Optimization of Non-Metallic Silica-Based Adsorbent of a $^{68}\text{Ge}/^{68}\text{Ga}$ Generator

Development of Cyclic Peptides (iRGD) for the Diagnostic and Goal-Oriented Personal Therapy for the Treatment of Oncological Diseases

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68Ge/68Ga Generators On the Market

All generators on the market are „not for human use“

<table>
<thead>
<tr>
<th></th>
<th>Cyclotron Co Ltd.</th>
<th>Eckert &amp; Ziegler IPL</th>
<th>I.D.B. Holland B.V.</th>
<th>ITG Isotope Technologies Garching GmbH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Origin</td>
<td>Russia</td>
<td>USA</td>
<td>South Africa</td>
<td>Germany</td>
</tr>
<tr>
<td>Matrix</td>
<td>Titanium-dioxide</td>
<td>Titanium-dioxide</td>
<td>Tin-dioxide</td>
<td>Silica matrix</td>
</tr>
<tr>
<td>Eluent</td>
<td>0.1 M HCl</td>
<td>0.1 M HCl</td>
<td>0.6 M HCl</td>
<td>0.05 M HCl</td>
</tr>
<tr>
<td>Elution Yield</td>
<td>60-75%</td>
<td>70-75%</td>
<td>80%</td>
<td>&gt; 80% *)</td>
</tr>
<tr>
<td>Breakthrough **)</td>
<td>&lt; 0.01%</td>
<td>&lt; 0.001% ***</td>
<td>&lt; 0.002% ****</td>
<td>&lt; 0.005%</td>
</tr>
</tbody>
</table>

*) Elution yield over shelf life
**) Breakthrough of 68Ge in % of eluted 68Ga at calibration
***) Breakthrough of 68Ge in % of loaded 68Ge on the column
****) Breakthrough of 68Ge at reference date
Inside the $^{68}$Ge/$^{68}$Ga Generator Column

Silica-based Ge-selective resin

- Formation of tris-chelate complex between $\text{H}_4\text{GeO}_4$ and ortho-diphenol rings of gallate in low pH
  - High affinity to $^{68}$Ge(IV)
  - Low affinity to $^{68}$Ga(III)
  - Elution of $^{68}$Ga with dilute acid (0.05 M HCl)
- Metal-free internal column/tubing system
- Low affinity of silica-based resin to metals such as Fe, Zn, Ti..
Research and Development Phase

Starting Point

- Stable and high $^{68}$Ga elution yield (> 80%)
- Relatively steady $^{68}$Ge breakthrough (0.001% - 0.005%)
  - European Pharmacopoeia limit: < 0.001% before and after labeling
- Rather high back pressure (elution convenience)
Improving the Generator Resin

Resin development
- To increase affinity ($K_D$) between resin and $^{68}$Ge
  - Enhance Ge/phenol ring complexation
  - Maximum capacity
  - Preparation methodology
- Stability in resin performance
  - One-phase chemical environment
  - Knowledge on behaviour of $^{68}$Ge in the resin
Next Generation $^{68}$Ge/$^{68}$Ga Generator

Optimized resin conditions: stable behaviour over the shelf life

- High affinity for $^{68}$Ge -> long shelf life
- Stable elution yield of $> 80\%$
- Low and stable $^{68}$Ge breakthrough
  - $\sim 0.0001\%$ or less depending on generator activity
- High chemical purity of eluate
- Smaller back pressure (lighter elution)
- GMP status in 2012
  - Generator fulfills the requirements for GMP production
Introduction

The mechanism of tumor-homing by iRGD

iRGD

CRGDK/R (CendR)

Cargo

αvβ3/β5

Neuropilin-1

Tumor blood vessel endothelial cells and tumor cells

Tissue and cell penetration

Cargo

Sugahara, Teesalu et al., Cancer Cell, 2009
Objective

- Synthesis of 5 different S-S cyclized iRGD peptides
- Addition of DOTA chelator for radiolabeling with $^{68}\text{Ga}$ and $^{177}\text{Lu}$
- Addition of PEGs to improve kinetic properties
- Variation in position and size of PEGs
- To study labeling kinetics, radiolysis and stability

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Formula</th>
<th>PEG size (Da)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptide-1</td>
<td>DOTA-PEG-[ CRGDKGPDC ]-CO-NH2</td>
<td>200</td>
</tr>
<tr>
<td>Peptide-2</td>
<td>DOTA-PEG-[ CRGDKGPDC ]-CO-NH2</td>
<td>5000</td>
</tr>
<tr>
<td>Peptide-3</td>
<td>DOTA-[ CRGDKGPDC ]-PEG-CO-NH2</td>
<td>200</td>
</tr>
<tr>
<td>Peptide-4</td>
<td>DOTA-[ CRGDKGPDC ]-PEG-CO-NH2</td>
<td>5000</td>
</tr>
<tr>
<td>Peptide-5</td>
<td>DOTA-Ahx-[ CRGDKGPDC ]-CO-NH2</td>
<td></td>
</tr>
</tbody>
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Results

- Position of PEG contributes to the stability of peptide
- Higher kinetics with PEG-C1 binding
- Higher mass (5000 Da) C9-bound PEG enhances retaining of iRGD to mouse kidneys
- C9-bound 200 Da PEG longer retention time in blood and slower rate of metabolism compared to mono-iRGD (no pegylation)
Results

- Different metabolic pattern depending on PEG size

![Bar graph showing the percentage of plasma and urine metabolization for different PEG sizes.](chart)
Conclusions and Future Studies

- C9-binding slows kinetics
- C9 200 Da PEG promising for less retention to kidneys than 5000 Da PEG
- Focus on iRGD with C9 bound 200 Da PEG
- Kinetics of enrichment to organs and tumor after intravenous injection with $^{68}$Ga-iRGD
- Radiopeptide therapy using $^{177}$Lu-iRGD
- Long-term therapy and dose optimization with $^{177}$Lu-iRGD-PEG(C9-200Da)
- Tumor enrichment with $^{68}$Ga-iRGD PET and immunohistochemistry ex vivo to find evidence of mechanism of $\alpha_v\beta_3$ & Neuropilin 1 binding and internalization
All Involved

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- Dr. J. Carlsen
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- S. Pfeiffer

ITM Group
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- Dr. R. Henkelmann
- M. Hutt
- Dr. J. Jernström
- T. Lipponen
- L. Nikula
- Dr. T. Nikula
- Dr. G. Schumacher
Thank you for your attention!