Production of Cu-64 and Synthesis and Biological Evaluation of $^{64}$CuATSM and second generation analogues

R.L.Paul, a P. Halsted, a J. Ballinger, a P.J. Blower, a P.K. Marsden, a A. Trivett A, c D. Lloyd, c M.J. O’Doherty b
K. Wood, d D.J. Honess, d M.I. Saunders, e

aGuy’s, King’s & St Thomas’ School of Medicine, London, UK
bGuy’s and St Thomas' NHS Trust, London, UK
cUniversity of Kent, Canterbury, UK
dGray Cancer Institute Northwood
eUniversity College, London, UK
## Copper Radionuclides

<table>
<thead>
<tr>
<th>Isotope</th>
<th>Half Life</th>
<th>Radiation</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{60}$Cu</td>
<td>20 min</td>
<td>$^+$ (93%), EC (7%)</td>
<td>cyclotron</td>
</tr>
<tr>
<td>$^{61}$Cu</td>
<td>3.3 hours</td>
<td>$^+$ (62%), EC (38%)</td>
<td>cyclotron</td>
</tr>
<tr>
<td>$^{62}$Cu</td>
<td>9.74 mins</td>
<td>$^+$ (98%), EC (2%)</td>
<td>generator/cyclotron</td>
</tr>
<tr>
<td>$^{64}$Cu</td>
<td>12.7 hours</td>
<td>$^+$ (18%), EC (41%), $^-$ (37%)</td>
<td>reactor/cyclotron</td>
</tr>
<tr>
<td>$^{66}$Cu</td>
<td>5.1 mins</td>
<td>$^-$ (100%)</td>
<td>reactor/cyclotron</td>
</tr>
<tr>
<td>$^{67}$Cu</td>
<td>62 hours</td>
<td>$^-$(100%) (52%)</td>
<td>reactor/cyclotron</td>
</tr>
</tbody>
</table>
Objectives

- Production of Cu-64

\[ ^{64}\text{Ni}(p,n)^{64}\text{Cu} \]

T1/2 12.7 h

+ (18%), - (37%), EC (41%)

- Synthesis of hypoxia-selective agents based on \[^{64}\text{Cu(ATSM)}\]
Production of Cu-64: 3 main steps

- Electroplating of Ni-64
- Irradiation of the Target
- Purification of Cu-64

⇒ tracer production
11.5 MeV proton beam
# The Chemistry

Requires separation of Cu-64 from other isotopes after target irradiation

<table>
<thead>
<tr>
<th>Isotope</th>
<th>Ni-58</th>
<th>Ni-60</th>
<th>Ni-61</th>
<th>Ni-62</th>
<th>Ni-64</th>
</tr>
</thead>
<tbody>
<tr>
<td>Content (%)</td>
<td>0.04</td>
<td>0.02</td>
<td>0.002</td>
<td>0.33</td>
<td>99.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Element</th>
<th>B</th>
<th>Mg</th>
<th>Si</th>
<th>Ca</th>
<th>Ti</th>
<th>Cr</th>
<th>Mn</th>
<th>Fe</th>
<th>Cu</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Content (ppm)</td>
<td>&lt;1</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>800</td>
<td>&lt;10</td>
<td>30</td>
<td>10</td>
<td>200</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>
64CuCl2 after Purification

Cu-64, 511 KeV
CuATSM and analogue

CuATSM (hypoxia)
E1/2 -0.59 V

CuPTSM (flow imaging agent)
E1/2 -0.51 V
ATSE: uptake at different oxygen levels *in vitro*

ATSE uptake switches on at higher O$_2$ levels (10x less hypoxic) than ATSM: more relevant to radiobiological hypoxia.
ATSE vs. ATSM: biodistribution

ATSM and ATSE
BALB/c mice, EMT6 tumour, 20 min

ATSE uptake higher in tumour, lower in liver and kidney
Mechanism of Hypoxia Selectivity

Cu(II)ATSM

O₂

fast O₂ + e⁻ fast

slow

“Cu” +
Biological Evaluation: Screening of ‘new’ Bis(thiosemi-carbazones)

<table>
<thead>
<tr>
<th></th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
</tr>
</thead>
<tbody>
<tr>
<td>GTS</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>GTSM</td>
<td>H</td>
<td>H</td>
<td>CH₃</td>
<td>H</td>
</tr>
<tr>
<td>PTS</td>
<td>CH₃</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>PTSM</td>
<td>CH₃</td>
<td>H</td>
<td>CH₃</td>
<td>H</td>
</tr>
<tr>
<td>PTSM₂</td>
<td>CH₃</td>
<td>H</td>
<td>CH₃</td>
<td>CH₃</td>
</tr>
<tr>
<td>PTSE</td>
<td>CH₃</td>
<td>H</td>
<td>C₂H₅</td>
<td>H</td>
</tr>
<tr>
<td>PTSP</td>
<td>CH₃</td>
<td>H</td>
<td>C₆H₅</td>
<td>H</td>
</tr>
<tr>
<td>ATS</td>
<td>CH₃</td>
<td>CH₃</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>ATSM</td>
<td>CH₃</td>
<td>CH₃</td>
<td>CH₃</td>
<td>H</td>
</tr>
<tr>
<td>CTS</td>
<td>C₂H₅</td>
<td>CH₃</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>CTSM</td>
<td>C₂H₅</td>
<td>CH₃</td>
<td>CH₃</td>
<td>H</td>
</tr>
<tr>
<td>DTS</td>
<td>C₂H₅</td>
<td>C₂H₅</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>DTSM</td>
<td>C₂H₅</td>
<td>C₂H₅</td>
<td>CH₃</td>
<td>H</td>
</tr>
</tbody>
</table>
Screening of ‘new’ Bis(thiosemi-carbazones)

![Chemical structure](image)

<table>
<thead>
<tr>
<th>Formula</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTS</td>
<td>CH₃</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>PTSM</td>
<td>CH₃</td>
<td>H</td>
<td>CH₃</td>
<td>H</td>
</tr>
<tr>
<td>PTSM2</td>
<td>CH₃</td>
<td>H</td>
<td>CH₃</td>
<td>CH₃</td>
</tr>
<tr>
<td>PTSE</td>
<td>CH₃</td>
<td>H</td>
<td>C₂H₅</td>
<td>H</td>
</tr>
<tr>
<td>ATS</td>
<td>CH₃</td>
<td>CH₃</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>ATSM</td>
<td>CH₃</td>
<td>CH₃</td>
<td>CH₃</td>
<td>H</td>
</tr>
<tr>
<td>CTS</td>
<td>C₂H₅</td>
<td>CH₃</td>
<td>CH₃</td>
<td>H</td>
</tr>
<tr>
<td>CTSM</td>
<td>C₂H₅</td>
<td>CH₃</td>
<td>CH₃</td>
<td>CH₃</td>
</tr>
<tr>
<td>DTS</td>
<td>C₂H₅</td>
<td>C₂H₅</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>DTSM</td>
<td>C₂H₅</td>
<td>C₂H₅</td>
<td>CH₃</td>
<td>H</td>
</tr>
</tbody>
</table>
Screening of ‘new’ Bis(thiosemi-carbazones)

\[
\begin{array}{cccc}
R_1 & R_2 & R_3 & R_4 \\
\end{array}
\]

<table>
<thead>
<tr>
<th>PTSE</th>
<th>CH₃</th>
<th>H</th>
<th>C₂H₅</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATS</td>
<td>CH₃</td>
<td>CH₃</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>ATSM</td>
<td>CH₃</td>
<td>CH₃</td>
<td>CH₃</td>
<td>H</td>
</tr>
<tr>
<td>DTS</td>
<td>C₂H₅</td>
<td>C₂H₅</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>DTSM</td>
<td>C₂H₅</td>
<td>C₂H₅</td>
<td>CH₃</td>
<td>H</td>
</tr>
</tbody>
</table>

Screening of ‘new’ Bis(thiosemi-carbazones)

ATSM

ATS
Preliminary evaluation of the effects of blood flow on PET detection of $^{64}$Cu-ATSM by dynamic gadolinium enhanced MRI in a rat tumour model

Honess DJ$^1$, Wood KA$^{1,2}$, Maxwell RJ$^3$, Wilson I$^1$, Paul RL$^4$, O’Doherty MJ$^4$, Marsden PK$^4$, Blower PJ$^4$, Sanghera B$^5$, Wong W$^5$, Saunders MI$^2$

1 University of Oxford Gray Cancer Institute, Northwood, Middlesex, UK
2 University College Hospital, London, UK
3 Northern Institute for Cancer Research, University of Newcastle, Newcastle, UK
4 Guy’s, King’s, St Thomas’ School of Medicine, London, UK
5 Paul Strickland Scanner Centre, Mount Vernon Hospital, Northwood, Middlesex, UK
Background

- Regional hypoxia within human tumours is a significant cause of treatment failure.

- Non-invasive identification of hypoxic regions could improve outcome by use of intensity modulated radiotherapy (IMRT).

- A reliable non-invasive hypoxia marker is not yet available.

- $^{64}\text{CuATSM}$ is a PET tracer being evaluated as a hypoxia marker. 

- Uptake of the tracer in the first few minutes may discriminate between hypoxia and normoxia (Lewis et al; J Nucl Med 1999;40:177-183).
Aim

To assess the degree of influence of blood flow on marker uptake into a rat tumour, using:

1. Gadolinium-enhanced dynamic MRI to monitor blood flow into the tumour immediately before....
2. PET scanning to monitor tumour \( ^{64}\text{CuATSM} \) uptake in the same slice.

Tumour methods

- P22 tumours s/c in left flank of BDXI rats, \( n = 8 \)
- Rats anaesthetised
- Rats placed in same jig for MR and PET scanning
MR methods:
- 4.7 T Varian with 6 cm RF coil
- 100 gradient echo images, one every 6 sec
- (TR 60 ms; TE 2.5 ms; 1.0 mm slice thickness)
- After 30 sec, Gd-DTPA given by i/v infusion for 5 sec
- AUC of the uptake curve for first 90 sec (AUC$_{90}$) calculated; this is a robust measure of blood flow in this tumour

PET methods:
- Concorde MicroPET Focus 220
- 10-60 MBq $^{64}$CuATSM given i/v; scanning for 60 min
- Images reconstructed in 5 minute segments for viewing tracer uptake and 10 minute segments for calculation of mean standard uptake values (SUVs) for the central tumour slice (0.8 mm thick)
CTI microPET Studies with $^{64}$Cu-ATSM

Non tumour bearing rat

10.09 MBq $^{64}$Cu-ATSM

Coronal view (head at top)

Uptake seen in liver, kidneys and bowel
P22 tumour bearing rat: 4.2 MBq $^{64}$Cu-ATSM

Axial View
Through tumour

Coronal view
Through tumour

Sagittal view, liver at top
**MR results:**

*Images of a typical tumour are shown below.*

**Before Gd-DTPA**

**At 90sec (60 sec after Gd-DTPA)**

Tumour Gd-DTPA uptake appears to be greater at the periphery, with less well-perfused central areas.
**PET results:**

*Transaxial PET images of the same slice after 20.3 MBq $^{64}$CuATSM.*

Mean activity for 0 - 5 min after $^{64}$CuATSM

Mean activity for 55 - 60 min after $^{64}$CuATSM

At early times $^{64}$CuATSM uptake is greater at the periphery than the centre, with a very similar pattern to Gd-DTPA distribution.

After $\sim$ 15 - 20 min this pattern disperses and uptake is distributed across the tumour.
Correlation of quantitative MR and PET data - early:

There is good correlation between MRI measurement of blood flow ($AUC_{90}$) and early $^{64}$CuATSM uptake (mean SUV 0-10 min):

$AUC_{90} = 6856$ mmol.sec for this tumour

Mean SUV 0-10 min = 1.31 for the same tumour

The correlation coefficient $R$ for all 8 tumours = 0.75 ($p = 0.04$)
Correlation of quantitative MR and PET data - later:

But there is no correlation between MRI measurement of blood flow ($AUC_{90}$) and later $^{64}$CuATSM uptake (mean SUV 50-60 min):

$AUC_{90} = 6856$ mmol.sec for this tumour

Mean SUV 50-60 min = 3.49 for the same tumour

The correlation coefficient $R$ for all 8 tumours = 0.44 ($p = 0.27$)
Conclusions:

- Early $^{64}$CuATSM uptake appears to be dominated by perfusion.
- Later, by 50-60 min, this quantitative correlation is lost.
- Hence later tracer retention in this model is determined by other factors, possibly hypoxia.

Further investigation is in progress using a vascular damaging agent to modify tumour hypoxia and pimonidazole, an immunohistochemical hypoxia marker.

- The data indicate that clinical PET imaging of $^{64}$CuATSM should not be carried out immediately after tracer administration, but time allowed for factors other than perfusion to determine tracer retention.
Acknowledgements

Guys’, Kings’, St Thomas’ School of Medicine
Mr Phil Halsted
Prof Phil Blower
Dr Paul Marsden
Dr Jim Ballinger
EPSRC for funding

The University of Kent
Ms Amanda Trivett
Dr Dan Lloyd

The Gray Cancer Institute
Dr Davina Honess
Dr Katie Wood
Prof Michelle Saunders
Purification of 64-CuCl₂ by Column Chromatography

Fraction no. (~ 2 ml)

Activity (MBq)
## ICP MS Analysis

<table>
<thead>
<tr>
<th>Fraction from Column (2ml)</th>
<th>[HCl]</th>
<th>Zn-64 (ppm)</th>
<th>Ni-60 (ppm)</th>
<th>Ni-61 (ppm)</th>
<th>Ni-64 (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8M</td>
<td>3.506</td>
<td>0.0034</td>
<td>-0.0000</td>
<td>-0.0012</td>
</tr>
<tr>
<td>2</td>
<td>6.758</td>
<td>0.0073</td>
<td>-0.0002</td>
<td>-0.0017</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>7820.09</td>
<td>0.3647</td>
<td>0.0138</td>
<td>428.6611</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>9777.94</td>
<td>0.5549</td>
<td>0.0200</td>
<td>606.4071</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>6M</td>
<td>880.026</td>
<td>0.0140</td>
<td>0.0005</td>
<td>61.3249</td>
</tr>
<tr>
<td>6</td>
<td>15.564</td>
<td>0.0077</td>
<td>-0.0002</td>
<td>0.0183</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.5M</td>
<td>12.752</td>
<td>0.0069</td>
<td>-0.0001</td>
<td>0.0055</td>
</tr>
<tr>
<td>8</td>
<td>6.607</td>
<td>0.0062</td>
<td>-0.0001</td>
<td>0.0048</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>10.098</td>
<td>0.0046</td>
<td>-0.0001</td>
<td>0.0007</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>25.843</td>
<td>0.0130</td>
<td>0.0004</td>
<td>-0.0020</td>
<td></td>
</tr>
</tbody>
</table>
CTI microPET Studies

- P22 carcinosarcoma is a tumour with clinically relevant hypoxia levels.

- CuATSM is clearly taken up in the tumours and distribution dependent on blood flow to an extent.

- Uptake is non-uniform and there are regions of hypoxia.

- Tumour blood flow is being monitored by Gd-enhanced MRI before the 64Cu-ATSM PET scanning and comparison with immunohistochemical pimonidazole staining.