

# Selective killing of hypoxic tumour cells with Cu-64-ATSM: potential for hypoxia-selective radionuclide therapy

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# Hypoxia

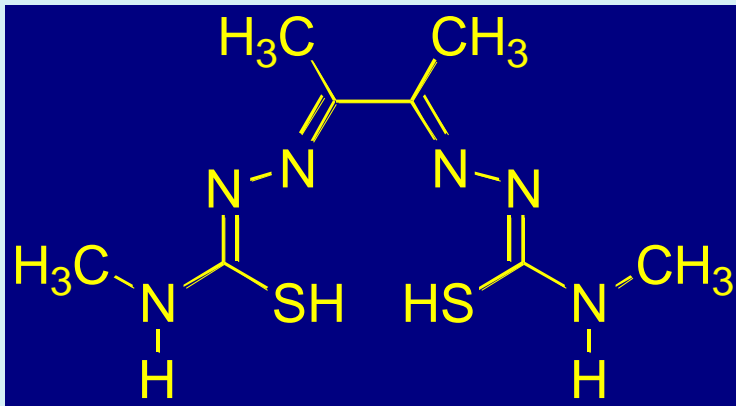
Hypoxia is a lack of oxygen in tissues usually due to poor perfusion

Hypoxia imaging maybe an important aid to “treatment planning” in solid tumours and cardiovascular disease

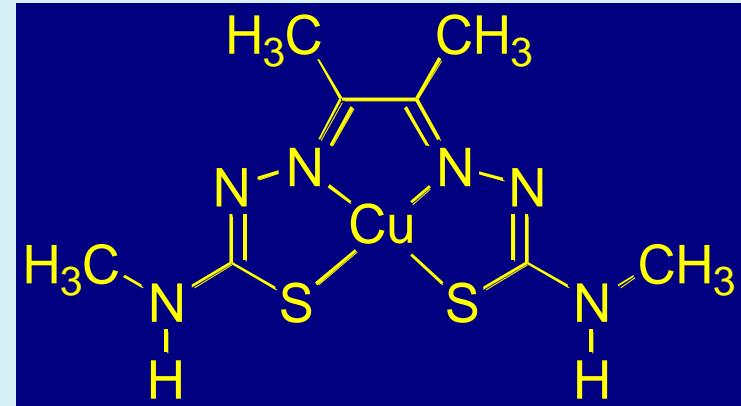
Hypoxic tumours are often resistant to conventional therapies

- Oxygen enhancement effect
- Requirement for alternative therapies
- Target hypoxic tumours

# Copper ATSM: a hypoxia selective radiopharmaceutical used for imaging tissue hypoxia



ATSM



CuATSM

# COPPER

| Isotope          | Half Life  | Radiation                                     | Source                  |
|------------------|------------|---|-------------------------|
| $^{60}\text{Cu}$ | 20 min     | $\beta^+$ (93%), EC (7%)                      | cyclotron               |
| $^{61}\text{Cu}$ | 3.3 hours  | $\beta^+$ (62%), EC (38%)                     | cyclotron               |
| $^{62}\text{Cu}$ | 9.74 mins  | $\beta^+$ (98%), EC (2%)                      | Generator/<br>cyclotron |
| $^{64}\text{Cu}$ | 12.7 hours | $\beta^+$ (18%), EC (41%),<br>$\beta^-$ (37%) | Reactor/<br>cyclotron   |
| $^{66}\text{Cu}$ | 5.1 mins   | $\beta^-$ (100%)                              | Reactor/<br>cyclotron   |
| $^{67}\text{Cu}$ | 62 hours   | $\beta^-$ (100%) $\gamma$ (52%)               | Reactor<br>/cyclotron   |

# Properties of Cu<sup>64</sup>ATSM

- ❑ Short half life (12.7 hours)
- ❑ Cu<sup>64</sup> can be produced in a cyclotron from Ni<sup>64</sup> plated onto a gold target (semi-automated)
- ❑ Lipophilicity of CuATSM permits diffusion across the cell membrane
- ❑ Redox activity of Cu(II) allows trapping of Cu<sup>64</sup> in hypoxic cells via a bioreductive mechanism
- ❑ Cu<sup>64</sup> emits gamma radiation and positrons for imaging and Auger electrons: potential for targeted therapy

# Aims of the project

- ❑ To develop an *in-situ* method of measuring uptake of  $^{64}\text{CuATSM}$  into cells cultured in 24 well plates
- ❑ To evaluate the radiobiological effects of  $^{64}\text{CuATSM}$  on cancer cells under hypoxic and normoxic conditions
- ❑ To establish whether increased cellular uptake in hypoxia is sufficient to overcome the reduced radiosensitivity of hypoxic cells

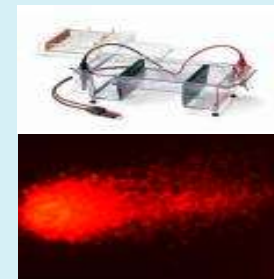
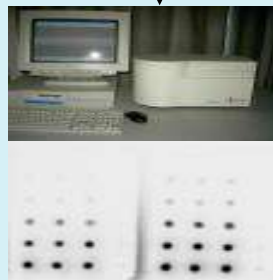
# Measuring cellular responses to $\text{Cu}^{64}\text{ATSM}$

Cells were treated with  $\text{Cu}^{64}\text{ATSM}$  in 24 well plates in normoxic or hypoxic conditions



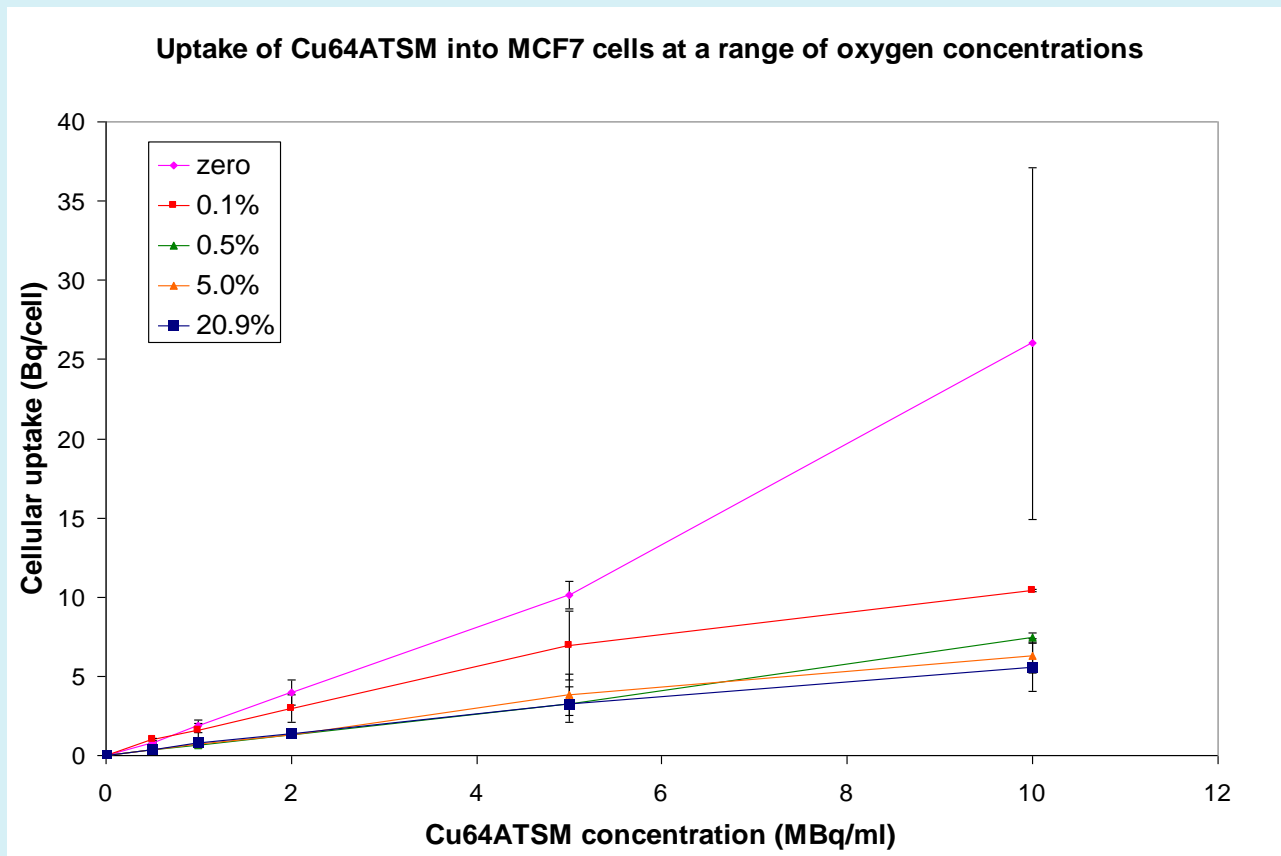
**Clonogenic survival assay**

**Measure cellular uptake using a phosphorimager or gamma counter**

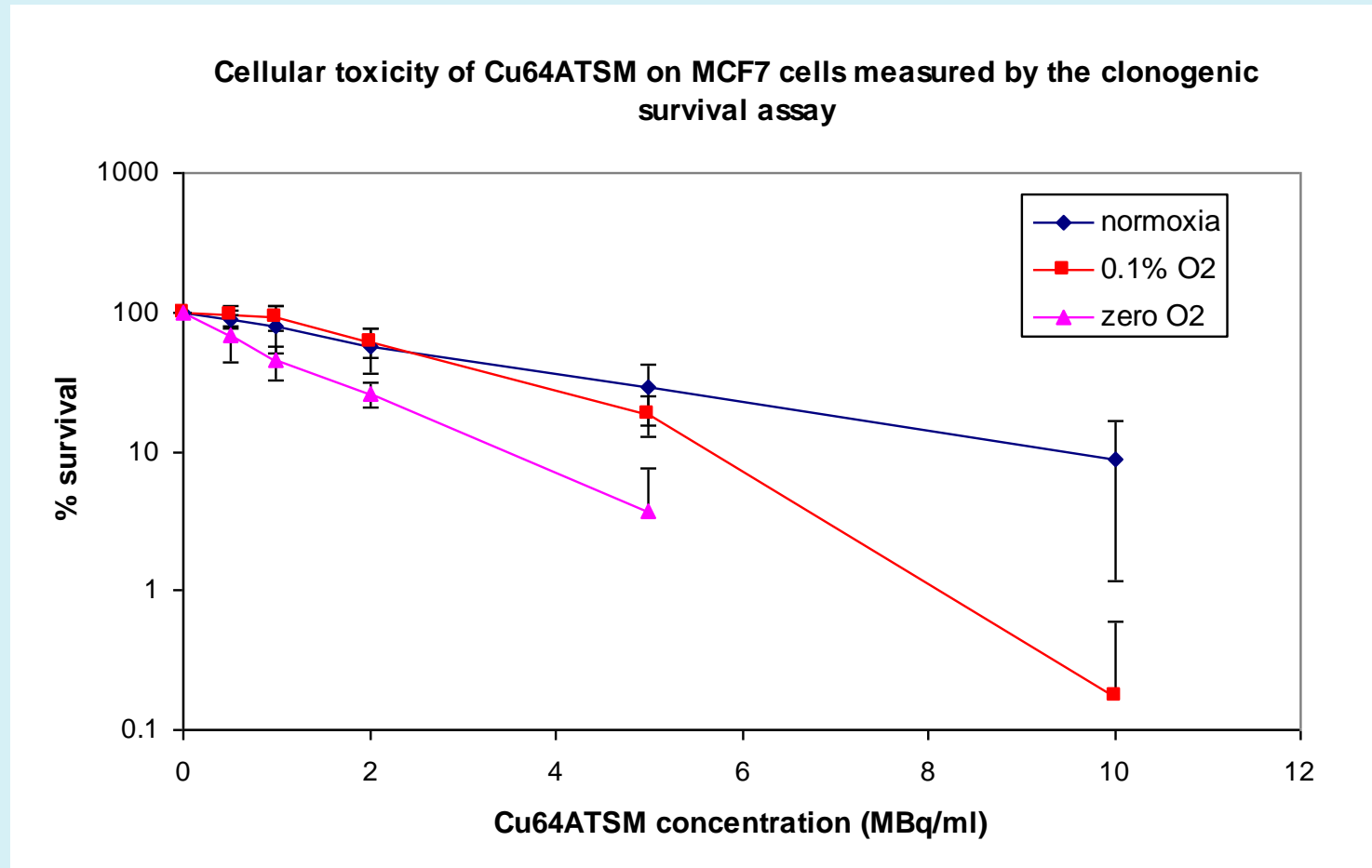


**Single cell gel electrophoresis (Comet) assay**

# Uptake of $\text{Cu}^{64}\text{ATSM}$ increases in hypoxia



# Toxicity of $\text{Cu}^{64}\text{ATSM}$ in MCF-7 cells increases with hypoxia



no detectable cytotoxicity associated with decayed Cu-ATSM

# Comet Assay

Cells treated with radiopharmaceutical



Cells are embedded on a microscope slide



Lysis of cells with mild detergent



Slides are PI stained and examined by fluorescence microscopy



Electrophoresis allows damaged DNA to migrate from the nucleus



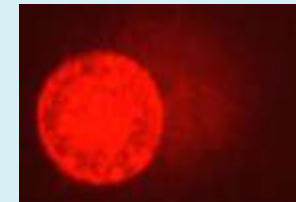
Alkali unwinding of DNA



Comets are observed emerging from nuclei of treated cells; whereas nuclei from untreated cells remain intact



A

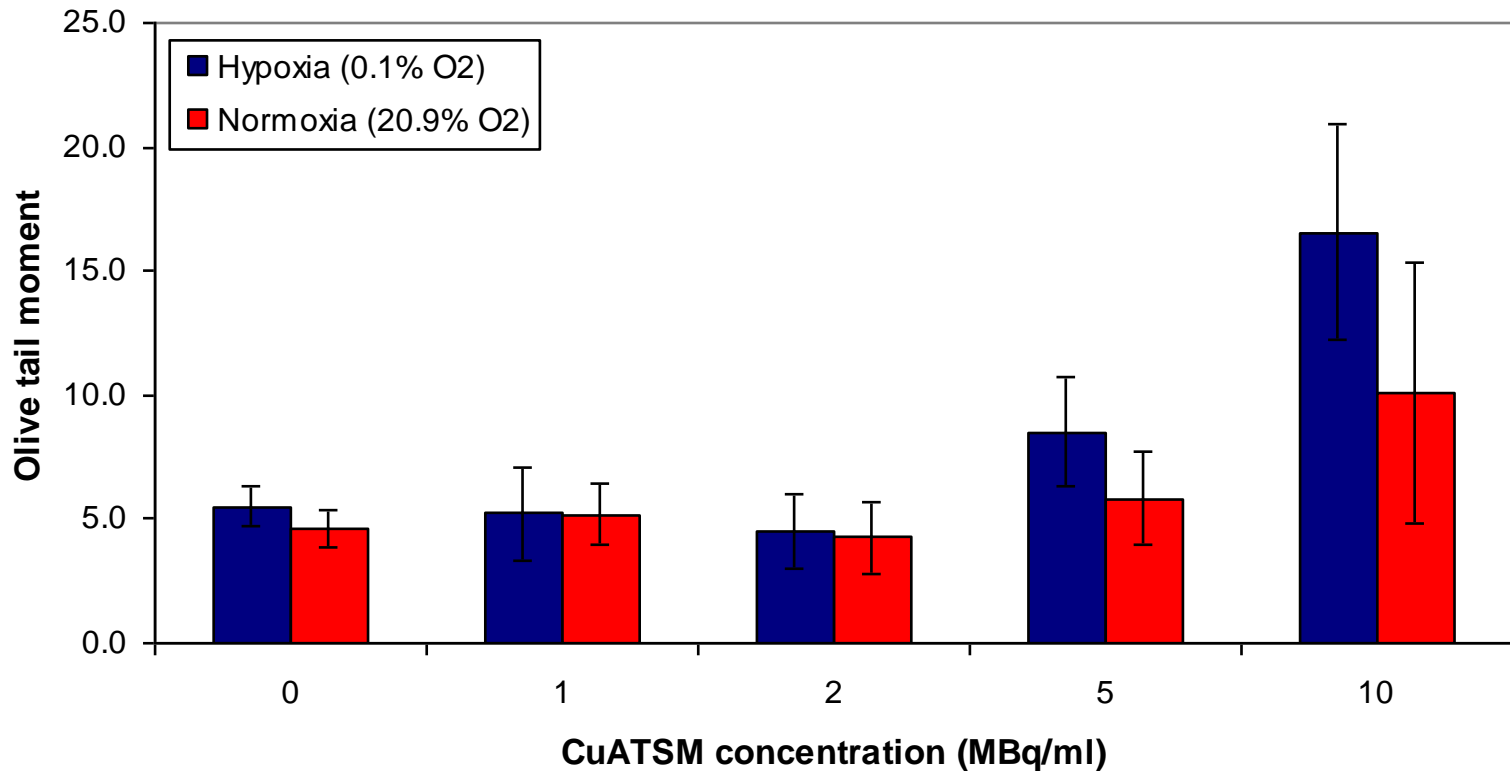


B

(A) Untreated nuclei (B) nuclei treated with 200uM H<sub>2</sub>O<sub>2</sub>

# DNA damage elicited by Cu<sup>64</sup>ATSM in MCF7 cells increases in hypoxia

DNA damage elicited by Cu<sup>64</sup>ATSM in MCF7 cells in normoxic and hypoxic conditions



# Summary

- ❑ Selective uptake of  $\text{Cu}^{64}\text{ATSM}$  into MCF-7 cells in hypoxia
- ❑ Increased toxicity in hypoxic MCF-7 cells
- ❑ Increased DNA damage in hypoxic MCF7 cells
- ❑ Increased uptake into hypoxic cells was sufficient to cause cellular toxicity and DNA damage, overcoming the reduced radiosensitivity of hypoxic cells
- ❑ Potential for hypoxia targeted therapy?

# Summary

- ❑ Producing Cu-61 and Cu-64 on a regular basis
- ❑ Two clinical trials
  - ❑ Oncology Trial (May 2008 in collaboration with UCLH)
  - ❑ Atherosclerosis (September 2008)
- ❑ Several preclinical projects
  - ❑ *In vitro* and preclinical screening of a library of ~90 ligands
    - ❑ Hypoxia uptake vs radiosensitivity (CRUK)
    - ❑ Hypoxia in Cardiovascular Disease (KCL/BHF)
    - ❑ Stroke (KCL)

# Acknowledgements

## University of Kent

- Dr. Dan Lloyd

## Kings College London

- Prof. Phil Blower

## St. Thomas's hospital (Clinical PET centre)

- Dr. Rowena Paul
- Dr. Paul Marsden
- Mr. Phil Halstead

## EPSRC