Wp6. Cancer Imaging with focus on breast cancer

Anikitos Garofalakis

‘Frederic Joliot Hospital Service(SHFJ), Institute of Biomedical imaging(I²BM), Atomic Energy Commission(CEA)’
The use of animal models for the evaluation of FMT under realistic conditions

**Objectives of Wp6:**

To provide cancer animal models

To provide key fluorescence probes

Quantitatively examine FMT performance to visualize disease processes in-vivo

To predict clinical utility  (48 month deliverable)
Animal models

- a. xenografted tumors
  - MDA-231
  - MCF-7
  - PC12-MEN2A
  - U87
  - PyMT
  - rat pheochromocytoma
  - human glioma

- b. spontaneous tumors
  - PyMT

Fluorescent probes

- a. commercial probes
  - Prosense
  - RGD-based optical probes (Integrisense680, Angiostamp680)
  - Angiosense

- b. custom-made probes
  - Aptamers
  - Nano-micelles

- Cathepsin activity
- Integrin avβ3
- Blood volume
fluorescent probes for tumor-related processes

- Integriν avβ3
- Neo-angiogenesis
- Cathepsin
- ECM degradation
- Blood volume
- FDG
Quantitatively examine FMT performance to visualize disease processes in-vivo

<table>
<thead>
<tr>
<th></th>
<th>μPET</th>
<th>FMT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type of information</strong></td>
<td>Molecular</td>
<td>Molecular</td>
</tr>
<tr>
<td><strong>Resolution</strong></td>
<td>~ 1 mm</td>
<td>~ 1 mm</td>
</tr>
<tr>
<td><strong>Kinetics</strong></td>
<td>Fast (~seconds)</td>
<td>5-15 min/scan</td>
</tr>
<tr>
<td><strong>Whole Body Imaging</strong></td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td><strong>Time range of follow up</strong></td>
<td>~ hours</td>
<td>~days</td>
</tr>
<tr>
<td><strong>Cost</strong></td>
<td>$$$</td>
<td>$</td>
</tr>
<tr>
<td><strong>Activatable probes</strong></td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td><strong>Labelling</strong></td>
<td>Need radiochemistry</td>
<td>Simple</td>
</tr>
<tr>
<td><strong>Quantification</strong></td>
<td>Linear from pM</td>
<td>?</td>
</tr>
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</table>
calibration of FMT

Use of tubes filled with fluorophores

Use of the quantification capacity of PET imaging in combination with a dual PET/Optical probe

vs [C]

PET

FMT

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FMT-PET-CT imaging

in vivo

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fDOT-PET-CT imaging/protocol

PET

FMT

PET

CT

0 min

30 min

60 min

90 min

CT contrast agent

targeted biological molecule
calibration/FMT-PET-CT imaging

PET/FMT/CT

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b./.calibration/fDOT-PET-CT imaging/volume validation
Sensitivity limit ~ 1 pmoles, 5nM(C)

**PET-FMT dual imaging**

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<td>Quantification</td>
<td>Linear from pM</td>
<td>Linear from nM</td>
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cancer imaging in combination with PET

Angiostamp680
ControlAngiostamp680
Angiosense750

FMT
PET
CT
FMT
day -1
day 0
day 1
day 2 ....

Prosense680
MDA-231/FDG-Cathepsin

Volume Mean

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Prosense_DAY_1</td>
<td>317.781</td>
<td>3.5</td>
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<tr>
<td>Prosense_DAY_2</td>
<td>412.45</td>
<td>3.5</td>
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<tr>
<td>Prosense_DAY_3</td>
<td>373.337</td>
<td>4.0</td>
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</table>

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MDA-231/FDG – Integrin αvβ3

RGD-based ligand

Control ligand

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models and probes

a. xenografted tumors

- MDA-231
- MCF-7
- PC12-MEN2A
- U87

  - MDA231/ Cathepsin activity
  - MDA231/ Integrin localization
  - MDA231/ Nanomicelle labelling

b. spontaneous tumors

- PyMT

  - PC12-MEN2A/ Cathepsin activity
  - PC12-MEN2A/ Cathepsin activity/ Integrin localization

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>MDA-231</th>
<th>MCF-7</th>
<th>PC12-MEN2A</th>
<th>U87</th>
</tr>
</thead>
<tbody>
<tr>
<td>% signal overlapping</td>
<td>6.6 ± 2.5</td>
<td>6.7 ± 5.0</td>
<td>34 ± 12</td>
<td>31.8 ± 12.0</td>
</tr>
<tr>
<td>% volume overlapping</td>
<td>37.6 ± 8.1</td>
<td>39.0 ± 18.6</td>
<td>40.03 ± 19.02</td>
<td>41.2 ± 12.5</td>
</tr>
</tbody>
</table>

HMGU@Munich Del 7.4

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MCFS7 xenografted tumor/aptamers ACE8

“Annexin II” as potential target?
To provide cancer animal models

**Del 6.3** To develop animal models of other cancer for studying FMT-XCT performance (U87 glioma cells)

To provide key fluorescence probes

2nd year deliverable (target of aptamer ACE8 has to be identified)

Quantitatively examine FMT performance to visualize disease processes in-vivo

**Del 6.4** To perform in-vivo imaging of key animal models of cancer and correlate the findings with standard laboratory tests and growth measures

To predict clinical utility (48 month deliverable)
**Acknowledgements**

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Frédéric Duongé, Dr

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Bertrand Czarny, Technician

**Group of radiochemistry et radio-pharmacy (GRR)**

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Frederic Dollé, Dr

**ex-members:**  
Agnes Cibiel, Dr  
Carine Pestourie, Dr  
Daniel Dupont, Dr  
Nicolas Mackiewicz, Dr  
Isabelle Jassens, Technician
a/fDOT-alone output

PC12-MEN2A with SentiDye® 700

coronal

sagittal

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fDOT is often combined with a structural modality
b./.cancer imaging in combination with PET/FDG-Two color fDOT

![Unmixed AngioStamp680](image1)

![Unmixed AngioSense750](image2)

- [F18]FDG segmented volume
- AngioStamp680 signal segmented volume
- AngioSense750 signal segmented volume

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b./calibration/fDOT-PET-CT imaging/protocol

PET  fDOT  PET  CT

0  30  60  90  min

Kidney contrast agent

Oligo

+ 

Known biodistribution

Deep seated organ

No degradation
b./.calibration/fDOT-PET-CT imaging
b./.calibration/fDOT-PET-CT imaging/volume validation

a) PET segmented volume
b) Optical signal segmented volume

PET/CT
fDOT/CT

PET segmented volume
Optical signal segmented volume
CT contrast
Limit ~ 1 pmoles, 5nM(C)
a/fDOT imaging

hCd2-GFP mouse

Garofalakis A et al
Diffuse light in a homogeneous medium

Continuous wave point source

\[ \nabla^2 U(\mathbf{r}) - \kappa^2 U(\mathbf{r}) = -\frac{S_0 \delta(\mathbf{r})}{D} \]

\[ \kappa = \sqrt{\frac{\mu_\alpha}{D}} \]

Solution for the energy density

\[ U(\mathbf{r}) \propto e^{-\kappa r} \]

\[ J_n \]

Source

Diffusive semi–infinite medium
b./.MDA-231/Integrisense and AngioStamp comparison

**Integrisense 0h**

- Tumor volume ~ 730 mm³
- 60.2% signal of optical in the surrounding
- 30.01% volume of tumor in the common area

**Integrisense 3h**

- 31.42% signal of optical in the surrounding
- 32.55% volume of tumor in the common area

**RAFT-RGD 0h**

- Tumor volume ~ 670 mm³
- 47.29% signal of optical in the surrounding
- 29.98% volume of tumor in the common area

**RAFT - RGD 2h**

- 36.2% signal of optical in the surrounding
- 36.48% volume of tumor in the common area
Discretization and weight matrix

- Sample the fluorescent concentration in \( N \) points (voxels)
- Convert integral equations into a system of linear equations

For \( M \) projections: \[
P[M \times 1] = W[M \times N] \cdot N[N \times 1]
\]
Commercial probes

- Prosense 680
- Integrinsense 680 (VisenMedical, USA)
- AngioStamp (Fluoptics, France)

Custom-made probes

- MPEG nano-micelles
b/./calibration

Use of fluorophores of controlled concentration

Use of the quantification capacity of PET imaging in combination with a dual PET/Optical probe

Low cost method but not realistic

High cost method but realistic