AGENTs D’IMAGERIE

17/07/14

Franck DENAT

Institut de Chimie Moléculaire de l’Université de Bourgogne
UMR CNRS 6302
Molecular imaging: a powerful tool for various applications

- Surgery assistance
- Caracterization of a tumor model
- Monitor the efficiency of a treatment
- Diagnosis agents / treatment
- Selection of drug candidates
- Biodistribution and PK studies of new drugs
- Development of new agents for RIT
- Proof of concept

... Needing transdisciplinarity

**CHEMISTRY**
- Synthesis

**BIOLOGY**
- Direct/indirect labeling
- in vitro validation

**PRECLINICAL IMAGING**
- in vivo validation

**PHASE 0 CLINICAL STUDIES**
Molecular imaging: a growing market

Growing market 5 years projection
$10 billion in 2012 to $14 billion in 2017

Contrast agents
This market has been segmented into indication (cardiovascular, neurology, oncology, respiratory, nephrology, gastrointestinal, and musculoskeletal) and segmented into medical procedure (X-ray, CT, MRI, ultrasound). Main contrast agents family are barium-, iodine- and gadolinium-based.

Radiopharmaceuticals
The market of radiopharmaceuticals is dominated by diagnostic radioisotopes, comprising of SPECT and PET radioisotopes. The lion's share of the SPECT market is taken up by Tc-99m, whereas the PET market is dominated by F-18FDG. Furthermore, the PET market will witness a double digit growth during the forecast period. The therapeutic segment contributes only 10% to the global radiopharmaceuticals market.

<table>
<thead>
<tr>
<th>Product</th>
<th>2012 (Billion $)</th>
<th>2017 (Billion $)</th>
<th>Growth rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contrast agents</td>
<td>6.2</td>
<td>8.6</td>
<td>6.8%</td>
</tr>
<tr>
<td>Radiopharmaceuticals</td>
<td>3.8</td>
<td>5.5</td>
<td>7.8%</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>14.1</td>
<td></td>
</tr>
</tbody>
</table>

http://www.marketsandmarkets.com/PressReleases/contrast-media.asp
http://www.marketsandmarkets.com/PressReleases/radiopharmaceuticals.asp
PET or MRI system equipments in developed countries
Growth on emerging markets (China, India, Brasil, …)
Generic agents with low margins (\(^{99m}\)Tc MIBI / Iodine / Gadolinium / \(^{18}\)F FDG)

Low number of Marketing approval: Lymphoseek, Amyvid, Neuraceq, Vizamyl
Low reimbursement rate: new Alzheimer PET agents can reach $50 Millions worldwide*
Reduced Innovation in big players: Siemens, GE, Guerbet, Bracco
Spin-off business: Lantheus (BMS), Malinckrodt (Covidien), Piramal (Bayer)
Biotech boom: Blue Earth Diag, Ground Fluor, Fluoropharma, imaginAb …

Growing market but changing innovation panorama: risk takers, low capital biotechs

* Initial projections were $500 millions:
Sustain innovation
Theranostic: biomarker positioning of imaging agents

- **Efficacy** - MI can provide earlier indications of the effectiveness of a compound by demonstrating if it has reached and bound to its target.

- **Response to therapy** - beyond simply confirming binding to a target, MI can provide indications that the target has been altered, reduced or eliminated.

- **Earlier detection of disease** - MI technologies with high sensitivity can identify indications of disease before the disease is apparent. This offers the opportunity to test compounds that may be effective at early stages of disease.

- **Drug delivery** - a probe that can reach and bind to a target to image it could potentially deliver a payload of a therapeutic compound that would affect only the target cells and cells nearby. This is particularly important for cancer, where the therapeutics are toxic, and in the brain, where a major challenge is getting drugs into the brain.

- **Pharmacokinetics and dosing** - a key step in a drug’s development is understanding how the drug is absorbed into the body, distributed to its target, metabolised and eliminated. The ability to more accurately track those processes can more quickly determine whether the drug is intact long enough to reach its target, and also assess the minimum required dose.

- **Toxicity** - the accurate tracking of a drug’s progress through the body can help identify potentially toxic compounds earlier.

- **Distinguishing between responders and non-responders** - MI can more clearly demonstrate mechanisms of action related to genetic or structural differences. Similarly, identifying subsets of patients who have an unwanted reaction allows the drug to be better targeted and reduces the chances of failure in clinical trials.

- **Surrogate end-points for drug approval** - the standard by which drugs are approved is their effect on mortality and morbidity. These are long-term measurements and morbidity has a subjective component. The results of imaging studies (surrogate end-point) enable a faster and more objective demonstration of results that can, if validated and qualified, speed clinical trials and drug approvals.
## Molecular imaging

**A MOLECULAR IMAGING PRIMER: MODALITIES, IMAGING AGENTS, AND APPLICATIONS**

Michelle L. James and Sanjiv S. Gambhir

<table>
<thead>
<tr>
<th>Type</th>
<th>Small molecule</th>
<th>Peptide</th>
<th>Affibody</th>
<th>Aptamer</th>
<th>Antibody</th>
<th>Nanoparticle</th>
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<td>~10–200 nm</td>
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<td></td>
<td></td>
<td>(no larger than 1,000 nm)</td>
</tr>
<tr>
<td><strong>Example</strong></td>
<td><img src="image" alt="FDG" /></td>
<td>RGD-Cy5.5</td>
<td><img src="image" alt="Beacon" /></td>
<td><img src="image" alt="Aptamer" /></td>
<td><img src="image" alt="Antibody" /></td>
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</tr>
</tbody>
</table>

- [F]FDG
- RGD-Cy5.5
- [Ga]DOTA-MUT-DS
- Molecular Beacon
- ICG-Trastuzumab
- RGD-SWNT

### Notes
- Quencher
- Donor
- Signaling component
**A MOLECULAR IMAGING PRIMER: MODALITIES, IMAGING AGENTS, AND APPLICATIONS**

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### DIRECT LABELING

<table>
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<td></td>
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<td>~0.5–2 kDa</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Example**

- $[^{18}F] $FDG
- $[^{18}F] $FDG
- RGD-Cy5.5
- $[^{68} $Ga] $DOTA-MUT-DS
- Molecular Beacon
- ICG-Trastuzumab
- RGD-SWNT

**Legend**

- Green: Quencher
- Red: Donor
- Orange: Signaling component
Small molecules: Direct labeling

$[^{18}\text{F}]$ FDG

$[^{18}\text{F}]$ methionine

Onco/cardio/neuro

Brain tumor imaging

$[^{18}\text{F}]$ FDG

$[^{11}\text{C}]$ methionine

$[^{11}\text{C}]$ methionine

$[^{11}\text{C}]$ methionine
Molecular imaging

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</tr>
<tr>
<td>Example</td>
<td>[¹⁸F]FDG</td>
<td>RGD-Cy5.5</td>
<td>[⁶⁸Ga]-DOTAMUT-DS</td>
<td>Molecular Beacon</td>
<td>ICG-Trastuzumab</td>
<td>RGD-SWNT</td>
</tr>
</tbody>
</table>

Diagram: Green: Quencher, Red: Donor, Orange: Signaling component
Peptide labeling

**Direct** $^{18}$F labeling of 4-nitro-3-trifluoromethylbenzoyl-c(RGDfk)

- $^{18}$F 110 min $\beta^+$ 635 keV

- Imaging of $\alpha\nu\beta$ 3 integrin expression

**Indirect** $^{68}$Ga labeling of RGD-PEG-DOTA

- $^{68}$Ga 67.7 min $\beta^+$ 1899 keV

- Need for a bifunctional chelating agent

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O. Jacobson et al., *Bioconjugate Chem.*, 2011, 422-428

Peptide labeling

Imaging Agents targeting NET (octreotide analogs)

\[ ^{111}\text{In-DOTA} \quad \text{(Octreoscan®)} \quad \text{vs} \quad ^{111}\text{In-DTPA} \quad \text{(ant)} \]

Design of radiolabeled bioconjugates

- Affinity – specificity for the target
- Stability of the immunoconjugate under physiological conditions
- Blood half life
- Permeability
- Rate of excretion

Biological targeting molecule: PK Affinity Specificity Clearance

Selection of the radiometal: Imaging method Half life Availability

Selection of the optimized CHELATOR: coordination properties, synthesis

Bioconjugation: Grafting function Linker properties Pretargeting

Radiolabeling Imaging
Design of radiolabeled bioconjugates

Carrier Vector
- Antibody Fragment
- Peptide
- Nanoparticle

Chelator BFC

Grafting function
- NCS
- Activated ester
- Maleimide
- Click chemistry

Linear Macrocyclic

SPECT: $^{111}$In, $^{99m}$Tc, ...

PET: $^{64}$Cu, $^{89}$Zr, $^{68}$Ga, ...

RIT: $^{90}$Y, $^{177}$Lu, $^{212}$Pb, ...

## Design of radiolabeled bioconjugates

<table>
<thead>
<tr>
<th>Common emitters: SPECT imaging</th>
<th>Generator</th>
<th>Worldwide GMP</th>
<th>Leas expensive radionuclide</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{99m}$Tc</td>
<td>(99mMo from reactor)</td>
<td>Worldwide GMP</td>
<td>Least expensive radionuclide</td>
</tr>
<tr>
<td>$^{131}$I</td>
<td>Cyclotron</td>
<td>Limited supply</td>
<td>Very expensive, high energy, limited manufacturing capacity.</td>
</tr>
<tr>
<td>$^{201}$Tl</td>
<td>Cyclotron</td>
<td>Very limited supply</td>
<td>Dedicated network of high energy cyclotrons is needed.</td>
</tr>
<tr>
<td>$^{111}$In</td>
<td>Cyclotron</td>
<td>Worldwide GMP</td>
<td>Expensive radionuclide – use presently limited by the number of applications</td>
</tr>
<tr>
<td>$^{67}$Ga</td>
<td>Reactor</td>
<td>Worldwide GMP</td>
<td>Same chemistry as technetium, but much more expensive – number of applications presently limited</td>
</tr>
</tbody>
</table>

**Positron emitters: PET imaging**

<table>
<thead>
<tr>
<th>Common emitters: PET imaging</th>
<th>Generator</th>
<th>Specific equipped locations</th>
<th>Still not available worldwide. Needs further investment in cyclotrons and GMP manufacturing sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{18}$F</td>
<td>Cyclotron</td>
<td>Worldwide GMP</td>
<td>Highest potential – several companies are presently developing GMP grade generators. MA will come later</td>
</tr>
<tr>
<td>$^{82}$Br</td>
<td>Cyclotron</td>
<td>Worldwide GMP</td>
<td>Worldwide supply is easy to secure</td>
</tr>
<tr>
<td>$^{11}$C</td>
<td>Cyclotron</td>
<td>Available only locally</td>
<td>Easy to produce, but large investment needed to supply at a worldwide level as existing cyclotrons are not adapted for this radionuclide production. Potential future in therapy (Auger electron)</td>
</tr>
</tbody>
</table>

**Beta and alpha emitters: therapeutic agents**

<table>
<thead>
<tr>
<th>Common emitters: Therapeutic agents</th>
<th>Generator</th>
<th>Worldwide GMP</th>
<th>Probably therapeutic radionuclide with the best profile. Care: two quantities of $^{177}$Lu available and high specific activity will have preference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{177}$Lu</td>
<td>Reactor</td>
<td>Worldwide GMP</td>
<td>Several sources available – no investment required, no risk of shortage</td>
</tr>
<tr>
<td>$^{90}$Y</td>
<td>Reactor</td>
<td>Worldwide GMP</td>
<td>Generator still needs to get its marketing approval – GMP generator under development</td>
</tr>
<tr>
<td>$^{169}$Tb</td>
<td>Reactor</td>
<td>Worldwide GMP</td>
<td>In the future major applications will probably be limited to thyroid diseases. Long half-life, high energy, highly contaminating</td>
</tr>
<tr>
<td>$^{125}$I</td>
<td>Reactor</td>
<td>Worldwide GMP</td>
<td>Presently only one reliable source. Not approved outside of South Korea</td>
</tr>
<tr>
<td>$^{123}$I</td>
<td>Reactor</td>
<td>Worldwide GMP</td>
<td>GMP manufacturing site under construction</td>
</tr>
</tbody>
</table>

### Selection of the radiometal:

- **Imaging method**
- **Half life**
- **Availability**

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Design of the BFC

Acyclic and cyclic BFC

- High affinity and selectivity
- Fast complexation kinetics relative to $T_{1/2}$
- Formation of inert complexes to avoid transmetallation and transchelation in vivo
- High specific activity
- Formation of non reducible complexes (Cu)

Coordinating groups
Donor atoms
Macrocyclic effect
Attachment site
Common acyclic and cyclic BFC

“The chelate makes the difference”

PET of Somatostatin Receptor–Positive Tumors Using $^{64}$Cu- and $^{68}$Ga-Somatostatin Antagonists: The Chelate Makes the Difference

Melpomeni Fani$^{1,2}$, Luigi Del Pozzo$^{1,2}$, Keelara Abiraj$^1$, Rosalba Manz$^{1,2}$, Maria Luisa Tamma$^1$, Renzo Cescato$^3$, Beatrice Waser$^3$, Wolfgang A. Weber$^2$, Jean Claude Reubi$^2$, and Helmut R. Maecke$^{1,2}$

J. Nucl. Med., 2011, 52, 7, 1110-1118

$^{64}$Cu
12.7 h
$\beta^+$ 653 keV

$^{68}$Ga
67.7 min
$\beta^+$ 1899 keV

NODAGA-LM3 = CB-TE2A-LM3 = DOTA-LM3

68Ga

\[
\begin{align*}
\text{NODAGA-LM3} & \quad \text{CB-TE2A-LM3} \\
\text{DOTA-LM3} & 
\end{align*}
\]
In this case, attempts to SPAAC has been rapidly adopted by many and even exploited for their con-
This strain-promoted AAC incorporating azide groups at the terminal ends of the presence of and mammalian cells,
Thus, quantitative conversions were accompanied by and van Hest and van Delft [dibenzoazacyclooc-
out the limitations of CuAAC for the functionalization of CS-
g) lower depolymerizations were found in reactions of CS
PEG (CS-
acrylate] and poly(
PEG. [69]
More recently, similar observations have been made by Fernandez-Megia and Riguera. In their program towards the development of PEG-grafted chitosan (CS-
MA)–
g]-PNIPAM [71]
OH radical when it is produced closer to the
This is in agreement with the stronger deleterious effect of the
The first report on the depolymerization of polysaccha-
H2O with various polysaccharides
was interpreted as resulting from oxidative degrada-
severe depolymerization of the CS backbone as revealed by size-exclusion chromatography. This depolymerization has been rationalized as resulting from
which has stimulated the development of CuAAC bioconju-
Cu has often been found to be detrimental to living cells,
against oxidative damage is well known.
Biorthogonal chemistry

Cu-catalyzed

N3
N
N

Copper free

N3
N
N

# Molecular Imaging

**A Molecular Imaging Primer: Modalities, Imaging Agents, and Applications**

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## Indirect Labeling

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| Example | [\(^{18}\text{F}\)]FDG | RGD-Cy5.5 | \(^{68}\text{Ga}\)-DOTA-MUT-DS | Molecular Beacon | ICG-Trastuzumab | RGD-SWNT |

---

- **Quencher**
- **Donor**
- **Signaling component**
Coupling Strategies - Labeling

RANDOM LABELING

\[ \text{BIOVECTOR} + (\text{amino acid residue}) \rightarrow \text{RADIOLABELING INJECTION} \]

SITE-SPECIFIC LABELING

\[ \text{BIOVECTOR} + \text{PRE-MODIFICATION} \rightarrow \text{INJECTION} \rightarrow \text{PRE-TARGETING} \rightarrow \text{RADIOLABELING INJECTION} \]

PRE-MODIFICATION

(insertion of natural or unnatural amino acids, specific linker)

RADIOLABELING
**Coupling Strategies - Labeling**

**RANDOM LABELING**

- **BIOVECTOR** + (amino acid residue) → **RADIOLABELING INJECTION**

**SITE-SPECIFIC LABELING**

- **BIOVECTOR** + **PRE-TARGETING** → **RADIOLABELING INJECTION**
- **PRE-MODIFICATION** (insertion of natural or unnatural amino acids, specific linker) → **INJECTION** → **RADIOLABELING**
Labeling of Antibodies

Labeling of trastuzumab with DOTAGA-Anhydride

PBS 0.1 M, pH 7.4
30 min, 25°C


QC MALDI-TOF MS. Degree of conjugation of 2.6 macrocycles per antibody.
Random labeling

Labeling of trastuzumab with DOTAGA-Anhydride

\[ \text{Labeling of trastuzumab with DOTAGA-Anhydride} \]

**Chemical Reaction:**

\[ \text{AcONH}_2 \quad 0.1 \text{M pH 5.7} \]

\[ ^{111}\text{InCl}_3 \text{in HCl 0.05 N} \]

\[ 1-3 \text{ h, } 37^\circ\text{C} \]

- Radiolabeling yield: 75%
- Radiochemical purity: 99%

**Ex vivo biodistribution of \(^{111}\text{In-DOTAGA-trastuzumab}\):**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>%ID/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td></td>
</tr>
<tr>
<td>Kidneys</td>
<td></td>
</tr>
<tr>
<td>Tumor</td>
<td></td>
</tr>
</tbody>
</table>


Subcutaneous BT-474 human breast tumor imaging by SPECT/CT (24h/48h/72h post-injection of \(^{111}\text{In-radiolabeled compound}\)).
Labeling of Antibodies

Labeling of pertuzumab with DOTAGA-Anhydride

Subcutaneous BT-474 human breast tumor imaging by SPECT/CT (5h/24h/48h/72h post-injection of $^{111}$In-radiolabeled pertuzumab).

* High accumulation of antibody in HER2+ breast tumors
* An excess of non-radiolabeled corresponding antibody significantly shifted down tumor-targeting

Ex vivo biodistribution of $^{111}$In-DOTAGA-Pertuzumab

Collin et al., SABCS 2012 – Oudot et al. EORTC 2012
**Coupling Strategies - Labeling**

**RANDOM LABELING**

- **SITE-SPECIFIC LABELING**
  - **PRE-MODIFICATION** (insertion of natural or unnatural amino acids, specific linker)

**PRE-TARGETING**

- **RANDOM LABELING**
  - (amino acid residue)

**INJECTION**

- **RADIOLABELING**
Site-specific labeling of affibodies

Selective labeling of pre-reduced Affibody with Maleimide-NOTA derivative

Affibody / «pre-reduced» strategy

Influence of DOTA Chelator Position on Biodistribution and Targeting Properties of $^{111}$In-Labeled Synthetic Anti-HER2 Affibody Molecules

Anna Perols,† Hadis Honaryar,‡ Joanna Strand,‡ Ramkumar Selvaraju,§ Anna Orlova,§ Amelie Eriksson Karlström,† and Vladimir Tolmachev†§*

Bioconjugate Chem., 2012, 23, 1661-1670

V. Tolmachev:

« Chemical properties and position of chelator/linker for labelling of Affibody molecules influence:

• Predominant excretion pathway;
• Uptake and retention in excretory organs;
• Blood clearance rate »

« Careful studies concerning influence of labeling chemistry on biodistribution and targeting may make a difference between success and failure ! »
Site-specific labeling of antibodies

Bridging Disulfides for Stable and Defined Antibody Drug Conjugates

George Badescu, Penny Bryant, Matthew Bird, Korinna Henseleit, Julia Swierkosz, Vimal Parekh, Rita Tommasi, Estera Pawlisz, Kosma Jurlewicz, Monika Farys, Nicolas Camper, XiaoBo Sheng, Martin Fisher, Ruslan Grygorash, Andrew Kyle, Amrita Abhilash, Mark Frigerio, Jeff Edwards, and Antony Godwin®

PolyTherics Ltd, The London Bioscience Innovation Centre, 2 Royal College Street, London NW1 0NH, United Kingdom
Site-specific labeling of antibodies

MAbs/ click strategy

Enzyme and click chemistry-mediated methodology for site-specific labeling of antibodies on the heavy chain by incorporation of azide-modified N-acetylgalactosamine monosaccharides

Coupling Strategies - Labeling

**RANDOM LABELING**

- **BIOVECTOR**
- **INJECTION**
- **RADIOLABELING**
- (amino acid residue)

**SITE-SPECIFIC LABELING**

- **BIOVECTOR**
- **INJECTION**
- **RADIOLABELING**
- PRE-MODIFICATION
  - (insertion of natural or unnatural amino acids, specific linker)

**PRE-TARGETING**

- **INJECTION**
- **RADIOLABELING**
Pre-targeting / Labeling of antibodies

Selective in vivo binding of radioactive tetrazine probe to trans-cyclooctene tag of antibody via bioorthogonal reaction, boosting target-to-nontarget ratios.

Weissleder, Lewis J. Nucl. Med. 2013, 54, 1389-1396
Pre-targeting / Labeling of antibodies

Pre-targeting / click strategy

FIGURE 3. Synthesis of $^{64}$Cu-Tz-Bn-NOTA.

TABLE 1
Biodistribution Data for In Vivo $^{64}$Cu Pretargeting Experiment

<table>
<thead>
<tr>
<th>Organ</th>
<th>1 h</th>
<th>4 h</th>
<th>12 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>3.5</td>
<td>2.8</td>
<td>2.3</td>
<td>2.1</td>
</tr>
<tr>
<td>Tumor</td>
<td>4.1</td>
<td>4.1</td>
<td>4.2</td>
<td>4.0</td>
</tr>
<tr>
<td>Heart</td>
<td>1.1</td>
<td>0.9</td>
<td>0.9</td>
<td>0.8</td>
</tr>
<tr>
<td>Lung</td>
<td>1.6</td>
<td>1.6</td>
<td>1.1</td>
<td>1.0</td>
</tr>
<tr>
<td>Liver</td>
<td>2.2</td>
<td>1.3</td>
<td>0.9</td>
<td>1.1</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.6</td>
<td>0.5</td>
<td>0.6</td>
<td>0.4</td>
</tr>
<tr>
<td>Stomach</td>
<td>0.5</td>
<td>0.3</td>
<td>0.5</td>
<td>0.2</td>
</tr>
<tr>
<td>Large Intestine</td>
<td>0.5</td>
<td>0.3</td>
<td>1.1</td>
<td>1.0</td>
</tr>
<tr>
<td>Feces</td>
<td>11.9</td>
<td>8.8</td>
<td>3.4</td>
<td>1.4</td>
</tr>
<tr>
<td>Small Intestine</td>
<td>0.0</td>
<td>0.4</td>
<td>0.8</td>
<td>0.5</td>
</tr>
<tr>
<td>Kidney</td>
<td>1.3</td>
<td>0.9</td>
<td>0.9</td>
<td>0.3</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.2</td>
<td>0.1</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>Bone</td>
<td>0.3</td>
<td>0.3</td>
<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Skin</td>
<td>0.3</td>
<td>0.3</td>
<td>0.4</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Values are %ID/g ± SD. Mice (n = 4) bearing subcutaneous SW1222 xenografts (100-150 mm³, arrow) were administered A33-TCO (100 μg) via tail vein injection. After 24 h, the same mice were administered $^{64}$Cu-Tz-Bn-NOTA (10.2-12.0 MBq [275-325 μCi], 1.2-1.4 μg, for 2.5-2.8 Tzto-A33 ratio) via tail vein injection. Transverse (top) and coronal (bottom) planar images intersect the center of the tumors.

Weissleder, Lewis J. Nucl. Med. 2013, 54, 1389-1396
Pre-targeting / Labeling of antibodies

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**SITE SPECIFIC LABELING**

- **$^{89}\text{Zr}$**
  - $78.4\ h$
  - $\beta^+\ 902\ \text{keV}$

- **$^{64}\text{Cu}$**
  - $12.7\ h$
  - $\beta^+\ 653\ \text{keV}$

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**Pre-targeting / click strategy**

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**Bioconjugate Chemistry**

Modular Strategy for the Construction of Radiometalated Antibodies for Positron Emission Tomography Based on Inverse Electron Demand Diels–Alder Click Chemistry

Brian M. Zeglis, Priya Mohindra, Gabriel I. Weissmann, Vadim Divitov, Scott A. Hilderbrand, Ralph Weissleder, and Jason S. Lewis

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**PET Images**
Pre-targeting / Labeling of antibodies

SITE SPECIFIC LABELING

Pre-targeting / hapten strategy

1. Injection of bsAb
2. Tumor targeting
3. Clearance of the bsAb from circulation
4. Peptide labeled with therapeutic or diagnostic radionuclide with 2 hapten moieties is administered
5. The bivalent hapten can cross-link 2 closely bsAb on tumor cell surface
## A MOLECULAR IMAGING PRIMER: MODALITIES, IMAGING AGENTS, AND APPLICATIONS

Michelle L. James and Sanjiv S. Gambhir

<table>
<thead>
<tr>
<th>Type</th>
<th>Small molecule</th>
<th>Peptide</th>
<th>Affibody</th>
<th>Aptamer</th>
<th>Antibody</th>
<th>Nanoparticle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size</td>
<td>&lt;1 nm</td>
<td>~1–4 nm</td>
<td>~5–10 kDa</td>
<td>~5–15 kDa</td>
<td>~150 kDa</td>
<td>~10–200 nm (no larger than 1,000 nm)</td>
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<tr>
<td></td>
<td>&lt;0.5 kDa</td>
<td>~0.5–2 kDa</td>
<td></td>
<td></td>
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<tr>
<td>Example</td>
<td>[(^{18})F]FDG</td>
<td>RGD-Cy5.5</td>
<td></td>
<td></td>
<td></td>
<td>RGD-SWNT</td>
</tr>
</tbody>
</table>

**MODIFICATION OF NPs**

- Quencher
- Donor
- Signaling component
Decorating Nanoparticles

Review Article
Advanced Nanomaterials in Multimodal Imaging: Design, Functionalization, and Biomedical Applications
Zhe Liu, Fabian Kiessling, and Jessica Gätjens  J. Nanomater., 2010

Copper-64 Radiopharmaceuticals for PET Imaging of Cancer: Advances in Preclinical and Clinical Research
Carolyn J. Anderson and Riccardo Ferdani
CANCER BIOThERAPY AND RADIOPHARMACEUTICALS
Volume 24, Number 4, 2009
Decorating Nanoparticles

Ultrasmall rigid siloxane Nps as multimodal probes (AGUIX®)

Gd₂O₃ polysiloxane APTES Np + Dissolution of the oxide core, captation of the gadolinium by the chelator

Ultrasmall size 4±1 nm, MW 8.5±2 kDa

Multimodal imaging
Renal excretion

Bioconjugates for OPTICAL imaging

Fluorescent probes: OPTICAL IMAGING AGENTS

Chemical tools for:
- Preclinical in vivo imaging
- Surgery assistance

Design of fluorescent organic probes
- Cy 5.5, Cy 3, ICG, FITC, Rhodamine, Bodipy, Alexa Fluor, …
Bioconjugates for OPTICAL imaging

Fluorescent Guide Surgery with a Fluorophore-conjugated antibody

Translational Optical imaging

Clinical Studies on NIR fluorescence image-guidance in cancer surgery

DOI 10.1245/s10434-014-3495-y

SMART PROBES
Ex : OFF/ON system after binding

Fluorescence imaging in vivo Rao, Dragulescu-Andrasi and Yao

Toward Bimodal imaging agents

Combination of two complementary imaging methods of similar sensitivities

Sequential introduction of the two probes vs. Single Attachment Point approach

FLUO DYE vs. MOMIA

CHELATOR
Toward multimodality

How to prepare a MOMIA?

Metal chelator (acyclic or cyclic) for Imaging and RIT

LINKER
Multimodal PLATFORM
Organic fluorophore


Toward multimodality

Multimodal radio- (PET/SPECT) and fluorescence imaging agents based on metallo-radioisotopes: current applications and prospects for development of new agents
Flora L. Thorp-Greenwood and Michael P. Coogan*
Dalton Trans., 2011

Multimodal Tumor-Targeting Peptides Functionalized with Both a Radio- and a Fluorescent Label
Joeri Kuil,† Aldrik H. Velders,* and Fijs W. B. van Leeuwen* Biocjugate Chem. 2010, 21, 1709–1719
Toward multimodality

Efficient Construction of PET/Fluorescence Probe Based on Sarcophagine Cage: An Opportunity to Integrate Diagnosis with Treatment

Shuanglong Liu,¹ Dan Li,¹ Chiun-Wei Huang,¹ Li-Peng Yap,¹ Ryan Park,¹ Hong Shan,² Zibo Li,¹ Peter S. Conti¹

DOI: 10.1007/s11307-012-0557-z

a/ PET images

8% ID/g
0% ID/g

1 h 4 h 20 h

c/ In vivo fluorescence imaging

d/ ex vivo fluorescent imaging of major organs (20h p.i.)
DUAL-LABELING of bacterial lipopolysaccharides (LPS) for studying the elimination pathway of these endotoxins

Liver uptake (after 24h) visualized in the whole animal by SPECT/CT imaging

Histological analyses with fluorescence microscopy


Imaging Agents DATABASE

Molecular Imaging and Contrast Agent Database (MICAD)
Bethesda (MD): National Center for Biotechnology Information (US); 2004-2013.
Copyright and Permissions  Cite this Page

http://www.ncbi.nlm.nih.gov/books/NBK5330/

- **111**In-britumomab tiuxetan.
- **111**In-Capromab pendetide.
- **111**In-Diethylenetriaminepentaacetic acid-trastuzumab.
- **111**In-Diethylenetriaminepentaacetic acid-bevacizumab.
- **111**In-Diethylenetriaminepentaacetic acid-2-(p-isothiocyanatobenzyl)-6-methyl-B3 monoclonal antibody.
- **111**In/125I-Labeled anti-mucin-1 murine, chimeric or humanized antibody hPAM4.

- **89**Cu-Labeled NOTA-conjugated anti-CD105 (endoglin) chimeric monoclonal antibody linked to near-infrared dye IRDye 800CW.

**Method of detection:**
- Multimodal imaging
- Optical and radionuclides
- SPECT

**Source of signal/contrast:**
- Indium-111
- Antibodies
- In vitro
- Rodents
- Non-primate non-rodent mammals
- Non-human primates
- Humans
- Any
Choice for the BFC chelators depends on the radiometal of interest
→ reduce the risk of release radioactive isotopes in vivo

BFC nature and labeling site influence the properties of the imaging / theranostic agent
→ selectivity and affinity

Trend: random labeling → site-specific approach
Used for therapeutic purpose (ADC, RIT) but also diagnostic (imaging, diagnostic companion)

Optical imaging: optimized fluorophores
preclinical imaging, surgery assistance, theranostic (RIT/optical imaging)

Chemists should play a key role in the future of imaging agents