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]

[a]Guy's, King's & St Thomas' School of Medicine, London, UK
[b]Guy's and St Thomas' NHS Trust, London, UK
[c]University of Kent, Canterbury, UK
[d]Gray Cancer Institute Northwood
[e]University College, London, UK

COPPER RADIONUCLIDES

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

Objectives

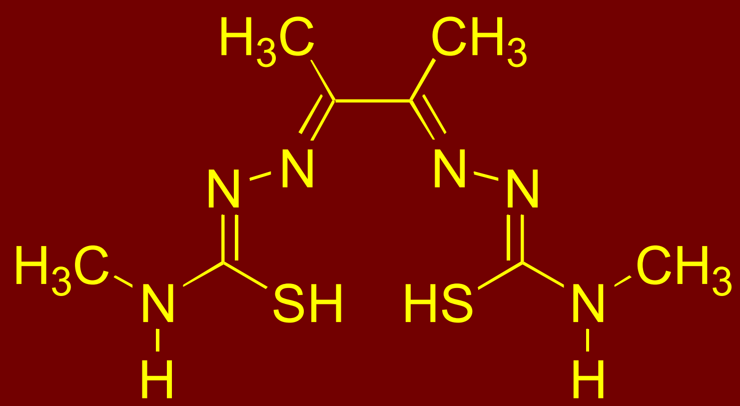
- Production of Cu-64

$^{64}Ni(p,n)^{64}Cu$

T1/2 12.7 h

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

- Synthesis of hypoxia-selective agents based on [^{64}Cu(ATSM)]

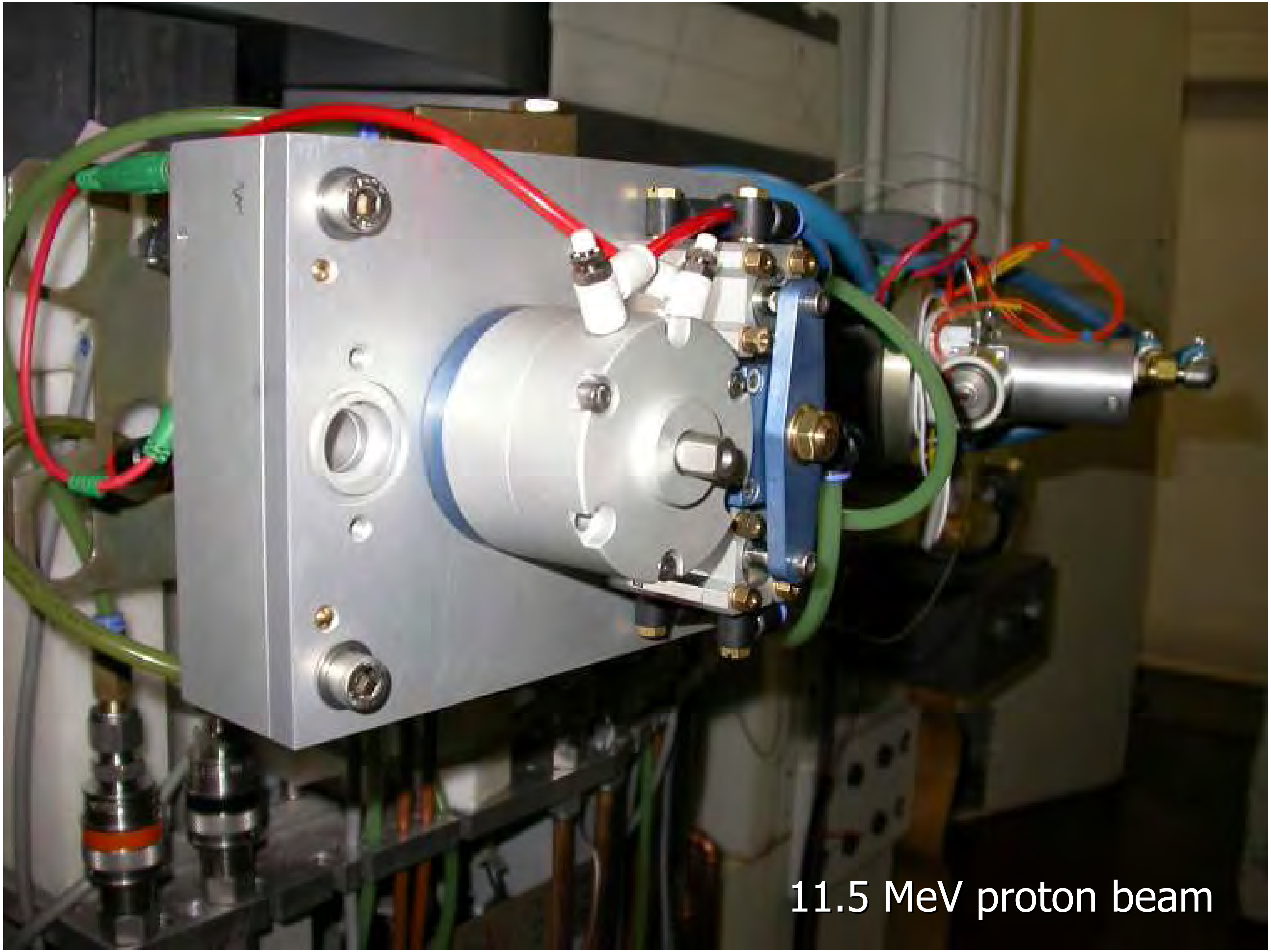


ATSM

Production of Cu-64 : 3 main steps

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

↳ tracer production

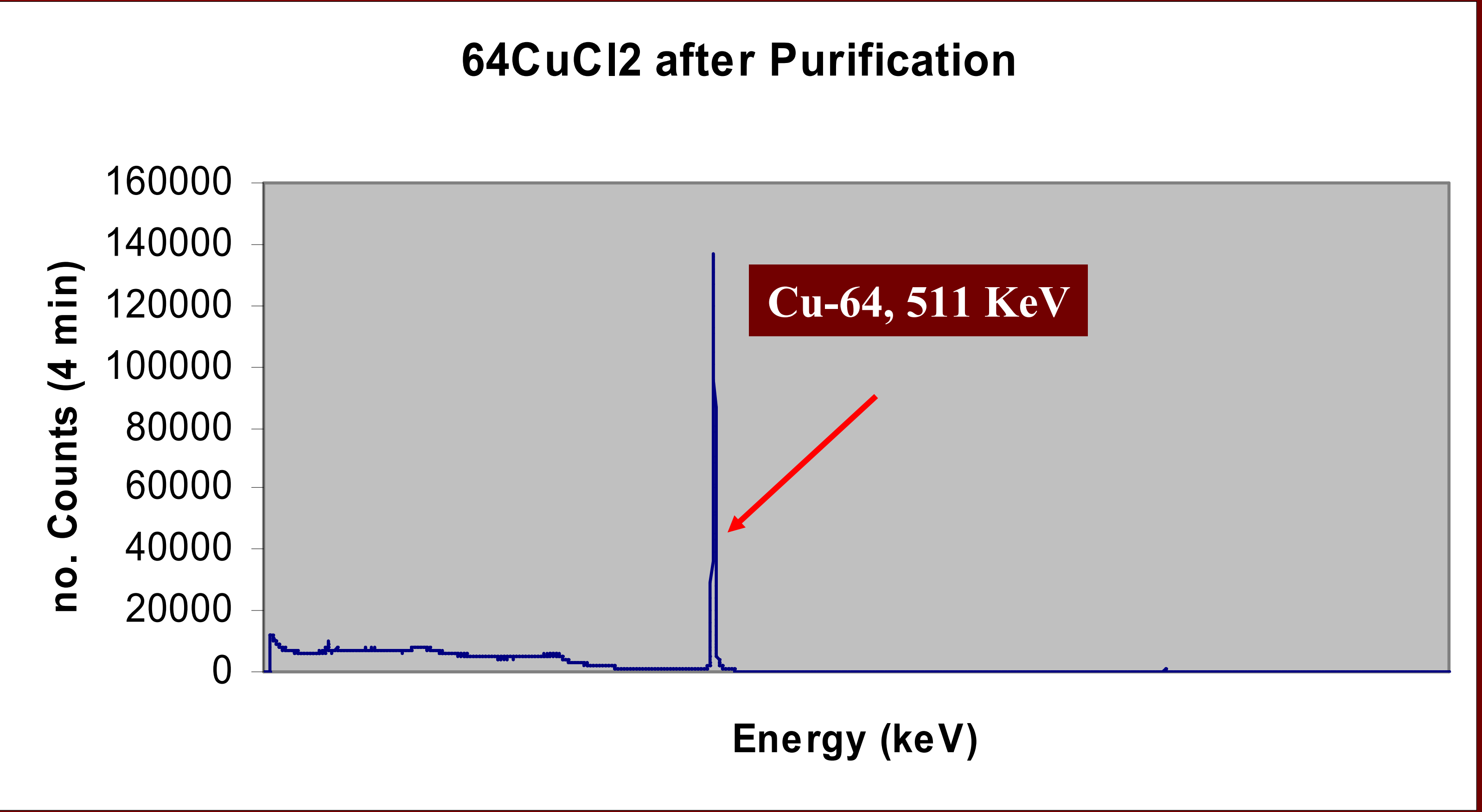

11.5 MeV proton beam

The Chemistry

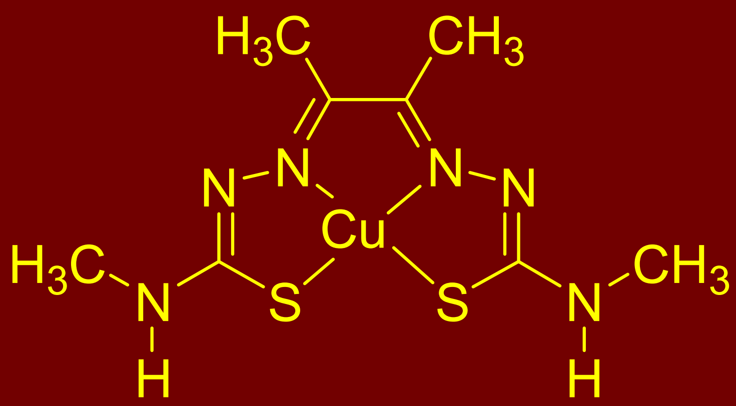
Requires separation of Cu-64 from other isotopes after target irradiation

Isotope	Ni-58	Ni-60	Ni-61	Ni-62	Ni-64
Content (%)	0.04	0.02	0.002	0.33	99.6

Element	B	Mg	Si	Ca	Ti	Cr	Mn	Fe	Cu	Zn
Content (ppm)	<1	<10	<10	800	<10	30	10	200	<10	<10

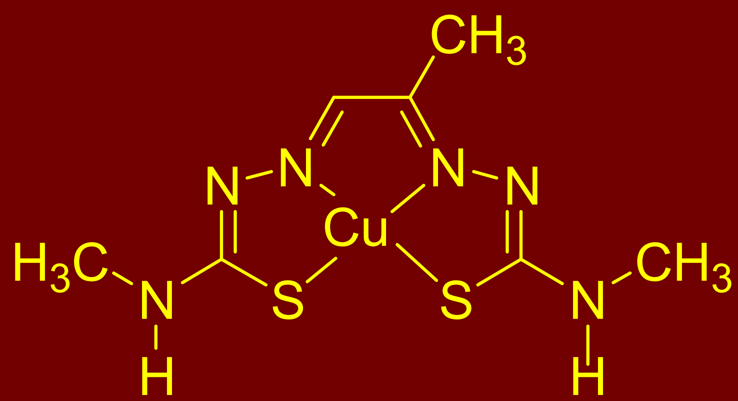

64CuCl2 after Purification
160000
140000
120000
100000
80000
60000
40000
20000
0
no. Counts (4 min)
Energy (keV)
Cu-64, 511 KeV

CuATSM
and analogue



CuATSM
(hypoxia)

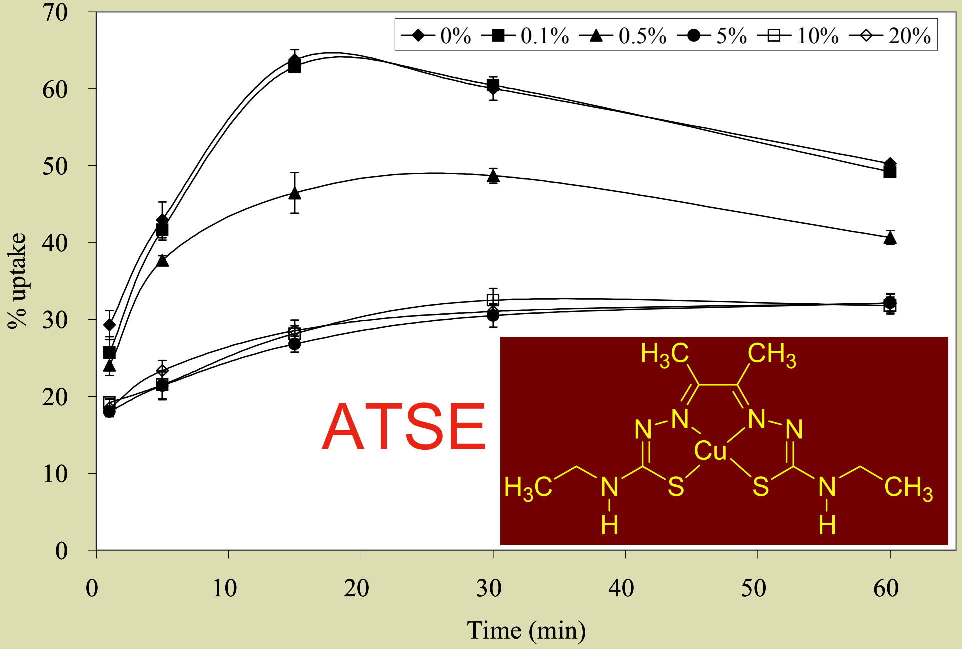
E1/2 -0.59 V

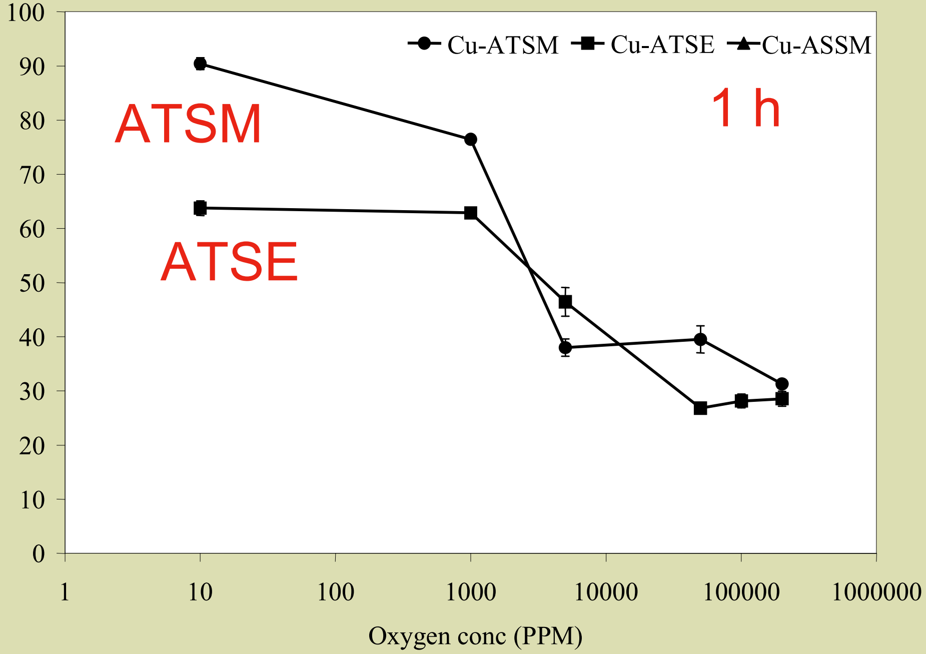


CuPTSM
(flow imaging agent)

E1/2 -0.51 V

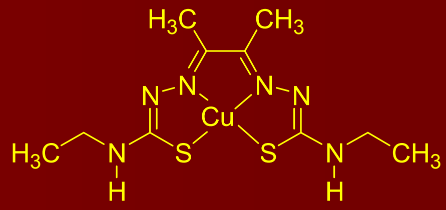
ATSE: uptake at different oxygen levels *in vitro*



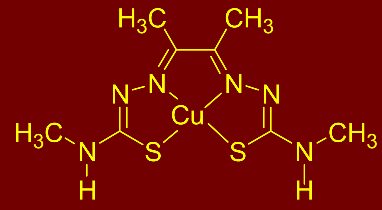


ATSE uptake switches on at higher O_2 levels (10x less hypoxic) than ATSM: more relevant to radiobiological hypoxia

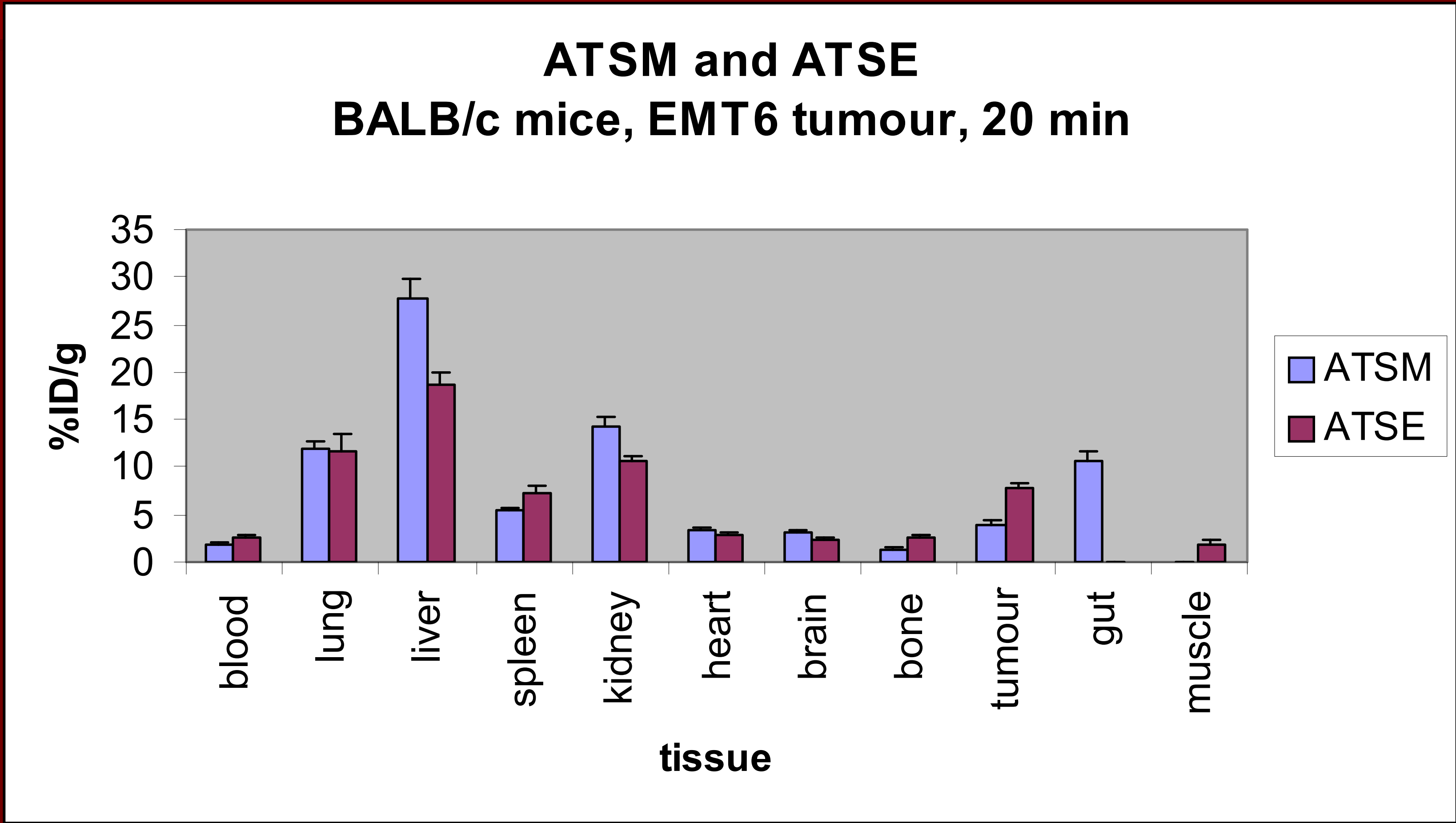
ATSE vs. ATSM: biodistribution



ATSE

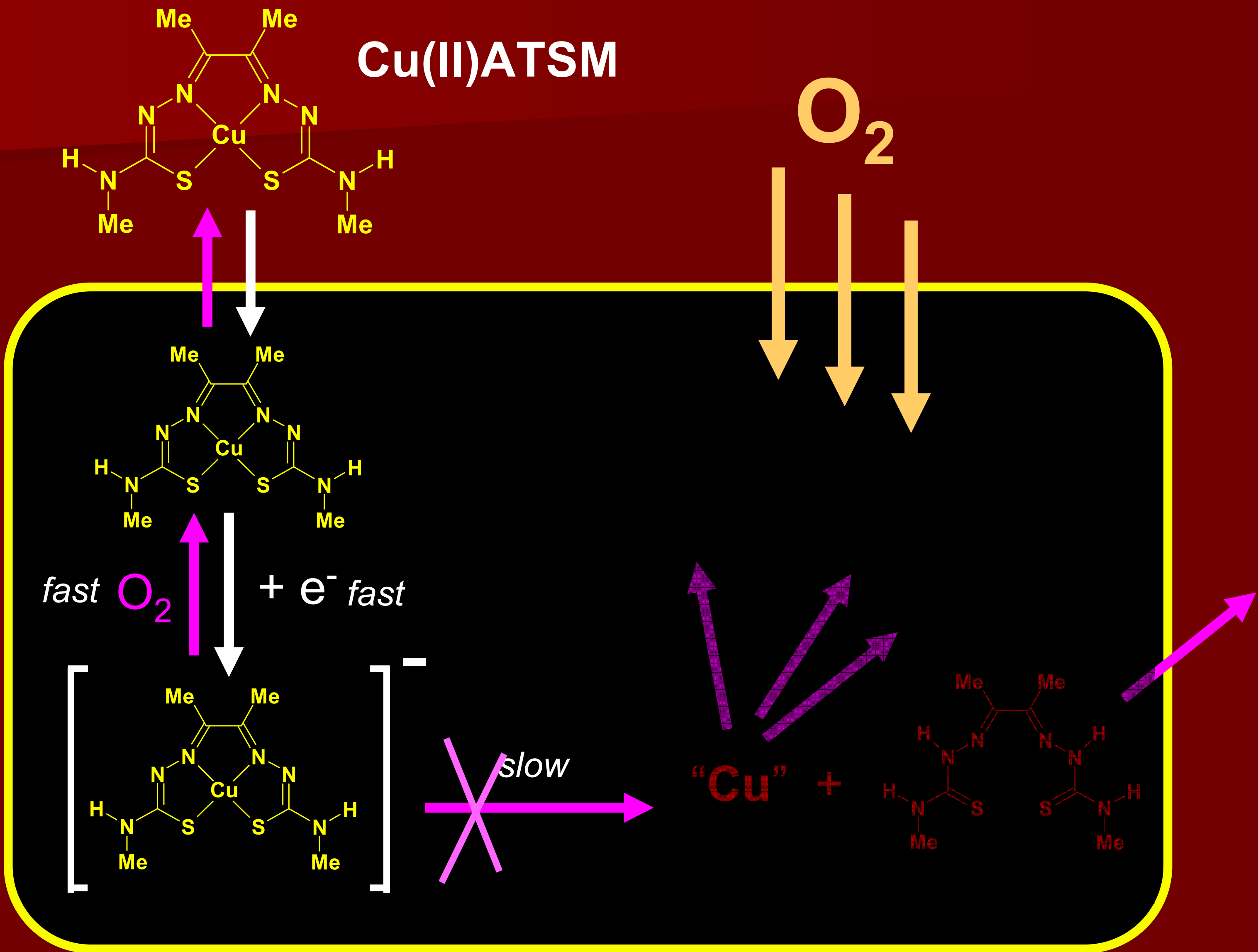


ATSM

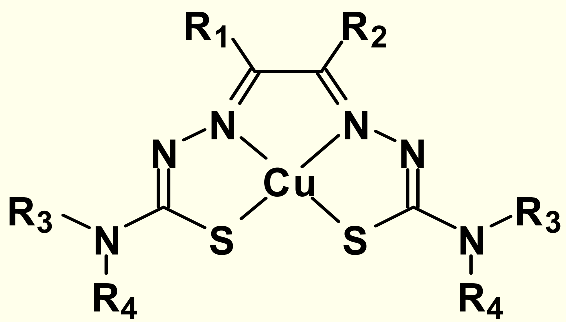


ATSE uptake higher in tumour, lower in liver and kidney

Mechanism of Hypoxia Selectivity

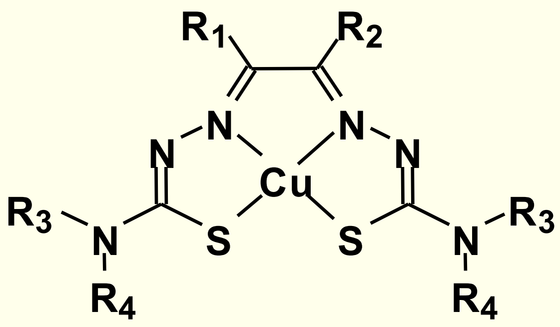


Biological Evaluation: Screening of 'new' Bis(thiosemi-carbazones)



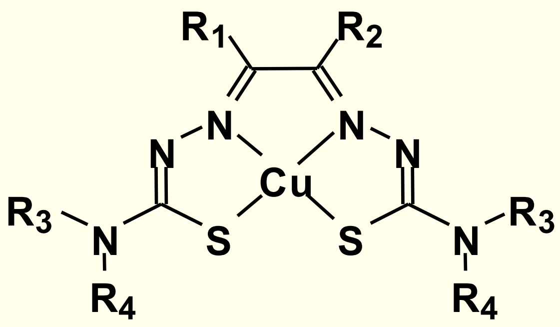
	R1	R2	R3	R4
GTS	H	H	H	H
GTSM	H	H	CH_3	H
PTS	CH_3	H	H	H
PTSM	CH_3	H	CH_3	H
$PTSM_2$	CH_3	H	CH_3	CH_3
PTSE	CH_3	H	C_2H_5	H
PTSP	CH_3	H	C_6H_5	H
ATS	CH_3	CH_3	H	H
ATSM	CH_3	CH_3	CH_3	H
CTS	C_2H_5	CH_3	H	H
CTSM	C_2H_5	CH_3	CH_3	H
DTS	C_2H_5	C_2H_5	H	H
DTSM	C_2H_5	C_2H_5	CH_3	H

Screening of 'new' Bis(thiosemi-carbazones)



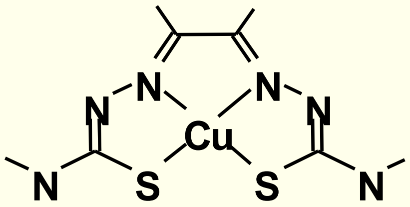
	R1	R2	R3	R4
PTS	CH_3	H	H	H
PTSM	CH_3	H	CH_3	H
PTSM2	CH_3	H	CH_3	CH_3
PTSE	CH_3	H	C_2H_5	H
ATS	CH_3	CH_3	H	H
ATSM	CH_3	CH_3	CH_3	H
CTS				
CTSM	C_2H_5	CH_3	CH_3	H
DTS	C_2H_5	C_2H_5	H	H
DTSM	C_2H_5	C_2H_5	CH_3	H

Screening of 'new' Bis(thiosemi-carbazones)

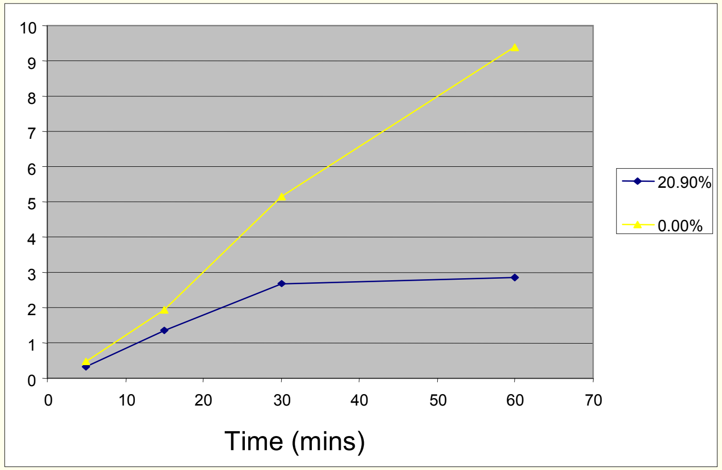


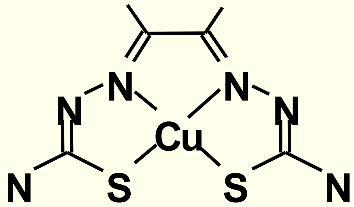
	R1	R2	R3	R4
PTSE	CH_3	H	C_2H_5	H
ATS	CH_3	CH_3	H	H
ATSM	CH_3	CH_3	CH_3	H
DTS	C_2H_5	C_2H_5	H	H
DTSM	C_2H_5	C_2H_5	CH_3	H

Screening of 'new' Bis(thiosemi-carbazones)

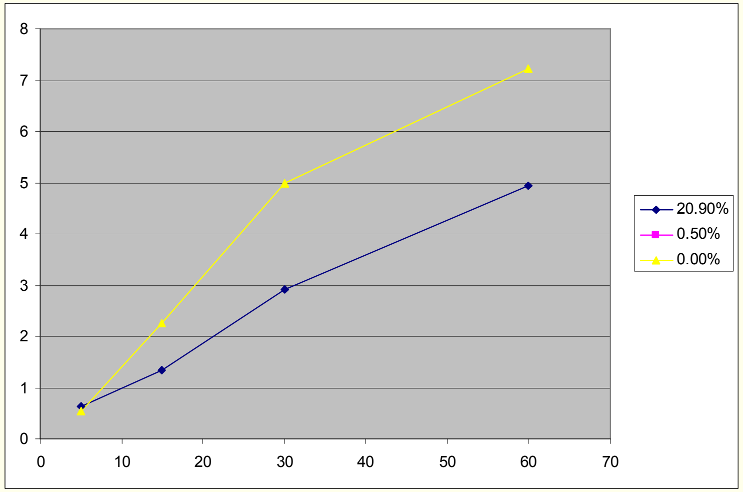


ATSM





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]

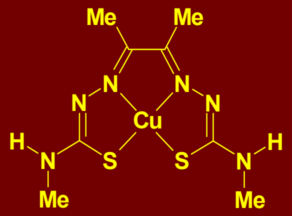
[1] University of Oxford Gray Cancer Institute, Northwood, Middlesex, UK
[2] University College Hospital, London, UK
[3] Northern Institute for Cancer Research, University of Newcastle, Newcastle, UK
[4] Guy's, King's, St Thomas' School of Medicine, London, UK
[5] Paul Strickland Scanner Centre, Mount 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}CuATSM is a PET tracer being evaluated as a hypoxia marker.

Cu(II)ATSM

^{64}Cu $t_{1/2}$ = 12.7h



- 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}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**

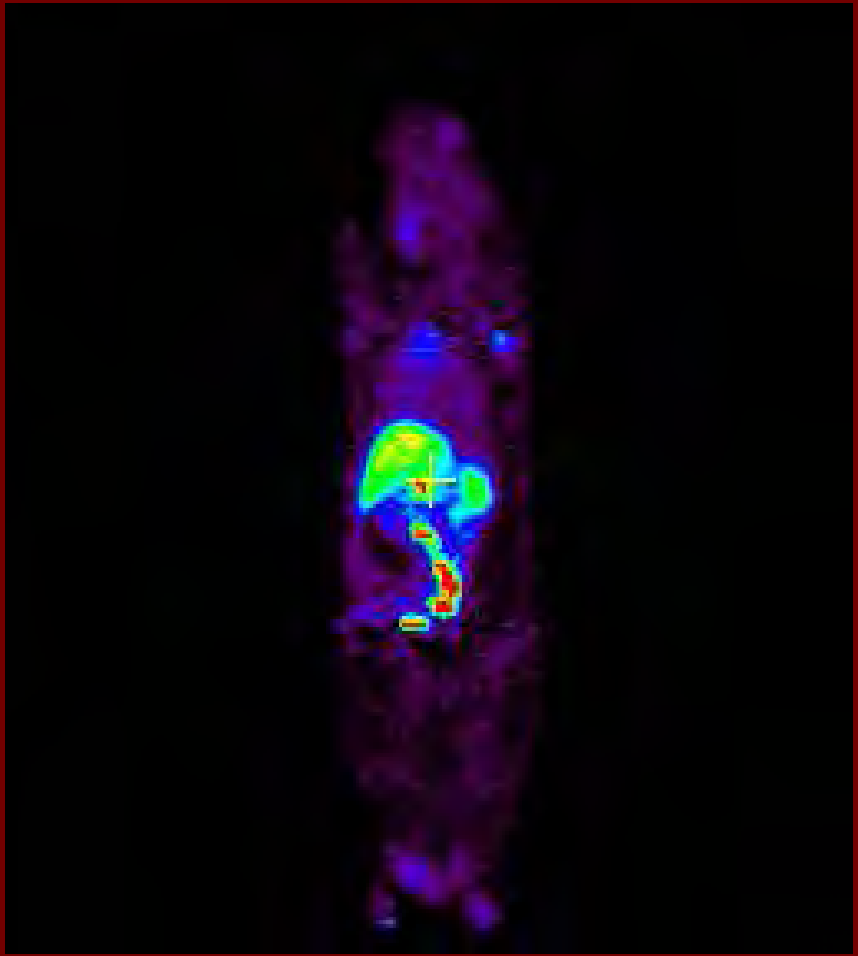
MR methods:

- 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

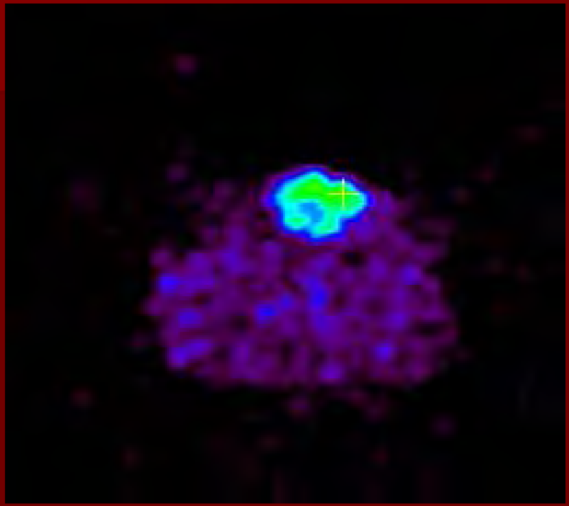
10.09 MBq 64Cu-ATSM

Coronal view (head at top)

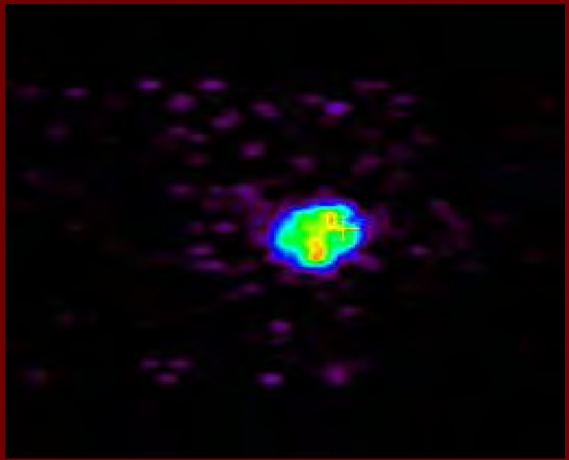
Uptake seen in liver, kidneys and bowel

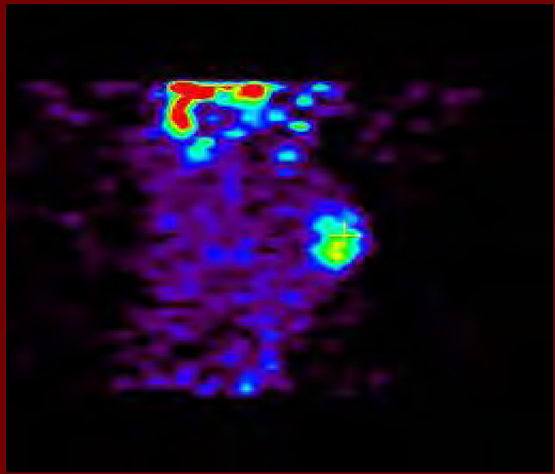
P22 tumour bearing rat: 4.2 MBq ^{64}Cu-ATSM

Axial View
Through tumour



Coronal view
Through tumour





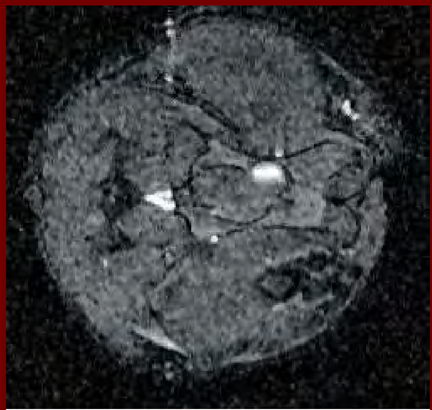
Sagittal view, liver at top

MR results:

Images of a typical tumour are shown below.

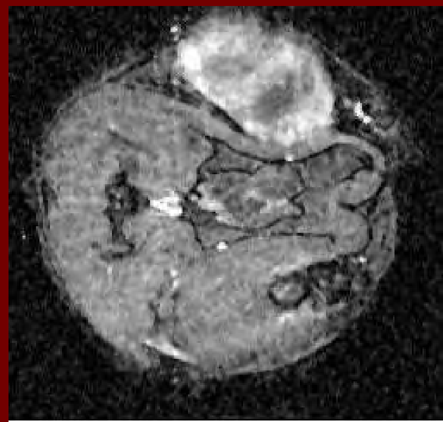
Before Gd-DTPA

tumour



At 90sec (60 sec after Gd-DTPA)

tumour

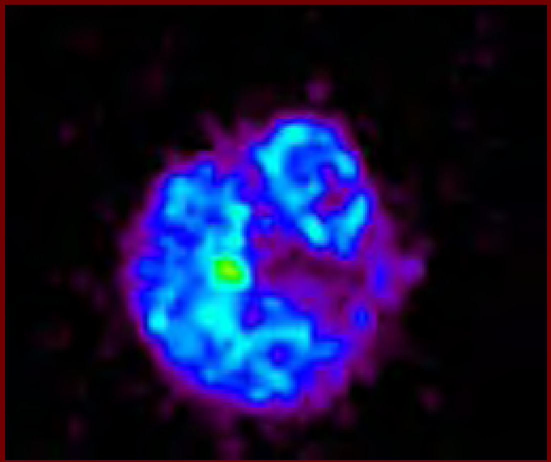


Tumour Gd-DTPA uptake appears to be greater at the periphery, with less well-perfused central areas.

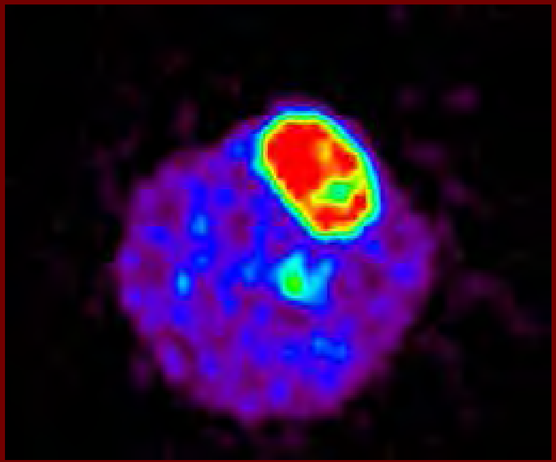
PET results:

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

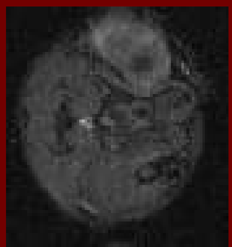


At early times ^{64}CuATSM uptake is greater at the periphery than the centre, with a very similar pattern to Gd-DTPA distribution.

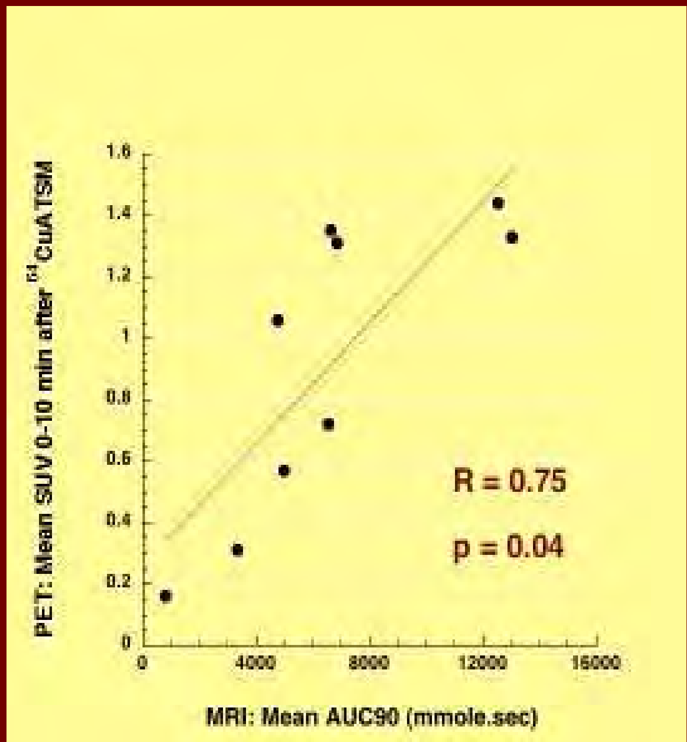
After ~ 15 -20 min this pattern disperses and uptake is distributed across the tumour

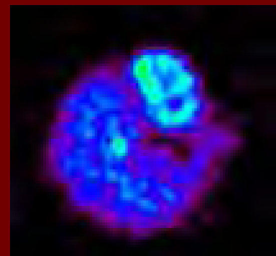
Correlation of quantitative MR and PET data - early:

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



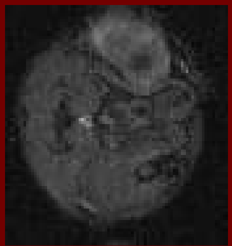


Mean SUV 0 -10 min = 1.31 for the same tumour

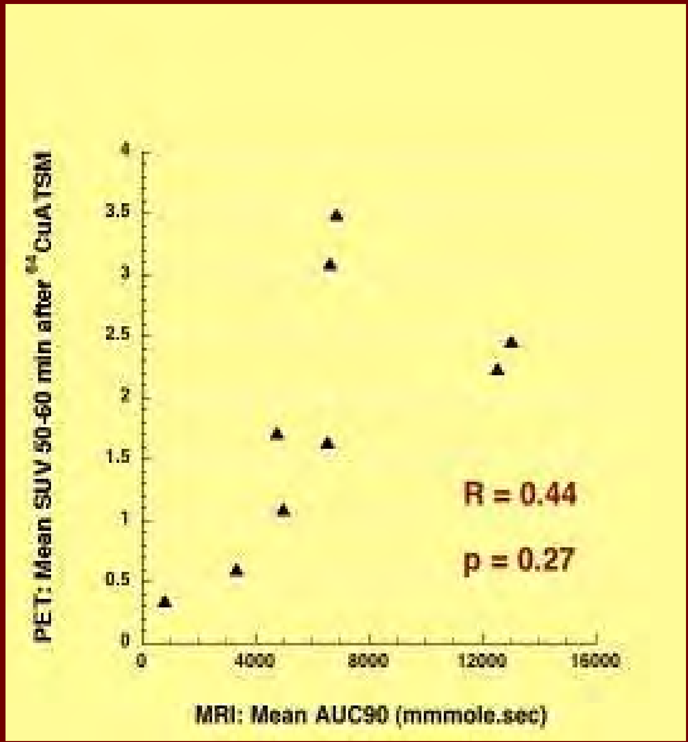
The correlation coefficient R for all 8 tumours = 0.75 (p = 0.04)

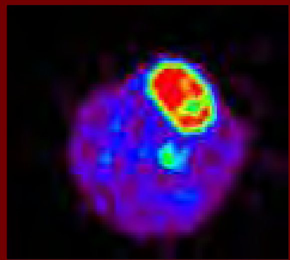
Correlation of quantitative MR and PET data - later:

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.

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Dr Jim Ballinger

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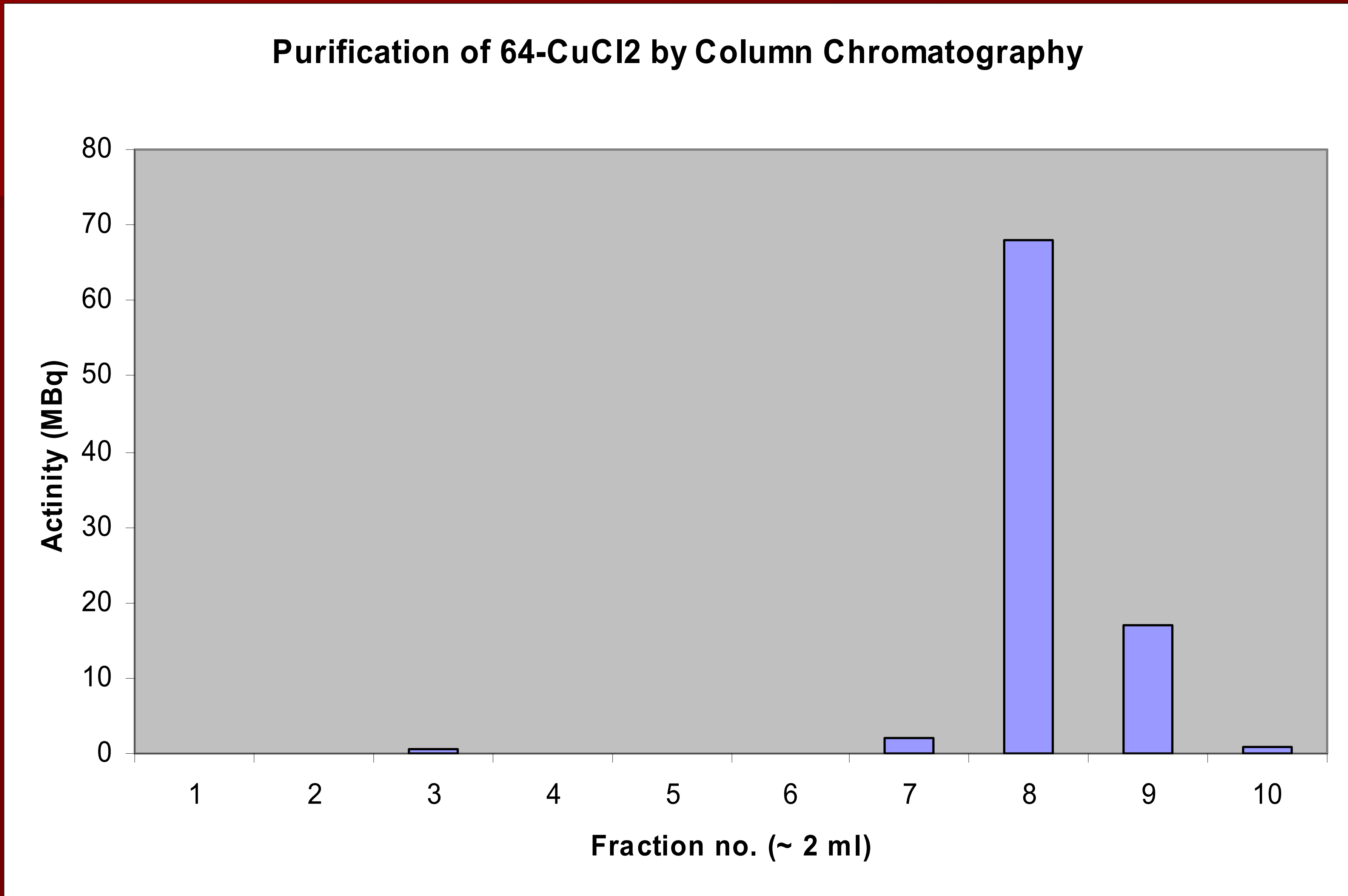
Dr Davina Honess

Dr Katie Wood

Prof Michelle Saunders


Purification of 64-CuCl2 by Column Chromatography
Actinity (MBq)
0
10
20
30
40
50
60
70
80
1
2
3
4
5
6
7
8
9
10
Fraction no. (~ 2 ml)

ICP MS Analysis

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

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.