Astatine-211 and Astatinated Radiopharmaceuticals

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Range of $\alpha$- and $\beta$-Particles

**Mean range**

- **Y-90 $\beta$**: 215 cells
- **I-131 $\beta$**: 40 cells
- **At-211 $\alpha$**: 3 cells

20 $\mu$m tumor cells
Potential Applications of $\alpha$-Particle Therapy

- Micrometastases
- Tumors of Circulation
  - Lymphoma
  - Leukemia
- Compartamental Tumors
  - Cystic
  - Ovarian
  - Neoplastic Meningitis
Radiobiological Advantages of Alpha Particles

- High relative biological effectiveness (RBE)
- Low oxygen enhancement ratio
- Absence of dose rate effects
- Not dependent on cell cycle
Astatine-211 Decay Scheme

At-211 α particles

\[ \text{LET}_{\text{mean}} = 99 \text{ keV/μm} \]
\[ E_{\text{ave}} = 6.79 \text{ MeV} \]
range = 55-70 μm

Imaging:
\[ ^{211}\text{Po} \text{ X-rays (EC decay; 77-92 keV)} \]

\( ^{211}\text{At} \rightarrow 7.2 \text{ h} \)

\( ^{211}\text{Po} \rightarrow 0.52 \text{ s} \)

\[ 58\% \text{ EC} \]

\[ 42\% \]

\[ E_\alpha = 5.87 \text{ MeV} \]

\( ^{207}\text{Bi} \rightarrow 38 \text{ y} \)

\[ 100\% \]

\[ E_\alpha = 7.45 \text{ MeV} \]

\( ^{207}\text{Pb} \rightarrow \text{stable} \)
Relative Biological Effectiveness vs. LET

![Graph showing RBE vs. LET with isotopes 211At, 90Y, and 131I]
• Astatine-211 Production

• Astatinated Monoclonal Antibodies

• Meta-$^{211}\text{At}$ Astatobenzylguanidine

• 5-$^{211}\text{At}$ Astato-2′-deoxyuridine

• Astatinated octreotide Analogues
211At-Internal Target

- Grazing angle at 1.5\(^\circ\) using curved target face
- Increases beam strike length from 2.5 to 10 cm
- Improves heat transfer
At-211 Internal Target

• Al vs. Cu backing material
  - yield
  - background activity
  - distillation efficiency

• Target Configuration
  - yield
  - still size, yield
$^{211}_{\text{At}} \text{Internal Target}$

$^{209}_{\text{Bi}}(\alpha,2n)^{211}_{\text{At}}$

- Beam currents of 50-60 $\mu$A, 28 MeV $\alpha$-particles, and 1.5-4.5 hr runs
- $0.8 \pm 0.1 \text{ mCi/µA} \cdot \text{h}$
- Maximum to date:
  \[ 55 \mu\text{A} \times 4.0 \text{ h} = 178 \text{ mCi} \]
- $67 \pm 16\%$ distillation yield
• Astatine-211 Production

• Astatinated Monoclonal Antibodies

• $Meta-[^{211}\text{At}]$Astatobenzylguanidine

• $5-[^{211}\text{At}]$Astato-2’-deoxyurididine

• Astatinated octreotide Analogues
N-Succinimidyl 3-[211At]Astatobenzoate ([211At]SAB) for mAb Labeling

COOH

BuLi

Bu3SnCl

COOR

N-Hydroxysuccinimide, DCC

COO-N

SnBu3

COO-N

SnBu3

mAb

N

NH2

CO

pH 8.5

20 min

BuLi

Bu3SnCl

mAb

mAb
Clinical Batches of Chimeric 81C6 mAb labeled with $^{211}$At Using $[^{211}\text{At}]$SAB

- 17 batches (2.8 – 14.0 mCi)
- $[^{211}\text{At}]$SAB yield: $54 \pm 10\%$ (30% EtOAc fraction from Sep-pak)
- Conjugation yield: $76 \pm 10\%$
- Radiochemical purity: $96.0 \pm 2.5\%$ (Size exclusion HPLC)
- Immunoreactive fraction: $83.3 \pm 5.3\%$ (Lindmo method)
Compartmental Administration: Surgically Created Resection Cavity (SCRC)
At-211 Labeled Chimeric 81C6: Clinical Protocol

- Thyroid blocking with SSKI and Cytomel beginning 48 hr prior to therapy
- Dose administration via indwelling catheter
  - Single dose of 10 mg of ch81C6
  - Escalating doses of $^{211}$At \{2 (n=5), 4 (n=6), 6.7 (n=5), and 10 mCi (n=1)\}
- Blood sampling at 1, 2, 4, 8, 12, 18 and 24 hr
- SPECT of head and whole body imaging at 2, 4, 8, 18 and 24 hr
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Median Age (years)</strong></td>
<td>50</td>
</tr>
<tr>
<td>Range</td>
<td>28-76</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>6 (35%)</td>
</tr>
<tr>
<td>Female</td>
<td>11 (65%)</td>
</tr>
<tr>
<td><strong>Karnofsky Performance Score</strong></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>11 (65%)</td>
</tr>
<tr>
<td>90</td>
<td>2 (12%)</td>
</tr>
<tr>
<td>80</td>
<td>3 (18%)</td>
</tr>
<tr>
<td>70</td>
<td>1 (6%)</td>
</tr>
<tr>
<td><strong>Histology</strong></td>
<td></td>
</tr>
<tr>
<td>GBM</td>
<td>14 (82%)</td>
</tr>
<tr>
<td>AO</td>
<td>3 (18%)</td>
</tr>
</tbody>
</table>
Serial whole-body gamma camera images after injection of $^{211}$At-labeled 81C6 in surgical resection cavity

% ID Remaining in Cavity (decay corrected)

<table>
<thead>
<tr>
<th>Time Post Injection</th>
<th>Location of intracranial cavity</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.2 h</td>
<td>![Image 36x36 to 46x46]</td>
</tr>
<tr>
<td>3.8 h</td>
<td>![Image 193x101 to 517x187]</td>
</tr>
<tr>
<td>6.2 h</td>
<td>![Image 193x226 to 517x313]</td>
</tr>
<tr>
<td>17.4 h</td>
<td>![Image 193x352 to 517x439]</td>
</tr>
<tr>
<td>25.2 h</td>
<td>![Image 193x605 to 517x692]</td>
</tr>
</tbody>
</table>
At-211 Activity Distribution in Patients

- Median cavity biological clearance half time: 218 hr
- Percent decays in cavity: 99.1 ± 0.9%
- Blood pool activity (decay corrected):
  - 0.032 ± 0.025% ID at 2 hr
  - 0.26 ± 0.43% ID at 24 hr
# At-211 Chimeric 81C6: Radiation Dosimetry

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Dose (Gy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRC Margin</td>
<td>1041</td>
</tr>
<tr>
<td>Bone Marrow</td>
<td>0.040</td>
</tr>
<tr>
<td>Brain</td>
<td>0.020</td>
</tr>
<tr>
<td>Liver</td>
<td>0.017</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.016</td>
</tr>
</tbody>
</table>
Phase I $^{211}$At-labeled Chimeric 81C6: Outcome

Survival Fraction

Median Survival: 54 weeks
\( N\text{-Succinimidy}l\ 3\text{-}[^{211}\text{At}]\text{Astatato-4-Guanidinomethylbenzoate (}[^{211}\text{At}]\text{SAGMB}) \)

\[
\begin{align*}
\text{COOH} & \quad \text{Several Steps} & \quad \text{COO} & \quad \text{SnBu}_3 & \quad \text{COO}\text{N} & \quad \text{NH} \\
\text{CH}_3 & & & & & \text{NH}_2
\end{align*}
\]

- Radiochemical yield: \( 61.7 \pm 13.1\% \)
- L8A4 (anti-EGFR \( \text{vIII} \) mAb) conjugation yield: \( 36.1 \pm 1.9\% \)
- Immunoreactive Fraction: \( 65.2 \pm 1.5\% \)
In vitro internalization by U87MGE Glioblastoma Cells: L8A4-[\(^{211}\)At]SAGMB vs. [\(^{131}\)I]L8A4 (Iodogen and [\(^{131}\)I]SGMIB)
Paired-label Tumor Uptake of $[^{131}\text{I}]$SGMIB-L8A4 and $[^{211}\text{At}]$SAGMB-L8A4 in U87MGΔEGFR Xenografts

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>%ID per gram $^{131}\text{I}$</th>
<th>%ID per gram $^{211}\text{At}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>5</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>7</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>14.5</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>24</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

*Significant difference
$^{211}\text{At}/^{131}\text{I}$ Uptake Ratios

Relative $^{211}\text{At}/^{131}\text{I}$ selectivity over 24 h: Lung 5-9; Spleen 5-17; Stomach 1-3
• Astatine-211 Production

• Astatinated Monoclonal Antibodies

• Meta-$^{211}$At Astatobenzylguanidine

• 5-$^{211}$At Astato-2’-deoxyuridine

• Astatinated octreotide Analogues
**Meta-[^{211}\text{At}]Astatobenzylguanidine**

([^{211}\text{At}]MABG)

\[
\text{Meta-Iodobenzylguanidine (MIBG)}
\]

\[
\text{Meta-[}^{211}\text{At}]\text{Astatobenzylguanidine ([}^{211}\text{At}]\text{MABG)}
\]

\[
\text{Several steps}
\]

\[
^{211}\text{At, NCS, TFA, 70}\,^\circ\text{C}
\]

\[
\text{RCY = 85%}
\]
[\textsuperscript{211}At]MABG

- Uptake in SK-N-SH neuroblastoma cells \textit{in vitro} and tissue distribution in mice \textit{in vivo} very similar to MIBG
- Minimal \textit{in vivo} dehalogenation
- Exquisite cytotoxicity (more than 1000-fold compared to n.c.a. [\textsuperscript{131}I]MIBG and equivalent to only a few \textsuperscript{211}At decays per cell in monolayers and multi-cellular spheroids)
Gene Therapy plus Targeted $\alpha$-Particle Radiotherapy

- *Hypothesis* – Alpha particle emitting compounds can be targeted to tumors via gene expressed markers, and effectively compensate for the heterogeneous nature of gene therapy via Bystander Effect
MABG and Gene Therapy

hTERT

hTERC

NET
$[^{211}\text{At}]\text{MABG}$ and Gene Therapy: Uptake in Glioma cells transfected with NET Gene Under the Control of Various Promoters
[\textsuperscript{211}At]MABG and Gene Therapy: Clonogenic Survival of UVW Spheroids: 100\% Cells Transfected with NET
[\textsuperscript{211}At]MABG and Gene Therapy: Clonogenic Survival of Mosaic Spheroids: Various Percent of Cells Transfected with NET
Solid-phase synthesis of $^{211}$At[MABG]

$^{211}$At, H$_2$O$_2$, HOAc, MeOH
[211At]MABG from Polymer-supported Precursor: Radiochemical yield vs time

Yield (%) vs Time (Minutes)

Resin: 5 mg

211At: 200-300µCi in 50 µl MeOH

H₂O₂ (30%):HOAc 17:10 (v/v): 10 µl

Room temp.
[\(^{211}\text{At}\)]MABG: Production at Higher Levels by A Kit Method

- 10 mg resin
- 1.0 – 8.0 mCi or \(^{211}\text{At}\) in ~100 µl MeOH
- 20 µl of \(\text{H}_2\text{O}_2/\text{HOAc}\) mixture
- 10 min @ RT
- Purification by C18 solid-phase extraction

- Radiochemical yield: 56.4 ± 9.8% (n = 5)
- Maximum \([^{211}\text{At}]\)MABG produced: 5 mCi
HPLC of $^{211}$At MABG from Kit Method

Waters X-Terra RP18 (4.6 × 250 mm; 5μ) column
0.1%TFA in 20/80 CH$_3$CN/Water; 1 ml/min
• Astatine-211 Production

• Astatinated Monoclonal Antibodies

• Meta-[\(^{211}\text{At}\)]Astatobenzylguanidine

• 5-[\(^{211}\text{At}\)]Astato-2’-deoxyuridinie

• Astatinated octreotide Analogue
5-Iodo-2'-deoxyuridine (I UdR)

\[
\begin{align*}
\text{Dioxane} & \quad (\text{Me}_3\text{Sn})_2, (\text{Ph}_3\text{P})_2\text{PdCl}_2 \\
\end{align*}
\]

5-\[^{211}\text{At}\]Astatito-2'-deoxyuridine

\[
\begin{align*}
\text{CHCl}_3, \text{Sonication 20 sec} & \quad ^{211}\text{At}, \text{H}_2\text{O}_2, \text{HOAc}, \\
\text{RCY} = 85-90\%; \text{Max produced: 2.7 mCi} \\
\end{align*}
\]
[\textsuperscript{211}At]AUdR: Rationale

Two potential zones of high-LET kill:

1) a-recoil nuclei (range <0.1 \mu m) for cells in S-phase taking up AUdR
2) \alpha\text{-}particles (range 55-70 \mu m) for killing of non S-phase cells by cross fire
Uptake of $^{211}$AtAUdR and $^{131}$I IUdR in D-247 MG human glioma cells

Blocking with excess IUdR demonstrates saturability of uptake.
Radiotherapy with $^{211}$At]AUdR in Rat Neoplastic Meningitis Models

- **Experiment 1:**
  - D341 Med human medulloblastoma
  - 43 mCi AUdR
  - 45 mCi free $^{211}$At]astatide or saline

- **Experiment 2:**
  - A431p human epidermoid carcinoma
  - 28 mCi AUdR
  - 61 mCi AUdR or saline

- **Experiment 3:**
  - D341 Med human medulloblastoma
  - $3 \times 30$ mCi AUdR
  - $3 \times 57$ mCi AUdR or 3 × saline
  - 1 injection daily for 3 days
Results: Experiment 1

**Median Survival**

- **saline**: 18 d
- \[^{211}\text{At}]\text{astatide}**: 20 d
  - (p=0.56)
- **AUdR**: 21.5 d
  - (p=0.02)
  - (p=0.07)
Results: Experiment 2

Median Survival

Saline 10 d
AUdR (28 mCi) 17 d
(p=0.01)
AUdR (61 mCi) 16 d
(p=0.004)
(p=0.7)
Results: Experiment 3

**Median Survival**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Median Survival</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>saline</td>
<td>22 d</td>
<td></td>
</tr>
<tr>
<td>AUdR (3×30 mCi)</td>
<td>27 d</td>
<td>0.02</td>
</tr>
<tr>
<td>AUdR (3×57 mCi)</td>
<td>31 d</td>
<td>0.49</td>
</tr>
</tbody>
</table>

(p=0.02)

(p=0.08)

(p=0.49)
Intrathecal administration of $^{211}$AtAUdR significantly improved the median survival of athymic rats with medulloblastoma neoplastic meningitis.

The therapeutic efficacy of $^{211}$AtAUdR was specific and could be increased by multiple dose protocols.

There was no evidence of toxicity either in intact animals or on histopathological analysis of the neuroaxis.
• Astatine-211 Production
• Astatinated Monoclonal Antibodies
• Meta-$^{[211}\text{At}]$ Astatobenzylguanidine
• 5-$^{[211}\text{At}]$ Astato-2’-deoxyuridine
• Astatinated octreotide Analogues
\[
\text{NH}_2-(D)\text{Phe-Cys-Phe-(D)Trp}
\]

\[
\text{Thr(OL)-Cys-Thr-Lys}
\]

**OCTREOTIDE**

Cannot be radioiodinated directly

\[
\text{NH}_2-(D)\text{Phe-Cys-Tyr-(D)Trp}
\]

\[
\text{Thr(OL)-Cys-Thr-Lys}
\]

**Tyr}^3\text{-OCTREOTIDE**

Tyrosine residue can be radioiodinated directly; however, unstable \textit{in vivo}
STRUCTURES OF IBO, INO AND ABO

IODOBENZOYLOCTREOTIDE (IBO): $X = I$, $Y = \text{CH}$
IODONICOTINYLOCTREOTIDE (INO): $X = I$, $Y = \text{N}$
ASTATOBENZOYLOCTREOTIDE (ABO): $X = ^{211}\text{At}$, $Y = \text{CH}$
Structures of Octreotide, Glu-TOCA and GMIBO

Octreotide:

\[
\text{H}_2\text{N-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-CH}_2\text{OH}
\]

GluTOCA:

\[
\text{N-D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Cys-Thr-COOH}
\]

\[
\begin{align*}
\text{OC} & \quad \text{H} \\
\text{N-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-COOH} & \quad \text{NH} \\
& \quad \text{NH}_3 \\
& \quad \oplus \\
\text{I} & \quad \text{H}
\end{align*}
\]

\[N-(4-\text{Guanidinomethyl-3-iodobenzoyl})\text{Phe}^1-\text{Octreotate (GMIBO)}\]
Synthesis of the Tin Precursor (Boc-GMTMSBO), [*I]GMIBO and [211At]AGMBO

SPPS

H₂N-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-COOH

N°-Dde-Lys⁵-Octreotate

GMIBO

Boc-Dde-GMIBO; X = I, R = Boc
Boc-Dde-GMTMSBO; X = SnMe₃, R = Boc

[⁺I]GMIBO; X = *I (30-35%)

[^211At]AGMBO; X = ^211At (15-20%)

a = SGMIB; b = i) ethanolamine ii) TFA;
c = i) ethanolamine ii) radiiodine or ^211At, TBHP, HOAc, 70°C
The affinity of GMIBO was determined using the SSTR-expressing AR42 J rat pancreatic carcinoma cell membranes via a cold-saturation assay. The Scatchard analysis of the data yielded a $K_d$ value of 4.8 nM.
Paired-label Internalization of $\text{[^{131}I]}\text{Glu-TOCA}$ and $\text{[^{125}I]}\text{GMIBO}$ by D341 Med Cells

![Graph showing internalization over time for different conditions](image-url)
Figure 5. Paired-label Internalization of \(^{131}\text{I}\)GMIBO and \(^{211}\text{At}\)AGMBO by D341 Med Cells
Summary

- Astatine-211 in quantities sufficient for the synthesis of clinical batches of astatinated radiopharmaceuticals can be produced.
- Synthesis of a number of astatinated radiopharmaceuticals, some in quantities sufficient for clinical trials have been achieved.
- Radiolysis may be the biggest issue; split and pool approach may be necessary.
Typical Size-exclusion HPLC of $^{211}$At-ch81C6
SAB Yield And Immunoreactive Fraction Of Labeled ch81C6 As A Function Of Radiation Dose

- SAB Yield (%)
  - Radiation Dose (Gy)
  - $r^2 = 0.05$

- Immunoreactive Fraction (%)
  - Radiation Dose (Gy)
  - $r^2 = 0.29$
At-211 Production If Clinical Rationale

- 200 µA internal current
- 90% distillation efficiency
- 4 h decay during transportation

720 mCi vs. 160 mCi
At-211 Therapy: Future?

• Assume: 720 mCi/run
  5 runs/wk × 50 wk/yr
  50% radiochemical yield
  5-20 mCi/patient dose

One Cyclotron yields 4,500 to 18,000 patient doses per year

Ovarian  22,000 cases/yr
Brain     18,000 cases/yr
Astatine-211 Needs

- Research
  - 10 centers
  - 10 mCi/center/wk
- Phase I/II clinical:
  - 25 centers
  - 1 patient/wk
  - 10 mCi/dose
- Clinical:
  - 100 centers
  - 3 patients/wk
  - 10 mCi/dose

- 100 mCi
  - 1 cyclotron × 1 run
- 250 mCi
  - 1 cyclotron × 2 runs
- 3,000 mCi
  - 6 cyclotrons × 3 runs
Astatine-211 Rationale

- 7.2 h Half life
- $\alpha$-emission with each decay
- Po K x-rays for imaging

- Half life compatible with variety of carriers
- Chemistry amenable to biomolecules
- Minimal problem with transformation during decay
Increasing $^{211}$At Production

- Design of internal target system
- Development of bismuth electroplating method
- Design of induction heating system
- Design of specialized cyclotron for $^{211}$At production
At-211 Internal Target

209Bi (α, 2n) 211At

28 MeV α Beam

Bi-Coated Target

Leading Monitor

Bi-Coated Target

RAM

Trailing Monitor

306 mg Bi - JVD
32 in radius
2.5 / 36 / 0.1 μA
45 μA, 90 min

380 mg Bi - Hand
32 in radius
3.0 / 40 / 0.1 μA
50 μA, 90 min

551 mg Bi - Hand Flat
? / 42 / ? μA
50 μA, 60 min