

In vivo activation of the hypoxia-targeted cytotoxin AQ4N in human tumour xenografts

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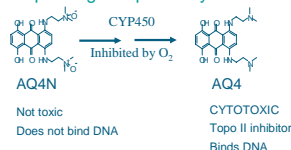
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Introduction

- The bioreductive drug AQ4N, banoxantrone, is metabolised into the cytotoxin AQ4 in hypoxic cells
- Hypoxia is prevalent in solid tumours
- Hypoxic cells are resistant to radiotherapy/chemotherapy and contribute to repopulation and treatment failure
- Preclinical studies show AQ4N can enhance efficacy of radiotherapy and chemotherapy
- Phase I clinical studies are underway to investigate AQ4N in man

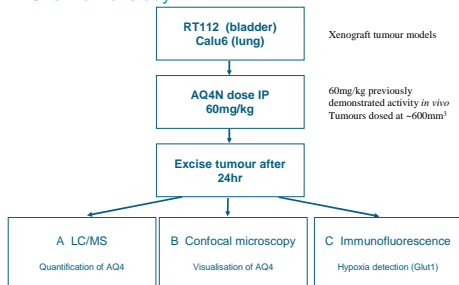
AQ4N: a prodrug of a potent cytotoxin



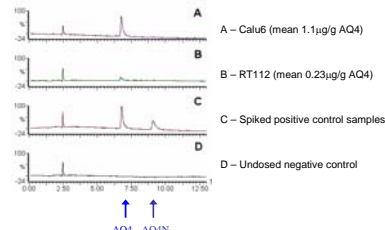
Aim of study

- Investigate activation of AQ4N in tumours *in vivo*
 - Detection and quantification of AQ4 in tumour
- Investigate extent of hypoxia in tumours
 - Detection of hypoxia in tumour
 - Co-localisation of AQ4 and hypoxia
- Correlate efficacy with tumour levels of drug and hypoxia
- Investigate and validate procedures for clinical pharmacodynamic study (Harris et al. abstract 2414)

Overview of study



A LC/MS

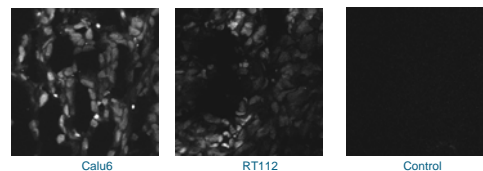


Reversed phase HPLC/mass spectrometry quantifies and discriminates AQ4N and AQ4

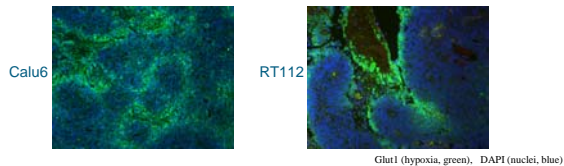
- AQ4 detected in all dosed samples
- AQ4N below LOD in all samples (expected from PK)

B Confocal microscopy

- AQ4 – bright nuclear fluorescence AQ4N – weak cytoplasmic fluorescence (PJ Smith et al. 1997)
- Heterogeneous distribution of nuclear fluorescence (AQ4) in RT112 and Calu6
- Consistent with activation in local regions of hypoxia



C Immunofluorescence



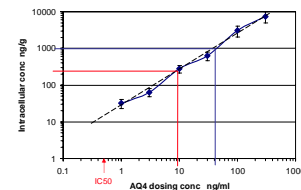
- Glucose transporter 1 (Glut-1) staining reveals hypoxic regions of xenograft tumours
 - Hypoxia present in both tumour types
 - Mean Glut1 scores
- | Tumour | Mean Glut1 score | Hypoxia prevalence |
|--------|------------------|--------------------|
| Calu6 | 2.8 | (10-20%) |
| RT112 | 1.8 | (5-10%) |

Cytotoxicity of tumour levels of AQ4

RT112 cells dosed with AQ4 for 1h.

After washing, AQ4 concentrations were quantified by LC/MS.

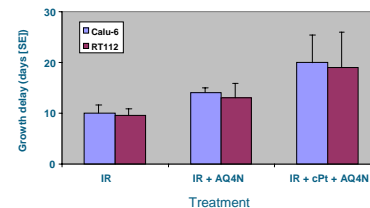
Intracellular concentrations of AQ4 (ng/g) proportional to dosing concentrations (ng/ml).



- Tumour levels of 0.25µg/g (eg RT112 xenograft) equivalent to dosing tissue culture cells at 10ng/ml for 1h (red)
- Tumour levels of 1µg/g (eg Calu6 xenograft) equivalent to dosing tissue culture cells at 40ng/ml for 1h (blue)
- IC50 *in vitro* values: RT112 ~0.5ng/ml Calu6 ~7ng/ml (clonogenic assay)
- IC90 *in vitro* values: RT112 ~2ng/ml Calu6 ~25ng/ml (clonogenic assay)
- Intratour concentrations are in excess of *in vitro* IC50
- AQ4 is heterogeneously distributed in tumours – local concentrations of AQ4 in hypoxic regions will be much higher and considerably above cytotoxic levels

60mg/kg AQ4N enhances efficacy of chemo-radiation

- RT112 (bladder) and Calu6 (lung) xenograft models
- Single dose of AQ4N, single dose cisplatin (cPt) and/or 5x2Gy fractions of radiation (IR)
- 60mg/kg AQ4N enhances efficacy of radiotherapy and chemo-radiation



Summary table

- AQ4 detected in all treated samples
- Glut1 staining correlates with AQ4 levels

Tumour	Treatment	AQ4 conc. (µg/g)	Confocal score	Hypoxia score
Calu6 (3)	AQ4N	1.07 (0.15 SD)	2.7	2.8
Calu6 (3)	Control	<0.1	0	2.3
RT112 (3)	AQ4N	0.23 (0.06 SD)	3.0	1.8
RT112 (2)	Control	<0.1	1 (not nuclear)	1.6

Conclusions

- AQ4N is activated to cytotoxic agent AQ4 *in vivo* in two xenograft models
- AQ4 can be quantified in tumours by LC/MS
- AQ4 can be directly visualised in tumours by confocal microscopy
- AQ4 levels in tumours correlate with hypoxia (Glut1)
- Activation of AQ4N in tumours has also been demonstrated in pancreatic tumours (Alters et al. abstract 3827)
- AQ4N enhances efficacy of radiation and chemo-radiation in lung and bladder models
- Intratour levels of AQ4 of 0.25-1µg/g are cytotoxic *in vitro* and sufficient to enhance efficacy *in vivo*
- Similar levels of AQ4 in clinical tumours may enhance efficacy of chemoradiation protocols in man

Phase I pharmacodynamic study

- Single dose 200mg/m² AQ4N ~24h before surgery
 - Head and Neck, Glioblastoma, Bladder, Breast, Cervix
- Analysis of tumour, normal, skin, lymph node samples
- 22/32 patient's tumours had >0.2µg/g AQ4
- 8/32 tumours >1µg/g AQ4
- Thus potentially efficacious doses delivered to tumours in man
- 22/30 tumours had >2-fold more AQ4 than adjacent normal tissue – proof of principle of selective tumour targeting
- Good correlation of AQ4 levels in tumours with Glut1 staining
- Talk by Harris et al. Monday 1-5pm, Clinical Research 8 (Abstract 2414)