studies**
  Performed by UCLA Division for Laboratory Animal Medicine under GLP
  - $50,000/probe

- **Dosimetry studies**
  Performed by Dr. David Stout at UCLA Crump Institute for Molecular Imaging

- **Probe manufacturing**
  By UCLA Biomedical cyclotron facility under USP

- **Clinical trials**
Potential metabolism of $^{18}$F-FAC

$^{18}$F-FAC $\xrightarrow{\text{CDA}}$ $^{18}$F-FAU

$^{18}$F-FAC $\xrightarrow{\text{dCK}}$ $^{18}$F-FAC-MP

dCK: deoxycytidine kinase
CDA: cytidine deaminase
$^{18}$F-FAC and 2\textsuperscript{nd} generation analogs

resistant to deamination by CDA

Shu, Campbell et al. JNM, 2010
Timeline of our approach for the FAC family of probes

Discovery and Pre-Clinical testing

- L-[\(^{18}\)F]-FAC
- D-[\(^{18}\)F]-FAC
- L-[\(^{18}\)F]-FMAC

Human studies

- Human Biodistribution studies using RDRC 2007-2011
- Obtained INDs for all 3 probes in 2011
- Phase 1 trials for all 3 probes 2012-
Biodistribution of $^{18}$F-FAC and analogs in healthy volunteers

Schwarzenberg et al; European J Nucl Med Mol Imaging 2010
Applications: FAC resembles gemcitabine and related prodrugs

- **Drug**: gemcitabine (dFdC)
- **PET probe**: ¹⁸F-FAC

Transporter (ENT1)

- Extracellular
- Intracellular

Antitumor effects

Accumulation in cancer cells detected by PET

Nucleoside Transporter (ENT1)

DNA

Nucleus
Tumor dCK expression is associated with prolonged survival after adjuvant gemcitabine in pancreatic adenocarcinoma.

Can PET be used to estimate dCK expression in tumors, and predict responses to gemcitabine?
Clinical studies in pancreatic cancer

Increased L-FMAC uptake in primary tumor...
Clinical studies in pancreatic cancer

*L-FMAC negative 2 cm metastatic lymph node (biopsy proven)*
Clinical studies in pancreatic cancer

*Known pancreatic head cancer without probe uptake (non-resectable)*
dCK protein correlates with $^{18}$F-FAC PET imaging in an ovarian cancer patient

Tumor site visualized on both scans

Pre-therapy imaging

L-FMAC Scan | FDG-Scan

Tumor histology | dCK expression is detected in epithelial tumor cells

H&E | α-dCK | α-dCK

IHC with anti-Hu-dCK monoclonal antibody
M. Riedinger (Witte Lab)
Diffuse Large B-cell Lymphoma
Ongoing translational studies

Patient

\(^{18}\text{F}\)-L-FAC PET/CT scan followed by biopsy/surgery

Tumor sample

tumor bank

dCK and CDA measurements

xenograft studies in mice
Conclusions

• Molecular imaging is a powerful diagnostic tool to assist treatment stratification and monitoring in cancer, as well as basic research

• Widely available PET probes such as $^{18}$F-FDG and $^{18}$F-FLT provide information about classic and emerging hallmarks of cancer; this information can be complemented by newer specialized probes such as the $^{18}$F-FAC tracers

• Significant challenges in molecular imaging still need to be addressed
>1600 PET probe have been synthesized, however 99.6% of clinical studies use one probe...
What explains the dominance of FDG?

1. FDG is very useful

2. The utility of the vast majority of probes has not been demonstrated. Why?

3. The cost of translating a new PET probe to the clinic can be substantial.

4. There may be many other useful probes, but the technology to radiolabel them does not exist.
$^{18}$F-glutamate derivative BAY94-9392 (left) vs. FDG (right)

brain tumor

head and neck tumor

Mitra et al; JNM 2011