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 this technology work?
2. Using PET for imaging tumor responses to therapy
3. Studying tissue pharmacokinetics by PET
“~70% of the decisions made by physicians in the USA are based on the results of a diagnostic test…

…yet only 2% of the US $2 trillion spent annually on healthcare goes into diagnostics”
Diagnostics and drugs

in vitro diagnostics 
&
in vivo diagnostics
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 *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
Question: What is PET?

Answer: A Molecular Camera
How does PET work?

- 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.
Classes of PET imaging probes

Small molecules
Typically drugs, drug & substrate analogs

Peptides

Antibodies

Aptamers

Nanoparticles
## 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>
</tr>
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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>
</tr>
<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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<tr>
<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>
</tr>
<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
General PET biomarkers

**PLASMA**

- Glucose
  - FDG → FDG Transporter
  - FDG

**TISSUE**

- Hexokinase
  - FDG-6-PO₄

- Glucose-6-phosphatase

- Glycolysis

- Pyrimidine
  - FLT → FLT Transporter
  - FLT

- Thymidine Kinase
  - FLT-5-PO₄

- Thymidine-dephosphorylase

- DNA
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

non-responder

SUV 4.8

responder

SUV 18.4

SUV 4.2

SUV 5.4

PET/CT monitoring of cancer treatment.

1st cycle

PET/CT

early follow-up

PET/CT

late follow-up pathology

chemo/radiation

PET/CT

surgery
NSCLC: an example of a partial response

prior to therapy

at three weeks

PET

CT

red arrows indicate the area of interest
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

time (days)

metabolic responders

metabolic non-responders
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

tissue pharmacokinetics
A new PET probe for visualizing DNA metabolism

1-(2’ deoxy-2’,-\(^{18}\)Fluoro-Arabinofuranosyl) Cytosine
\[^{18}\text{F}]\text{FAC}

Molecular imaging of lymphoid organs and immune activation by positron emission tomography with a new \[^{18}\text{F}]-labeled 2’-deoxycytidine analog

Caius G Radu\(^1\), Chengyi J Shu\(^1\), Evan Nair-Gill\(^1\), Stephanie M Shelly\(^1\), Jorge R Barrio\(^1\), Nagichettiar Satyamurthy\(^1\), Michael E Phelps\(^{1,2}\) & Owen N Witte\(^{1,3,4}\)
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
18F-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?
18F-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="image" alt="Cytarabine 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)</td>
<td><img src="image" alt="Fludarabine 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®)</td>
<td><img src="image" alt="Cladribine 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)</td>
<td><img src="image" alt="Gemcitabine 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)</td>
<td><img src="image" alt="Clofarabine Structure" /></td>
<td>64 studies in leukemia, NHL, AML, MDS. Indicated in pediatric ALL.</td>
</tr>
<tr>
<td>Decitabine (Dacogen®)</td>
<td><img src="image" alt="Decitabine Structure" /></td>
<td>32 studies in a variety of cancers. Indicated for myelodysplastic syndromes (MDS).</td>
</tr>
</tbody>
</table>
Translation of FAC to the clinic
FDA approval process for PET probes

Investigational New Drug Application
Submit IND

Clinical Research

New Drug Application
Submit NDA
NDA approval

Pre-Clinical Testing R&D

Phase I
Phase II
Phase III

“Phase 0”

Limited human studies can be done through:

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

Imaging probe
### Comparing RDRC, eIND and IND approaches

<table>
<thead>
<tr>
<th></th>
<th>RDRC</th>
<th>eIND</th>
<th>IND</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Purpose</strong></td>
<td>- Only for basic research</td>
<td>- For Micro-dose/limited dose and exposure studies</td>
<td>- For clinical investigation of radioactive probes</td>
</tr>
<tr>
<td></td>
<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>
</tr>
<tr>
<td></td>
<td>- Not intended for diagnostic/therapeutic</td>
<td>- Not intended for diagnostic/therapeutic</td>
<td>- For determining safety/efficacy</td>
</tr>
<tr>
<td><strong>Requirements</strong></td>
<td>- Determined by RDRC</td>
<td>- Determined by FDA</td>
<td>- Determined by FDA</td>
</tr>
<tr>
<td></td>
<td>- Clinical protocol</td>
<td>- Clinical protocol</td>
<td>- Clinical protocol</td>
</tr>
<tr>
<td></td>
<td>- Manufacturing under USP 823 guidelines</td>
<td>- Manufacturing under USP 823 guidelines</td>
<td>- Manufacturing under USP 823 guidelines</td>
</tr>
<tr>
<td></td>
<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>
</tr>
<tr>
<td></td>
<td></td>
<td>- No safety pharm studies</td>
<td>- Genotoxicity studies</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- No genotoxicity studies</td>
<td></td>
</tr>
<tr>
<td><strong>Approval</strong></td>
<td>Approval by RDRC</td>
<td>Approval by FDA</td>
<td>Approval by FDA</td>
</tr>
<tr>
<td><strong>Subject #</strong></td>
<td>Up to 30</td>
<td>Up to 30</td>
<td>No limit</td>
</tr>
</tbody>
</table>
Pre-clinical safety studies → Pre-IND meeting with FDA → IND submission → Initiate phase 1 clinical trials

**Toxicity 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

Imaging probe IND submission
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:

$50,000/probe

- **Pre-Clinical Safety/Tox studies**
  Performed by UCLA Division for Laboratory Animal Medicine under GLP

- **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

$dCK$: deoxycytidine kinase

$CDA$: cytidine deaminase
$^{18}$F-FAC and 2nd 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

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.
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

Pre-therapy imaging

L-FMAC Scan

Tumor site visualized on both scans

FDG-Scan

Tumor histology dCK expression is detected in epithelial tumor cells

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

Patient

$^{18}$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.
18F-glutamate derivative BAY94-9392 (left) vs. FDG (right)

brain tumor

head and neck tumor

Mitra et al; JNM 2011