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# Prediction of tumour hypoxia and radioresistance with nuclear medicine markers

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**Summary** Second-generation nuclear medicine markers of tumour hypoxia have been synthesised and screened for hypoxic marking activity in cell cultures and in mouse tumours (EMT-6). Markers of the iodinated azomycin nucleoside class with greater water solubility and faster plasma clearance rates relative to iodoazomycin arabinoside (IAZA) were of particular interest. The test systems used to characterise hypoxic marking activity of compounds included (1) covalent linkage of radiolabelled markers to cells in suspension culture equilibrated with specific $O_2$ concentrations; (2) biodistribution of radiolabelled markers in EMT-6 tumour-bearing mice; and (3) biodistribution in R3327-AT tumour-bearing rats by nuclear medicine procedures. Of the iodinated azomycin nucleosides produced to date, β-D-iodoazomycin galactoside (β-D-IAZG) and β-D-iodoazomycin xylopyranoside (β-D-IAZXP) exhibited high metabolism-dependent hypoxic cell uptake, rapid clearance kinetics from the blood and excellent tumour marking activity *in vivo*. Tumour-blood (T/B) ratio (a measure of tumour hypoxic fraction) was dependent upon EMT-6 tumour size and implantation site. The radioresistance of individual tumours was measured by *in vivo/in vitro* assay and correlated well with the T/B ratio of hypoxic marker. These studies have identified β-D-IAZG and β-D-IAZXP as effective hypoxic markers for planar and single photon emission computerised tomography (SPECT) imaging studies of tumour oxygenation.

**Keywords:** iodoazomycin arabinoside; β-D-iodoazomycin galactoside; β-D-iodoazomycin xylopyranoside; single photon emission computerised tomography

Although oxygenation status may predict for tumour radioresistance (Gray *et al.*, 1953), this tumour property is not routinely measured during patient work-up for radiotherapy prescription. Clinical studies performed over recent years with hypoxia-directed therapies, including hyperbaric oxygen therapy (HBO), neutron radiotherapy, hypoxic cell sensitiser trials and pretreatment transfusion of anaemic cancer patients did not determine if the tumours in the patients accessed to those studies did, indeed, contain hypoxic cells. Recent studies of human tumour oxygenation show this property to be extremely heterogeneous both within and between tumours of the same histological class and between tumours of different histology (Vaupel *et al.*, 1992; Chapman, 1991). These data indicate that if tumour oxygenation is to be employed as a predictor of radioresistance, it will have to be measured for each tumour. In that regard, at least two different procedures for measuring tumour oxygenation have now been tested in pilot studies with over 50 patients (Stone *et al.*, 1993). The Eppendorf $pO_2$ histograph is a computer driven microelectrode system which makes $pO_2$ measurements along spatially distinct tracks within tumours. Several studies with both rodent and human tumours have now demonstrated the accuracy, reproducibility and practicality of this device for quantifying tumour oxygenation status in accessible tumours (Vaupel *et al.*, 1991; Höckel *et al.*, 1993; Yeh *et al.*, 1995). *In vitro* studies of hypoxic radiosensitiser toxicity showed that metabolically activated forms of 2-nitroimidazole drugs can covalently link to cellular molecules at rates which are inversely proportional to intracellular oxygen (Wong *et al.*, 1978; Chapman *et al.*, 1981, 1983). The potential for sensitiser adducts to provide indirect measurements of tumour oxygenation status was recognised (Chapman 1979; Urtasun *et al.*, 1986). A clinical study with tritiated misonidazole was encouraging (Chapman *et al.*, 1989) and this procedure has now been used to predict tumour oxygenation status by non-invasive imaging techniques of positron emission tomography (PET) (Koh *et al.*, 1992) and single photon emission computerised tomography (SPECT) (Parliament *et al.*, 1992; Groshar *et al.*, 1993; Urtasun *et al.*, 1996). The PET procedure is suboptimal due to the short half-life of most positron-emitting isotopes which require isotope detection in tumours before adequate clearance of unbound marker from normal tissues and the unavailability of this diagnostic equipment to most cancer centres. The SPECT procedure with IAZA is suboptimal because the marker requires primary chemical syntheses, has a relatively long clearance half-life from plasma and tissue, undergoes *in vivo* dehalogenation and is relatively lipophilic which promotes non-hypoxia-specific labelling associated with hepatobiliary excretion.

A marker which could accurately measure tumour oxygenation status would find wide clinical utility if (1) it produced meaningful averages of this property over tumour volumes which present for radiotherapy; (2) it could be administered before, during and after fractionated radiotherapy treatment for investigation of tumour oxygenation and reoxygenation and (3) it was non-invasive and used equipment available to most modern cancer centres. Consequently, our research has synthesised second-generation nuclear medicine markers of tumour hypoxia of the iodinated azomycin nucleoside class with improved chemical properties relative to IAZA and azomycin-chelates which radiolabel with Te-99m or In-111. The hypoxic marking activity of some novel, water soluble markers of the iodinated azomycin nucleoside class are described along with animal tumour radiobiology data which demonstrates that marker avidity does, indeed, predict for tumour radioresistance.

## Materials and methods

Second-generation iodinated azomycin nucleosides with lipid-water partition coefficients near to 1.0 were synthesised using published procedures with minor modifications unique to each compound. The novel markers were purified by high performance liquid chromatography (HPLC) and chemically defined by melting point, UV/VIS spectroscopy and NMR spectroscopy. Techniques for exchange labelling markers with iodine-125 and/or iodine-123 were individually

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optimised. Details of these synthetic and radiolabelling procedures are similar to those for IAZA (Mercer *et al.*, 1990) and will be reported elsewhere. Hypoxic marking activity of β-D-iodoazomycin galactoside (β-D-IAZG), β-D-iodoazomycin lyxopyranoside (β-D-IAZLP), β-D-iodoazomycin glucoside (β-D-IAZGL), β-D-iodoazomycin xylopyranoside (α-D-IAZXP), α-L-iodoazomycin xylopyranoside (α-L-IAZXP) and α-D- iodoazomycin arabinoside (α-D-IAZA) are reported.

The bioreducible and hypoxic marking potential of various concentrations of each marker were investigated by measuring the covalent linkage of their radioactivity to EMT-6 tumour cells *in vitro*. EMT-6 tumour cells were grown in tissue culture flasks to mid-exponential growth phase, trypsinised from flasks, concentrated by centrifugation procedures and resuspended in Spinner minimal essential medium (S-MEM) plus 5% fetal calf serum and antibiotics. Cell cultures were incubated at 37°C in slowly stirred suspensions in the presence of known concentrations of radiolabelled marker and defined oxygen environments (Chapman *et al.*, 1991). Aliquots of cells were removed every 90 min and the radioactivity linked to whole cells and/or to cell macromolecular fractions were determined with a γ-well counter. The total amount of bound drug was computed in units of pmol $10^{-6}$ cells. Time (metabolism)-dependent binding of markers to cells was best fit by a linear function to yield adduction rates in units of pmol $10^{-6}$ cells $h^{-1}$. Absolute rates of marker binding were determined as a function of marker concentration and oxygen concentration.

Marker pharmacokinetics and avidity to hypoxic cells *in vivo* were investigated with EMT-6 tumours growing in C.B17/Icr *scid* mice. Iodine-125 labelled markers were injected i.v. into tumour-bearing mice and their distribution into and clearance from tumour and normal tissues were measured by scintigraphy of necropsied tissue specimens. Initial rates of clearance from plasma were derived from at least four different time measurements over the first three hours and activity levels in tissues at 18–24 h were used to define maximal tumour–normal tissue ratios. Tumour–blood (T/B) and tumour–muscle (T/M) ratios were used to estimate the hypoxic avidity of each novel compound.

Additional studies of tumour oxygenation were performed with β- D-IAZG which exhibited the fastest plasma clearance half-life and good tumour marking potential. It was administered either i.v. or i.p. since both routes were found to produce equivalent bioavailability of marker to the tumour and the normal tissues of interest. T/B ratios were measured as a function of time after marker administration, as a function of weight of tumours grown in the flank and on the back of animals and as a function of individual tumour radiosensitivity. Tumour-bearing animals which had been administered (I-125) β-D-IAZG 18–24 h earlier were irradiated with 20 Gy immediately before sacrifice. The EMT-6 tumours were resected *in toto* and dissected into several pieces. Tumour samples were well mixed to average any spatial heterogeneity of hypoxia and assayed for both incorporated radioactivity and cell plating efficiency *in vitro*. These assays produced numerical values of T/B ratio and cellular plating efficiency after 20 Gy for each tumour.

## Results

Figure 1 shows absolute rates of hypoxic marker adduction to the macromolecular fraction of severely hypoxic EMT-6 cells for different concentrations of the six iodinated azomycin nucleosides, five of which are novel structures and all with water solubilities greater than IAZA (Mannan *et al.*, 1991). Under these *in vitro* conditions, β-D-IAZXP exhibited the highest rate of marker binding with kinetics near to 1/2-order. α-L-IAZXP, α-D-IAZA and β-D-IAZLP had slightly lower binding rates with 1/2-order kinetics and β-D-IAZG and β-D-IAZGL exhibited the lowest rates of hypoxic binding but with kinetics near to 1-order. These *in vitro* data were used to estimate the maximal metabolism-dependent cell marking effectiveness of novel compounds. Binding rates of β-D-IAZG were measured for EMT-6 cells equilibrated with air plus 5% carbon dioxide and nitrogen plus 5% carbon dioxide with 1.0%, 0.3%, 0.1% and <0.001% oxygen. The ratios of binding rates to severely hypoxic and aerobic cells was ~30 and the $K_m$ of the oxygen dependency was 0.5% oxygen in the gas phase. These characteristics of oxygen inibition of the metabolic binding

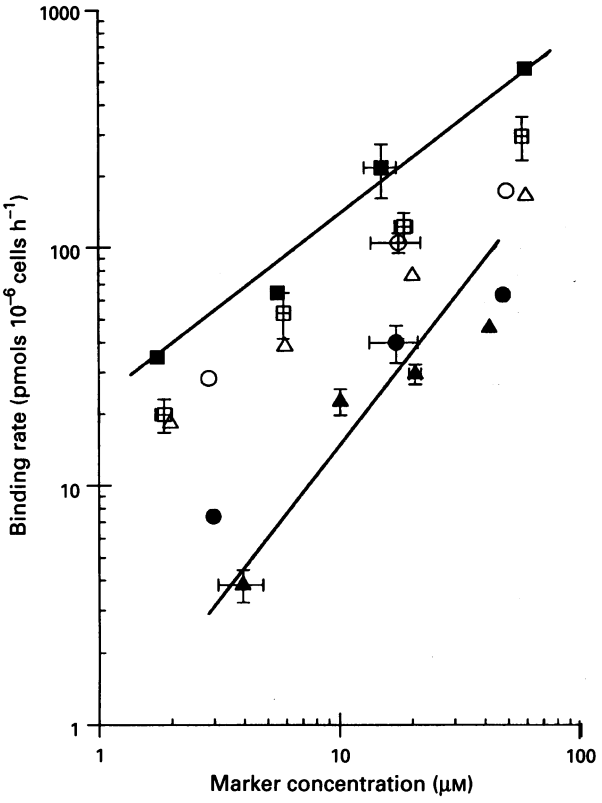


**Figure 1** Binding rates of iodoazomycin nucleosides to hypoxic EMT-6 cells *in vitro* as a function of marker concentration. Symbols designate specific markers as follows: β-D-IAZG (▲), β-D-IAZGL (●), β-D-IAZLP (△), α-L-IAZXP (○), α-D-IAZA (□) and β-D-IAZXP (■). Symbols represent mean values and bars represent s.e. for three or more determinations.

**Table I** Chemical and biological properties of novel hypoxic markers

| *Marker* | *MW* | *P Octanol/ water* | In vitro *binding rate at 10 μM (pmol $10^{-6}$ cells $h^{-1}$)* | *Plasma $T_{1/2}$ (h)* | *T/B 8–24 h* |
|---|---|---|---|---|---|
| β-D-IAZ[a] galactoside | 385.1 | 0.63 | 13.6 | 0.46 | 11.1 |
| β-D-IAZ lyxopryanoside | 355.1 | 1.00 | 51.5 | 0.79 | 7.3 |
| β-D-IAZ glucoside | 385.1 | 1.07 | 20.9 | 0.52 | 7.9 |
| β-D-IAZ xylopyranoside | 355.1 | 1.26 | 133 | 0.46 | 9.9 |
| α-L-IAZ xylopyranoside | 355.1 | 1.29 | 65.5 | 1.51 | 11.7 |
| α-D-IAZ arabinoside | 355.1 | 3.85 | 76.0 | 0.87 | 8.3 |

[a]IAZ, Iodinated azomycin.

of β-D-IAZG to EMT-6 cells are similar to those previously reported for misonidazole and some analogues (Chapman *et al.*, 1990) indicating that the azomycin substituent of these bioreducible markers governs their marking ability. The lines in Figure 1 are for the binding kinetics of β-D-IAZXP and β-D-IAZG.

Table I shows the initial plasma clearance rates of the hypoxic markers in C.B17/Icr *scid* mice along with their octanol–water partition coefficients and other properties. Since the clearance of unbound marker is essential for the optimal detection of marker linked to hypoxic tissue, the two markers with the fastest clearance rates were selected for additional studies. T/B ratios were measured as a function of time after marker administration for ~0.1 $cm^3$ tumours growing in animal flanks. Figure 2 shows that this ratio increases rapidly for both markers for 5–6 h and reaches maximum plateaux after 10 h. These data confirm our previous hypothesis that at least ten half-lives of marker clearance are required for the expression of maximal hypoxic tumour signal relative to marker bound to normal tissues (Parliament *et al.*, 1992). Maximum T/B ratios of hypoxic markers in EMT-6 tumours are significantly higher than those observed with other rodent tumours. These same markers produce maximum T/B ratios of only 3–5 in R3327-AT rat prostate carcinomas in Fischer X Copenhagen rats (Schneider *et al.* unplublished results).

β-D-IAZG was used to investigate marker avidity in tumours growing in the flanks and on the backs of mice. Figure 3 shows T/B ratios (at 18–24 h) for EMT-6 tumours as a function of tumour weight. Tumours growing subcutaneously in the flank exhibited T/B ratios which were independent of tumour weight between 0.05 and 0.6 g, indicative of a relatively uniform hypoxic fraction. In contrast to this result, EMT-6 tumours growing subcutaneously on the back, exhibited a marked rise in T/B ratios as tumour weight increased from 0.1 to 0.25 g. The skin over these tumours with volumes >0.1 $cm^3$ was extremely taut and showed evidence of varying degrees of necrosis. Increased pressures within tumours at this site may have led to reduced blood flow and increased hypoxic fraction as measured by hypoxic marker avidity.

Additional studies were performed to determine if β-D-IAZG avidity to EMT-6 tumours was predictive of individual tumour radioresistance. For these experiments, tumours were grown subcutaneously on the backs of mice to exploit the wide variation observed for T/B ratios (Figure 3). Animals with EMT-6 tumours of various volume were labelled *in vivo* with β-D-IAZG for 18–24 h, irradiated with 20 Gy of Cs-137 γ-rays and analysed for both marker avidity (T/B ratio) and tumour cell radiosensitivity measured by *in vivo/in vitro* clonogenic assays. A total of 44 tumours with volumes of 0.05 to 0.8 $cm^3$ were used in this study. For data presentation and analyses, animals were divided into five groups (four groups of nine animals and one group of eight animals) from smallest to largest tumours based upon tumour weight at resection. Figure 4 shows the plating efficiencies of cells derived from these tumours as a function of tumour weight. Over the range of 0.1–1.0 g, a positive correlation between increased plating efficiency (radioresistance) and tumour volume is observed. Tumours of ~50 mg consistently showed higher plating efficiencies than tumours of ~100 mg. This result may indicate that angiogenesis and vascularisation of the smaller tumour masses were incom-

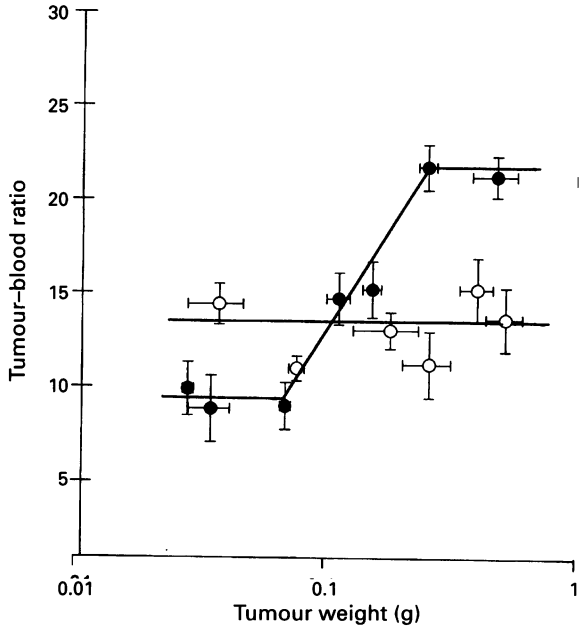


**Figure 3** EMT-6 tumour–blood ratios of β-D-IAZG 18–24 h after administration for tumours growing on the back (●) or in the flank (○) of C.B17/Icr *scid* mice as a function of tumour size. Mean values and s.e.. for $n=5$.

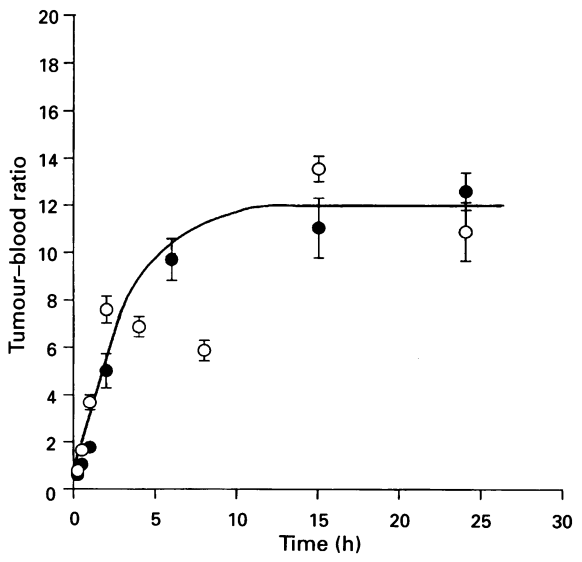


**Figure 2** EMT-6 tumour–blood ratios of β-D-IAZG (●) and β-D-IAZXP (○) at various times after i.v. administration for tumour volumes of ~0.1 $cm^3$. Mean values and s.e. for $n=5$.

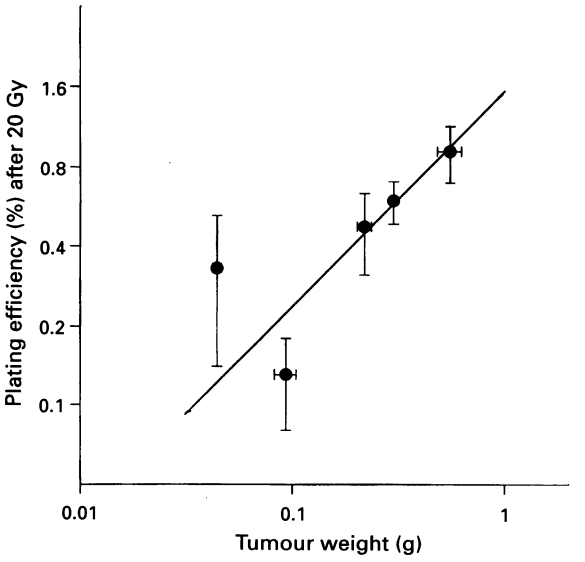


**Figure 4** Tumour radioresistance as measured by *in vivo/in vitro* assays of cell viability after 20 Gy as a function of tumour size. Mean values and s.e.. for $n = 9$.

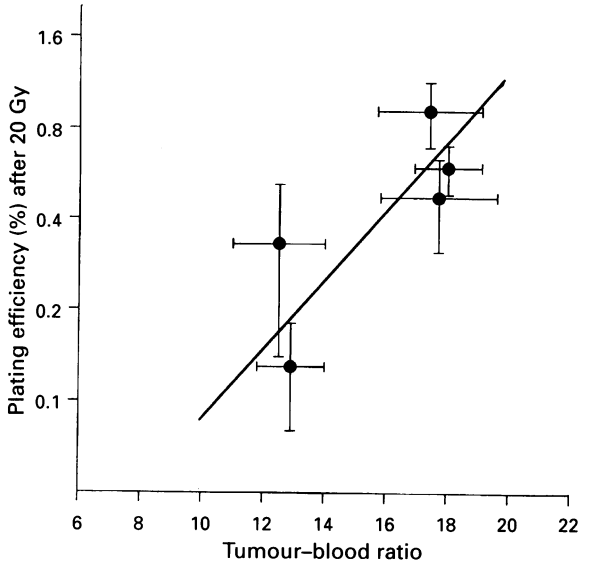


**Figure 5** Tumour radiosensitivity as measured by *in vivo/in vitro* assays of cell viability after 20 Gy as a function of hypoxic marker (β-D-IAZG) avidity (T/B ratio). Mean values and s.e. for $n=9$.

plete. When tumour plating efficiency (radiosensitivity) was plotted *vs* the T/B ratio of the same tumour group, a good correlation between hypoxic marker avidity and radioresistance was demonstrated (Figure 5).

## Discussion

Second-generation bioreducible nuclear medicine markers of the iodoazomycin nucleoside class have been synthesised, chemically characterised and radiolabelled with iodine-125 and iodine-123. Tumour biology data for EMT-6 tumours growing in mice indicate that hypoxic marker avidity is predictive of radioresistance in this animal tumour model. These studies have identified at least two markers with higher water solubility and faster plasma clearance rates relative to IAZA. The uptake of radiolabel into mouse thyroid tissue was not measured since previous studies with IAZA (Mannan *et al.*, 1991; Parliament *et al.*, 1992) suggested that the mouse model was not predictive of the dehalogenation observed in humans, probably because of the relatively rapid clearance rate of these compounds in the mouse. If the shorter plasma half-lives observed with β-D-IAZG and β-D-IAZXP in mice translate into a faster plasma clearance rate in humans, dehalogenation of these compounds in humans might be less than that observed for IAZA.

Ongoing studies in our laboratory will determine if planar and/or SPECT imaging of (I-123) β-D-IAZG in R3327-AT rat prostate tumours growing in Fischer X Copenhagen rats can predict for tumour radioresistance, non-invasively. As well, T/B ratios obtained for individual tumours by imaging and scintigraphy procedures will be correlated with median $pO_2$ measured with the Eppendorf $pO_2$ histograph, $P_i/\beta$-NTP ratios from NMR spectroscopy and *in vitro* assays of tumour cell clonogenicity after a dose of 20 Gy delivered to tumours *in vivo*. The establishment of any novel assay of individual tumour hypoxic fraction will require extensive validation by independent measurements of tumour oxygenation.

The power of nuclear medicine procedures for identifying and investigating tumour oxygenation status has already been demonstrated (Koh *et al.*, 1992; Parliament *et al.*, 1992; Urtasun *et al.*, 1986). The iodoazomycin nucleoside markers are produced by relatively laborious synthetic and radiochemistry procedures. Nuclear medicine practice performs most of its assays with chelates which link Te-99m (or In-111) to molecules of interest by so called 'shake and bake' chemistry. We have synthesised five hypoxic markers of the azomycin-chelate class using two distinct chelation groups. To date, those markers show unacceptable levels of non-specific labelling to cells and serum proteins which masks the component of marker bound after bioreductive activation in hypoxic cells. One hypoxic marker of the azomycin-chelate class has exhibited T/B ratios of ~5 in the EMT-6 mouse tumour model. The prospect of identifying an azomycin-chelate which can be linked to Te-99m for measuring tumour oxygenation status appears promising and is under active investigation in at least five different laboratories. If an appropriate Te-99m or I-123 labelled compound with good hypoxic marking activity can be identified, nuclear medicine procedures for monitoring tumour oxygenation would be available to most modern cancer centres. If this tumour property is found to be a strong predictor of radioresponse for some human tumours, such a nuclear medicine assay would find wide utility.

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## References

CHAPMAN JD. (1979). Hypoxic sensitizers: implications for radiation therapy. *N. Engl. J. Med.*, **301**, 1429–1432.

CHAPMAN JD. (1991). Measurement of tumor hypoxia by invasive and non-invasive procedures: a review of recent clinical studies. *Radiother. Oncol.*, **20**, 13–19.

CHAPMAN JD, FRANKO AJ AND SHARPLIN J. (1981). A marker for hypoxic cells in tumors with potential clinical applicability. *Br. J. Cancer*, **43**, 546–550.

CHAPMAN JD, BAER K AND LEE J. (1983). Characteristics of the metabolism-induced binding of misonidazole to hypoxic mammalian cells. *Cancer Res.*, **43**, 1523–1528.

CHAPMAN JD, URTASUN RC, FRANKO AJ, RALEIGH JA, MEEKER BE AND MCKINNON SA. (1989). The measurement of oxygenation status of individual tumors. In *Prediction of Response in Radiation Therapy: the Physical and Biological Basis*, Fowler JF, Herbert DE, Kinsella TJ and Orton CG (eds). pp.49–60. American Institute of Physics: New York.

CHAPMAN JD, LEE J AND MEEKER BE. (1990). Adduct formation by 2-nitroimidazole drugs in mammalian cells: optimization of markers for tissue oxygenation. In *Selective Activation of Drugs by Redox Processes*, Adams GE, Breccia A, Fielden EM and Warden P. (eds). pp. 313–323. Plenum Press: New York.

CHAPMAN JD, STOBBE CC, ARNFIELD MR, SANTUS R, LEE J AND MCPHEE MS. (1991). Oxygen dependency of tumor cell killing *in vitro* by light-activated Photfrin II. *Radiat. Res.*, **126**, 73–79.

GRAY LH, CONGER AD, EBERT M, HORNSEY S AND SCOTT OCA. (1953). Concentration of oxygen dissolved in tissues at time of irradiation as a factor in radiotherapy. *Br. J. Radiol.*, **26**, 638–648.

GROSHAR D, MCEWAN AJB, PARLIAMENT MB, URTASUN RC, GOLDBERG LE, HOSKINSON M, MERCER JR, MANNAN RH, WEIBE LI AND CHAPMAN JD. (1993). Imaging tumor hypoxia and tumor perfusion. *J. Nucl. Med.*, **34**, 885–888.

HÖCKEL M, KNOOP C, SCHLENGER K, VORNDRAN B, BAUSSMANN E, MITZE M, KNAPSTEIN PG AND VAUPEL P. (1993). Intratumoral $pO_2$ predicts survival in advanced cancer of the uterine cervix. *Radiother. Oncol.*, **26,** 45–50.

KOH WJ, RASEY JS, EVANS ML, GRIERSON JR, LEWELLEN TK, GRAHAM MM, KROHN KA AND GRIFFIN TW. (1992). Imaging of hypoxia in human tumors with [F-18] fluoromisonidazole. *Int. J. Radiat. Oncol. Biol. Phys.*, **22,** 199–212.

MANNAN RH, SOMAYAJI VV, LEE J, MERCER JR AND CHAPMAN JD. (1991). Radioiodinated 1-(5-Iodo-5-deoxy-D-arabinofuranoxyl)-2-nitroimidazole (Iodoazomycin Arabinoside: IAZA), a novel marker of tissue hypoxia. *J. Nucl. Med.*, **32,** 1764–1770.

MERCER JR, MANNAN RH, SOMAYAJI VV, LEE J, CHAPMAN JD AND WIEBE LI. (1990). Sugar-coupled 2-nitroimidazoles: Novel *in vivo* markers for hypoxic tumor tissue. In *Advances in Radiopharmacology*, Maddalena DJ, Snowdon GM and Boniface GR. (eds) p. 103–104. Int. Assoc. Radiopharmacol., Univ. Wollongong Printing Services: Wollongong, Australia.

PARLIAMENT MB, CHAPMAN JD, URTASUN RC, McEWAN AJ, GOLDBERG LE, MERCER JR, MANNAN RH AND WEIBE LI. (1992). Non-invasive assessment of human tumor hypoxia with $^{123}$I-Iodoazomycin arabinoside: Preliminary report of a clinical study. *Br. J. Cancer*, **65,** 90–95.

STONE HB, BROWN JM, PHILLIPS TL AND SUTHERLAND RM. (1993). Oxygen in human tumors: Correlation between methods of measurement and response to therapy. *Radiat. Res.*, **136,** 422–434.

URTASUN RC, KOCH CJ, FRANKO AJ, RALEIGH JA AND CHAPMAN JD. (1986). A novel technique for measuring human tissue $pO_2$ at the cellular level. *Br. J. Cancer*, **54,** 453–457.

URTASUN RC, PARLIAMENT MB, MCEWAN AJ, MERCER JR, MANNAN RH, WEIBE LI, MORIN C AND CHAPMAN JD. (1996). Measurement of hypoxia in human tumors by non-invasive SPECT imaging of iodoazomycin arabinoside. *Br. J. Cancer*, **73,** (suppl. 27).

VAUPEL P, SCHLENGER K, KNOOP C AND HÖCKEL M. (1991). Oxygenation of human tumors: Evaluation of tissue oxygen distribution in breast cancers by computerized $O_2$ tension measurements. *Cancer Res.*, **51,** 3316–3322.

VAUPEL P, SCHLENGER K AND HÖCKEL M. (1992). Blood flow and tissue oxygenation of human tumors: an update. In *Oxygen Transport to Tissue XIV*, Erdman W, Bruley DF. (eds) pp. 139–151. Plenum Press: New York.

WONG TW, WHITMORE GF AND GULYAS S. (1978). Studies on the toxicity and radiosensitizing ability of misonidazole under conditions of prolonged incubation. *Radiat. Res.*, **75,** 541–555.

YEH KA, BIADE S, LANCIANO RM, BROWN DQ, FENNING MC, BABB J, HANKS GE AND CHAPMAN JD. . (1995). Polarographic needle electrode measurements of oxygen in rat prostate carcinomas: accuracy and reproducibility. *Int. J. Radiat. Oncol. Biol. Phys.*, **33,** 111–118.