Rhenium-188 and Copper-67 Radiopharmaceuticals for the Treatment of Bladder Cancer

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Abstract: The favourable nuclear properties of copper-67 and rhenium-188 for therapeutic application are described, together with methods for the chemical synthesis of a number of derivatives. Survival from invasive bladder cancer has changed little over the past 20 years. The intravesicular administration of Cu-67 or Re-188 radiopharmaceuticals in the treatment of bladder cancer offers some promise for improvement in this situation.

Keywords: Rhenium-188, Copper-67, Bladder, Cancer, Radioimmunotherapy.

INTRODUCTION

The therapeutic application of parenterally administered radionuclides dates back more than 60 years to the administration of phosphorus-32 phosphate to a patient with chronic myeloid leukaemia. The effectiveness of this treatment arose from a favourable combination of the nature of the lesions treated, the availability of a suitable radionuclide (P-32) and the appropriateness of the radiopharmaceutical. For many years the development of therapeutic radiopharmaceuticals has been dictated by limitations in the availability of suitable radionuclides, and even now, most therapeutic regimes are based on the use of radiopharmaceuticals containing iodine-131, yttrium-90 and phosphorus-32.

Of late the number of radiopharmaceuticals for targeted therapy has increased significantly. Increased knowledge of radiochemistry has made possible the synthesis of ligands with improved targeting properties, and these in turn have made it possible to exploit the favourable nuclear properties of selected radionuclides for optimal therapeutic effect.

Rhenium-188 has the particular advantage of being generator produced, and therefore accessible to those centres who do not have ready access to nuclear production facilities. Copper-67 has many potentially useful chemical properties which make possible the synthesis of stable complexes with predictable biological properties.

BLADDER CANCER

This condition was originally reported in Germany in 1895 [1]. Interest in the subject originally focussed on occupational bladder cancer, particularly in dyestuffs workers. Since then, a number of causal agents have been identified including tobacco, drugs such as phenacetin, infection and ionising radiation. The majority of bladder cancers are malignant, and of these, most are transitional cell tumours. The survival from invasive bladder cancer has changed little over the last 20 years. 5-year tumour-free survival of muscle invasive bladder cancer remains at best 50% despite radical therapies such as cystectomy and radiotherapy

Diagnosis

The diagnosis of bladder cancer is ultimately made at cystoscopy, after histopathological examination of resected specimens. Cystoscopy can miss flat, high-grade lesions. One study of 1012 endoscopies [2] reported that 34% of all tumours were detected only by induced fluorescence with 5-aminolevulinic acid. All methods for staging bladder cancer have limitations. Ultrasound is unreliable for assessing deeply invasive lesions and nodal disease. X-Ray Computerised Tomography (CT) cannot estimate accurately the depth of tumour penetration, and can overestimate the stage in the presence of oedema and scarring from previous resection. Magnetic Resonance Imaging (MRI) can also overstage. Immunoscintigraphy has a proven role in the management of some forms of cancer. An indium-labelled antibody conjugate, Capromab pendetide, has recently been shown to outperform standard radiological technique in the detection of pelvic nodal metastases from prostate cancer [3]. The Type 1 transmembrane protein MUC1 is uprated and abnormally glycosylated in transitional cell bladder cancer, and can be regarded as a tumour-associated antigen. Antibodies raised against the protein core of MUC1 represent one way forward in the development of tumour markers for the detection and staging of bladder cancer.

Therapy

Treatment of bladder cancer falls into three main categories: intravesical chemotherapy and immunotherapy, surgery and radiotherapy. The main chemotherapeutic drugs in use are Thiotepa, Doxorubicin, Mitomycin, and Epirubicin. Use of these may prevent recurrence in the short term after transurethral resection but is less effective over longer periods [4]. Bacillus Calmette-Guerin has been widely used as an immunotherapeutic agent in the treatment of superficial bladder cancer and there is a view that its use prevents disease progression [5].

Surgical transurethral resection is the standard treatment for superficial tumour, although most tumours do recur. Radical cystectomy is the standard treatment for muscle invasive disease.

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1389-5575/04 $45.00+.00

Radiotherapeutic approaches have employed brachytherapy, in which implanted radium wires have been effective in the treatment of T1 tumours [6]. External beam therapy is a proven treatment for muscle-invasive disease, requiring a linear accelerator with energy of at least 4MeV. Unsealed source therapy has been applied by the use of intravesical yttrium-90 in gelatin [7]. A dose of 3000 cGy to the bladder has been effective in controlling superficial disease in the short term.

Therapeutic approaches using Re-188 and Cu-67 are being explored and some of the properties and uses of these two radionuclides will now be reviewed.

RHENIUM-188

Rhenium-188 (Re-188) is an important therapeutic radionuclide, which is obtained on demand as carrier-free sodium perrhenate by saline elution of the tungsten-188/rhenium-188 generator system. With a half-life of 16.9 hours and emission of a high-energy beta particle (maximal energy of 2.12 MeV) and a gamma photon (155 keV, 15%) for imaging, Re-188 offers the prospect of cost-effective preparation of radiopharmaceuticals for cancer treatment [8].

Radiochemistry

The production of stable complexes having predefined radiochemical properties and *in vitro* and *in vivo* stability is the main objective of numerous investigations. Direct radiolabelling of antibodies by reductive attachment of Re-188 in which free sulphydryl groups have been generated by reduction of the intramolecular S-S disulfide bonds is described by Iznaga-Escobar [9] as a promising approach. Preparation of the labelled monoclonal antibody Re-188-MAb-B43.13 by the addition of generator-produced perrhenate to a preformulated antibody in the presence of an optimal amount of stannous tartrate has been described [10]. The final radiolabelled product retained its biochemical purity (as determined by size-exclusion HPLC and R/NR-SDS-PAGE), and its immunoreactivity (as determined by immunoassay). Stability in the presence of serum and cysteine, and biodistribution in xenografted mice were as anticipated.

Dadachova [11] has studied the influence of Sn(II) during direct labelling of proteins with Re-188 by the use of Sn-117m in the presence of two transchelation buffers-sodium gluconate and sodium citrate. Sn(II) readily binds to the thiol groups on the protein, and the fraction of Sn bound to the protein was 5 to 10 times higher in citrate than in gluconate buffer for all Sn(II) concentrations studied. The amount of Sn(II) in reaction mixture must exceed a certain level in order to achieve high labelling yields, and this level of Sn(II) was found to be different for citrate and gluconate buffers.

Rhenium compounds have lower redox potentials than their technetium-99m analogues and therefore a greater tendency to reoxidize. Conditions for directly labelling B72.3 IgG with Re-188 via both mercaptoethanol and stannous ion antibody reduction have been investigated [12]. Reduced Re-188 was stabilized as the glucoheptonate complex and transchelated in the presence of excess stannous ion. Labelling efficiencies after about 15 minutes averaged 58.77% with as little as 4% non-specific binding. Specific activities of 15 μCi/microgram were achieved after 1.5 hours. Losses of Re-188 due to oxidation (16%) and to cysteine (7%) after incubation for 24 hours at 37ºC in serum were identical for both methods. Using rhenium-188 spiked with cold rhenium, it has been determined that approximately one rhenium atom per molecule of antibody can be conjugated directly. It has been suggested that only radiolysis concerns will limit the amount of rhenium-188 capable of being conjugated to antibody [13].

Rhenium-188 hydroxyethylidene diphosphonate (HEDP), (Fig. (**1**)), has been used to palliate the pain resulting from bone metastases. Alendronate, a new bisphosphonate, has been proposed as a promising new radiopharmaceutical for bone metastases pain palliation. Preparation of this material using stannous fluoride as reducing agent, together with chemical, spectroscopic and microscopic characteristics, quality control, rat bone uptake have been described [14]. Rhenium-186 HEDP has been shown to localize in metastatic foci within bone in a manner similar to Tc-99m bone-seeking agents. HEDP can be labelled at high efficiency with rhenium-188 in the presence of carrier levels of rhenium ranging between 10^{-8} and 10^{-3} M. Addition of carrier does, however, have an effect on the observed biodistribution in rats [15].

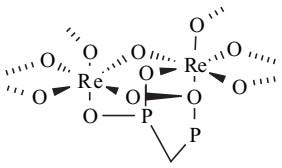

Fig. (1). Possible conformation of rhenium diphosphonate. Species formed is dependent on pH and concentration and is a mixture of oligomers, all of which have an affinity for bone.

Direct labelling via HEDP of a Re-188-somatostatin analogue peptide beta-(2-naphthyl)-D-Ala-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-amide complex has been developed [16]. The influence of reaction conditions such as pH, temperature, weak ligand concentration and stannous chloride concentration were investigated. A high radiochemical purity of 90% and a specific activity up to 1.8 GBq mg^{-1} without radiolytic degradation of the product were reported.

Direct labelling techniques are limited to compounds which themselves are ligands or which contain structures such as disulphide bonds which can be reduced to create labelling sites. Other compounds require conjugation with a suitable ligand. Studies by Prakash [17] have indicated that di and polydentate ligands such as ethylenediamine, or 1,4,8,11-tetraazacycloundecane offer an appropriate compromise between biological stability and ease of synthesis, and have potential as chelators for rhenium in radiopharmaceuticals. Rhenium-188 labelling of a 7-amino analogue of bombesin (Fig. (**2**)) has been achieved via a trisuccin conjugate [18]. The trisuccin was attached either directly or by connection through a 6-carbon linker (TrisC6BBN). Cell-binding assays performed with BNR-11 and PC-3 cell lines resulted in positive binding.

5-oxoPro-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-MetNH$_2$

Fig. (2). Amino acid sequence of Bombesin, a pharmacologically active tetradecapeptide.

Re-188 labelled dextran has been proposed as a template with therapeutic and diagnostic potential in nuclear oncology, either alone for local treatment or as a backbone in a tumour specific conjugate for systemic treatment. Dextran was oxidized with sodium periodate yielding reactive aldehyde groups and subsequently reacted with cysteine. The linkage was stabilized by reducing the Schiff bases with sodium cyanoborohydride, and the conjugate radiolabelled with Re-188 by using Re-188-gluconate as the transchelator, labelling the free thiols. Synthesis and radiolabelling were done in the absence of oxygen. The labelling efficiency was 60-70% and the radiochemical purity > 95%. [19].

A technetium labelling method, employing non-reducing conditions, has been described for the preparation of a histidine-tagged somatostatin-dextran conjugate using a Tc-99m(I)-carbonyl compound $[Tc\text{-}99m(OH_2)_3(CO)_3]^+$ [20]. It should be possible to use this method for labelling with rhenium-188 for therapeutic applications, starting from the analogous compound $[Re\text{-}188(OH_2)_3(CO)_3]^+$, shown in Fig. (**3**).

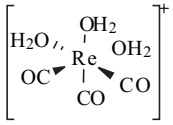

Fig. (3). Structure of the Rhenium carbonyl compound $[Re^{I}(OH_2)_3(CO)_3]^+$. This compound has been proposed as a precursor for labelling of biomolecules at high specific activity.

Preparation of lipophilic rhenium compounds with affinities for blood cells have been described. A dithiocarbamate nitride technetium compound $Tc\text{-}99mN(DTCX)_2$, where DTCX = $CH_3(CH_2)_8CS_2$, has high lymphocyte selectivity (69%). The synthesis of the analogous rhenium compound is described [21]. The same authors also describe the preparation of the nitride Re-188 $N(NOET)_2$, where NOET = $Et(EtO)NCS_2$, analogous to the known technetium-99m radiopharmaceutical [22] (see Fig. (**4**)).

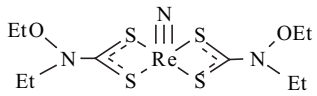

Fig. (4). Structure of Rhenium nitride $ReN(NOEt)_2$.

Biodistribution

Re-188-labeled monoclonal antibodies (Mabs) modified with 2-iminothiolane have been investigated for targeting small-cell lung cancer [23]. Radiolabelled NK1NBL1 and C218 recognizing neural cell adhesion molecules were injected i.v. into athymic mice inoculated with human tumours. NK1NBL1 localized in the tumours better than C218. Re-188-labeled MAbs cleared from the blood faster than I-125 labelled counterparts, resulting in higher tumour-to-blood ratios.

The potential of Re-188 anti-NCA antigen antibody BW 250/183 for adjuvant conditioning radioimmunotherapy of bone marrow before bone marrow transplantation has been assessed in 12 patients with advanced leukaemia [24]. Doses of between 6.5 and 12.4 GBq were administered.

Biodistribution of a Re-188 antibody has been determined in nude mice inoculated with human colorectal carcinoma LoVo [25]. B72.3, which recognises tumour antigen TAG-72, expressed on the surface membranes of colorectal cancer cells, was directly labelled using stannous tartrate and compared with I-125 labelled B72.3. Although the absolute tumour accumulation level of Re-188-B72.3 was lower than I-125-B72.3, the rhenium antibody demonstrated higher tumour-to-blood contrast because of fast clearance from the blood.

Pentavalent rhenium-188 dimercaptosuccinic acid (DMSA) is a beta-emitting analogue of Tc-99m(V)DMSA, a tracer that is taken up in a variety of tumours and bone metastases (Fig.(**5**)). The organ distribution of Re-188 in mice and in three patients with cancer of the prostate and three with cancer of the bronchus, all with bone metastases suggest that further clinical assessment for the treatment of painful bone metastases is warranted [26]. The agent should also be assessed in medullary thyroid carcinoma and other soft tissue tumours which have been shown to accumulate Tc-99m (V)DMSA.

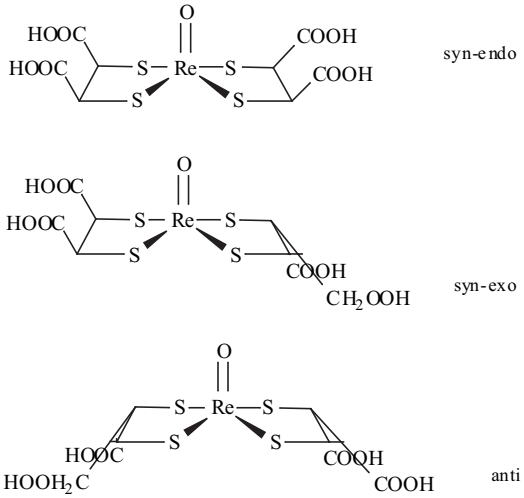

Fig. (5). Structure of isomers of Rhenium (V) DMSA.

The biodistribution and kinetics of biotinylated Re-188-chimeric BR96 have been investigated in colon carcinoma-isografted Brown Norwegian rats with the intention of comparing the enhancement of the tumor-to-normal tissue radioactivity ratio using extracorporeal immunoadsorption and an avidin "chase" [27]. Extracorporeal immunoadsorption, with direct removal of unbound circulating biotinylated Re-188-chiBR96, was shown to improve and maintain tumour/normal ratios without overloading the liver with radioactivity, in contrast to avidin chase. The potential of Re-188-Lipiodol as a radiopharmaceutical for the treatment of hepatic tumours in humans has been investigated [28]. Radioactivity accumulating in hepatic tumours in rats following intraarterial injection was very high throughout the study, with a biological half-life of 122.9h. Radioactivity in the normal liver tissue was also high, but was significantly lower in the tumour. The biological half-life in the normal liver tissue was 31.7h. The ratio of tumour concentration to the normal liver tissue concentration was 5.15 at 1h rising to 7.7 at 24h and 10.84 at 48h.

Applications/Biological Effectiveness

There are numerous reports about the use of Re-188 hydroxyethylidine diphosphonate (Re-188 HEDP) in the palliation of bone pain from skeletal metastases. Prompt and significant relief of bone pain has been reported in 80% of patients overall in a trial involving the use of Re-188 HEDP in 61 patients [29]. Of specific tumour types, pain relief was achieved in 77% of patients with lung cancer, in 80% with prostate cancer, in 83% with breast cancer, in 100% with bladder cancer, in 50% with renal cancer, in 50% with rhinopharyngeal cancer, and in 87% of patients with other tumour types, with no severe side effects or haematopoietic toxicity. The effects of dose escalation have been assessed in a study involving 22 prostate cancer patients with osseous metastases suffering from bone pain [30]. It was concluded that in this patient group, the maximum tolerated dose of Re-188-HEDP was 3.3 GBq if the baseline thrombocyte count was below $200x10^9$/l. In patients with thrombocyte counts significantly above $200x10^9$/l, a dose of 4.4 GBq might be tolerable. Thrombo- and leukopenia were the most important side effects observed. The biodistribution and radiation dosimetry characteristics of Re-188 HEDP appear similar to those of the Re-186 analogue and result in similar benefits and toxicities in patients with skeletal metastases [31]. Three other similar studies [32-34], which have included assessment of the Karnofsky performance index as an indicator of improvement, have concluded that the palliation of bone pain with Re-188 HEDP is effective and associated with minimal toxicity.

The therapeutic potential of rhenium-188 dimercaptosuccinic acid complex Re-188(V)DMSA in bone pain palliation has been explored by comparing tumour-to-normal tissue ratios and kidney-to-soft tissue ratios of the gamma emitter Tc-99m(V)DMSA and Re-188(V)DMSA. This would determine whether a scan with Tc-99m(V) DMSA could be used to identify patients for whom Re-188(V)DMSA treatment would be contra-indicated, and enable prediction of relative kidney and tumour radiation absorbed dose in Re-188(V)DMSA treatment. Only minor differences were seen between Tc-99m and Re-188 scans, and kidney-to-background ratios on Re-188 scans were not higher than on Tc-99m scans suggesting that the method could be used to estimate tumour and renal dosimetry and assess suitability of patients for Re-188(V)DMSA treatment.[35].

Rhenium-188 microspheres have been developed as a radiation synovectomy agent for the treatment of rheumatoid arthritis. It has been shown that the levels of unwanted extra-articular radiation are negligible with this agent. A histological study in rabbits has revealed transient inflammation of the synovium but no evidence of damage to articular cartilage [36]. In an experimental study in rabbit, the mean retention percentages of a Re-188 sulphur colloid in arthritic knees were 93.7% (+/- 1.4%), 90.8% (+/- 1.7%) and 87.2% (+/- 0.6%) at 1 h, 1 day and 2 days, respectively. A biodistribution study revealed that the highest activity outside the knees was in the liver and the kidneys [37].

Re-188 tin colloid [38] may have advantages over Re-188 sulphur colloid, due to higher labelling efficiency, control of the particle size, and lower residual activity in the injection syringes.

Other potential therapeutic applications have been explored. The conditioning regimen prior to stem cell transplantation in 36 patients with high-risk acute myeloid leukemia and myelodysplastic syndrome was intensified by treating patients with a rhenium 188-labelled anti-CD66 monoclonal antibody [39]. Radioimmunotherapy with the labelled antibody provided a mean of 15.3 Gy of additional radiation to the marrow; the kidney was the normal organ receiving the highest dose of supplemental radiation (mean 7.4 Gy). The sodium-iodide symporter (NIS), which transports iodine into the cell, is expressed in thyroid tissue and was recently found to be expressed in approximately 80% of human breast cancers but not in healthy breast tissue. The feasibility of using Re-188 in the form of perrhenate has been compared with I-131 iodide for the treatment of NIS-expressing mammary tumours [40], using a xenografted breast cancer model induced by the ErbB2 oncogene in nude mice. Dosimetry calculations in the tumour demonstrate that Re-188-perrhenate is able to deliver a dose 4.5 times higher than I-131 iodide. The clinical potential of radiolabelled peptides such as octreotide and vasoactive intestinal peptide (VIP) is already widely established for tumour localization. Rhenium-188 coupled to the analogue RC-160 has been used to establish the feasibility of treating tumours with radiolabelled peptides in three different experimental tumour models (human prostate, mammary gland, and small cell lung carcinomas) in nude mice. Treatment resulted in significant reduction or elimination of tumour burden [41].

COPPER-67

Copper-67 (Cu-67) has physical properties which are very well suited for use in radiotherapy. It is a beta-emitting radionuclide with a half-life of 61.9 hours and emission energies distributed between 577 and 395 keV. These emissions are very suitable for treatment of tumours up to 5 mm in diameter. In addition gamma radiation in the energy range 91 – 184 keV allows pretherapy imaging on a gamma camera [42]. The use of Cu-67-labelled antibodies for the treatment of cancer has been advanced to the clinical trial phase. Copper-67 can be prepared by bombarding zinc-67 with neutrons in a high flux reactor or by irradiating zinc with protons. Quantitation of Cu-67 radiopharmaceuticals is complicated by the presence of the radioimpurity of Cu-64 in Cu-67 supplies. A method of assay for Cu-67 and Cu-64 in a mixed sample using an ionization chamber dose calibrator has been reported [43]. The presence of copper-64 also affects the radiation dose to tissue. A study based on Medical Internal Radiation Dose (MIRDose) formalism [44] has shown the tumour radiation dose per unit of activity (cGy/GBq) from Cu-67 to be five times greater than that from Cu-64 while the marrow dose (CGy/GBq) from Cu-67 was only three times greater than that from Cu-64. Therefore, the therapeutic index was diminished by the presence of Cu-64. When Cu-64 radioimpurity was less than 25% of the total activity, there was less than 10% decrease in the therapeutic index.

Radiochemistry

Many methods are described for the incorporation of copper into immuno- or other conjugates for therapeutic use.

The synthetic porphyrins, *N*-benzyl-5,10,15,20-tetrakis (4-carboxyphenyl) porphine and *N*-4-nitrobenzyl-5-(4-carboxyphenyl)-10,15,20-tris(4-sulfophenyl) porphine (N-bzHCS3P), are excellent radiocopper chelating agents for potential use in antibody-mediated therapy. N-bzHCS3P has been conjugated to an anti-renal cell carcinoma antibody, A6H, and labelled with copper-67. Biodistribution studies in xenograft-bearing nude mice produced tumor/blood ratios greater than 16:1 after 45 h, although unwanted localization also occurred in the liver and spleen [45]. The bifunctional chelating agent, 6-[p-(bromoacetamido)benzyl]-1,4,8,11-tetraazacyclotetradecane- *N,N′,N″,N*‴-tetraacetic acid (BAT), Fig. (**6**), has been shown to be an effective reagent for linking copper to proteins. An improved synthesis has been reported by Moran [46]. Other studies designed to optimise preparation of immunoconjugates based on the BAT macrocycle have demonstrated higher kinetic stabilities *in vitro* in human serum for the 6-BAT isomer compared with the 2-BAT isomer [47].

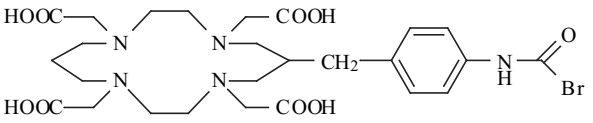

Fig. (6). Structure of 6-[p-(bromoacetamido)benzyl]-TETA (BAT).

The basis of many methods for the incorporation of copper is the 4N macrocycle 1,4,8,11- tetraazacyclodecane-*N,N′,N″,N*‴-tetraacetic acid (TETA) developed by the Meares group [48,49]. The DO3A derivative, in which a linkage group is attached to the acetate-chelating arm via a C-N bond, has been the most used. The nature of the peptide linker has a marked effect on stability as shown during a study of four tripeptide derivatives coupled to $F(ab')_2$ fragments of anti-colon carcinoma MAb35. *In vitro*, the Cu-67-labeled antibody fragments were fully immunoreactive and stable in human serum. *In vivo* in nude mice bearing human colon carcinoma xenografts the triglycyl and glycyl-prolyl-glycyl conjugates showed improved tumour uptake and lower levels of radioactivity in the liver compared with the other conjugates. Radioactivity was released more slowly from the triglycine linker [50].

Biological Fate

Copper-67 has ideal properties for radioimmunotherapy. The 62-hours half-life is similar to the residence time of antibodies in tumour, and the therapeutic beta emission of Cu-67 is comparable to that of I-131. In addition, Cu-67 has gamma emissions similar to technetium-99m that are favourable for imaging. The safety, efficacy, and practicality of Cu-67 attached via a BAT macrocycle to Lym-1, a mouse monoclonal antibody that preferentially targets malignant lymphocytes, has been assessed in a phase I/II clinical trial for patients with non-Hodgkin's lymphoma who had failed standard therapy [51]. Up to four doses of Cu-67-2IT-BAT-Lym-1, 0.93 or 1.85-2.22 GBq/m^2/dose, respectively, were administered; the lower dosage being used when disease was detected in the bone marrow. Imaging was possible, and response was observed in 7 of 12 patients (58%). Haematological toxicity was dose limiting, but no other significant toxicity was observed.

The same radiopharmaceutical has been administered to lymphoma patients [52]. It was observed that the beta phase of blood clearance, when corrected for Cu-67 decay, was positive or flat, a phenomenon not observed in similar patients treated with I-131 Lym-1. Results suggested recycling of Cu-67 to another plasma protein, identified by affinity-purified polyclonal antibodies as ceruloplasmin. Albumin levels correlated negatively with recycled copper ($r = -0.745$, $p < 0.05$). The data suggest that the liver metabolises Cu-67-2IT-BAT-Lym-1 and recycles a small fraction of the Cu-67, transferring it to ceruloplasmin.

ChCE7 is an internalising, neuroblastoma-specific monoclonal antibody. Biodistribution studies of copper-67 conjugates of this antibody and its $F(ab')_2$ have been performed in nude mice bearing neuroblastoma xenografts [53]. Fragments were derived with the bifunctional ligand 4-(1,4,8,11-tetraazacyclotetradec-1-yl)-methyl benzoic acid tetrahydrochloride (CPTA). The intact antibody showed high tumour uptake, stable over 4 days postinjection (33.7% +/- 2.8% ID/g), with tumor/blood ratios increasing from 4.4 on Day 1 to 23.0 on Day 7 postinjection and low levels of radioactivity in other tissues. Radioactivity in the blood was exclusively in the form of intact antibody, while radioactivity in the liver was found to consists of intact antibody, fragments and the lysine-CPTA metabolite. In the case of the $F(ab')_2$ fragments, high accumulation of radioactivity in the kidneys was observed due to rapid accumulation of the lysine-CPTA complex.

Patients with non-Hodgkin's lymphoma have been treated with the antilymphoma mouse monoclonal antibody Lym-1, labelled either with Cu-67 or I-131. Mean tumour radiation dose delivered by Cu-67-2IT-BAT-Lym-1 (2.8 Gy/GBq range 0.8-6.7) was twice that of I-131 Lym-1 (1.4 Gy/GBq range 0.4-35). A favourable therapeutic index and the better imaging characteristics suggest that Cu-67-2IT-BAT-Lym-1 may be superior to I-131 Lym-1 for radioimmunotherapy [54].

The biological fate of the monoclonal antibody chCE7 has been investigated in human neuroblastoma (SKN-AS) cells [55]. The antibody was derivatized with the macrocyclic amine ligand 4-[(1,4,8,11-tetraazacyclotetradec-1-yl)-methyl] benzoic acid tetrahydrochloride and labelled with Cu-67. A progressive increase in the accumulation of radioactivity was due to the intracellular accumulation of a degradation product consisting of the Cu-67-4[(1,4,8,11-tetraazacyclotetradec-1-yl)-methyl] benzoic acid complex, possibly with a short peptide attached to it.

The Cu-67-labeled Lym-1 antibody has been observed to remain in lymphomatous tissue longer than I-131-Lym-1 and, consequently, results in higher absorbed radiation doses to tumours. In a study in athymic mice implanted with Raji xenografts Cu-67-2IT-BAT-Lym-1 produced a therapeutic and frequently curative effect at tolerated doses. In addition it was demonstrated that the therapeutic effectiveness might have been enhanced by recombinant interleukin-2 [56].

Clinical Applications

A study designed to define the maximum tolerated dose of Cu-67-2IT-BAT-Lym-1 has been performed in patients with Ann Arbor stage IVB non-Hodgkin's lymphoma, resistant to standard therapy, including multiple

chemotherapy regimens. Each dose of Cu-67-2IT-BAT-Lym-1 was given after a preload of unmodified Lym-1. Dose-limiting toxicities were grade 3-4 thrombocytopenia and neutropenia. Favourable radiation dosimetry and a remarkably high therapeutic index were observed. The non-myeloablative maximum tolerated dose for each of 2 doses was 2.22 GBq per square meter of body surface area [57].

The nadir and duration of thrombocytopenia predicted by a model were similar to those observed in experimental mice following administration of Cu-67-2IT-BAT-Lym-1. Predicted information could be useful for planning the dose and timing of fractionated radionuclide therapy [58]. Further endorsement of the therapeutic potential of Cu-67 antibodies is reported in a study using Cu-67-2IT-BAT-Lym-1 in nude mice bearing human Burkitt's lymphoma tumours [59]. The authors concluded that the conjugate provided a therapeutic and frequently curative dose of radiation with modest toxicity.

Radioimmunotherapy of colorectal carcinoma using a Cu-67-labeled anti-CEA monoclonal antibody has been studied in six patients scheduled for the surgery of primary colorectal cancer [60]. Results indicated that the copper-67-labeled antibody is more favourable than its iodine-131 counterpart due to higher tumour-to-blood ratios, but the problem of non-specific liver and bowel uptake must first be overcome. The absolute accumulation of activity in tumour was low, however. The authors suggest that the probability of cure with this compound alone is questionable and that the use of Cu-67 as one component of a multimodality adjuvant treatment seems to remain the most appropriate application for radioimmunotherapy.

A number of studies have progressed to the stage of clinical trials, particularly in the treatment of patients with B-cell non-Hodgkin's lymphoma. In one such study [61] Cu-67-2IT-BAT-Lym-1 produced a response rate of 58% (7/12) with no significant non-haematologic toxicity. Thrombocytopenia was dose limiting. A further 70 patients with B-lymphocytic non-Hodgkin's lymphoma have been studied using Cu-67-2IT-BAT-Lym-1, I-131-Lym-1, or In-111-2IT-BAD-Lym-1; indium being used as a surrogate for yttrium-90 to estimate pharmacokinetics and radiation dosimetry [62]. The therapeutic indices (ratios of radiation doses to tumour and normal tissues) for Cu-67-2IT-BAT-Lym-1, and less generally for Y-90-2IT-BAD-Lym-1, were more favourable when compared to those for I-131-Lym-1.

In preclinical and clinical trials, Cu-67-2IT-BAT-Lym-1 has shown an exceptionally long tumour residence time associated with substantial cumulated radiation doses. In a study of mice bearing Raji lymphoma xenografts Cu-67-2IT-BAT-Lym-1 RIT induced an overall response rate of 50% with tolerable toxicity, and 29% of the tumours were cured at cumulated tumour radiation doses of about 1800 cGy. Apoptosis was convincingly demonstrated to be a major mechanism for the effectiveness of radioimmunotherapy and occurred by p53-independent mechanisms [63].

RE-188 AND CU-67 IN THE TREATMENT OF BLADDER CANCER

The high-molecular-weight glycoprotein MUC1 mucin is overexpressed on bladder tumours and represents a useful target for radioimmunoscintigraphy and radioimmunotherapy. Serum MUC1 is not as useful as a tumour marker for screening, as it has a low sensitivity although MUC1 levels are high in advanced disease and serum MUC1 levels may be useful for disease monitoring [64]. In a study of 87 patients with transitional cell carcinoma of the bladder and in 31 controls undergoing cystoscopy for benign conditions, 47% of patients with T4 tumours had MUC1 levels above the normal range ($P<0.001$). Patients with T3 tumours also had significantly higher MUC1 levels than controls, but the overall sensitivity was only 24% for all tumours when the upper limit of normal was defined as 4.8 U/mL. The anti-MUC1 monoclonal antibody C595 has been radiolabelled with technetium-99m, using a direct, reduction-mediated technique [65]. The resultant conjugate was shown to be highly immunoreactive, stable and to bind specifically to MUC1. The ability of the conjugate to localise MUC1-expressing tumours was demonstrated in a nude mouse xenograft model. The ability of the conjugate to bind to bladder tumours has been demonstrated in an *ex vivo* model where the mean tumour:normal urothelial uptake was 5.7:1. *In vivo*, following intravesical administration to patients with bladder cancer, the mean tumour:normal urothelial uptake was 20.4:1. The same antibody conjugate has been used in an imaging study in twenty-one patients with invasive or metastatic transitional cell carcinoma [66]. 14 patients subsequently underwent cystectomy, four underwent radiotherapy and the remaining three had histologically confirmed metastatic disease. The results of immunoscintigraphy were compared with surgical findings and conventional radiology. Of the 20 patients who were found to have tumour at the time of the study, positive localization of antibody in tumour was apparent in 16. Of the remaining four patients, false-positive localization of antibody in presumed nodal tissue was detected in two. The remaining scan results were equivocal. In three patients, histologically confirmed pelvic nodal metastases that had not been detected on preoperative computed tomography were identified. To develop the therapeutic potential of this antibody a rhenium-188 complex has been developed [67]. C595 antibody was reduced with 2-mercaptoethanol and was labelled with Re-188 perrhenate in the presence of stannous tartrate. Tumour localization was investigated using an *ex vivo* model in human cystectomy specimens. Tracer amounts of the complex were also administered intravesically to three patients with bladder cancer, who were then imaged by gamma scintigraphy. The complex was immunoreactive (70% +/- 17%) and specific for MUC1 antigens. Binding to bladder tumours was observed in an *ex vivo* model in which tumours were successfully imaged in four specimens. The mean tumour-to-normal tissue ratio in *ex vivo* bladders was 7:1. Tumour uptake after intravesical administration was confirmed in three patients with bladder cancer (mean tumour-to-normal tissue ratio, 4:1).

C-595 can also be successfully labelled with copper-67 using the neutral tetra-aza macrocycle CTPA [68]. The radioimmunoconjugate has been shown to be stable and to maintain high immunoreactivity. Pilot studies on cystectomy specimens in an *ex vivo* system and in one patient confirmed the ability of this conjugate to localise to tumour after intravesical administration. In a pilot therapy study, approximately 20 MBq of Cu-67-C595 monoclonal

antibody has been administered intravesically to 16 patients with a clinical indication of superficial bladder cancer [69]. After 1 hour, the bladder was drained and irrigated. Tissue uptake was assessed by imaging and by the assay of tumour and normal tissues obtained by endoscopic resection. Tumour was correctly identified in the images of 12 of 15 patients. Assay of biopsy samples at 2 hours showed a mean tumour-to-normal tissue ratio of 14.6:1 (SD = 20). After 24 hours (n = 5), this decreased to1.8:1 (SD = 0.8). It was concluded that the approach had promise for the treatment of superficial bladder cancer although greater retention of the cytotoxic radionuclide in tumour tissue would be required.

REFERENCES

[1] Rehn, L. *Archiv Klin Chir.,* **1895**, *50*, 588.

[2] Zaak, D.;. Kriegmair, M.; Stepp, H.; Baumgartner, R.; Oberneder, R.; Schneede, P.; Corvin, S.; Frimberger, D.; Knuchel, R.; Hofstetter, A. *Urology,* **2001**, *57*(4), 690.

[3] Manyak, M.J.; Hinkle, G.H.; Olsen, J.O.; Chiaccherini, R.P.; Partin, A.W.; Piantadosi, S.; Burgers, J.K.; Texter, J.H.; Neal, C.E.; Libertino, J.A.; Wright, G.L. Jr.; Maguire, R.T. *Urology,* **1999**, *54*(6),1058.

[4] Lamm, D.L.; Riggs, D.R.; Traynelis, C.L. *J. Urol.,* **1995**, *153*, 1444.

[5] Lamm, D.L.; Blumenstein, B.A.; Crissman, J.D.; Montie, J.E.; Gottesman, J.E.; Lowe, B.A.; Sarosdy, M.F.; Bohl, R.D.; Grossman, H.B.; Beck, T.M.; Leimert, J.T.; Crawford, E.D. *J. Urol.,* **2000**,*163*, 1124.

[6] Van der Werf-Messing, BHP. *Urol. Clin. North Am.,* **1984**, *11*, 659.

[7] Alcock, C.J.; Durrant, K.R.; Smith, J.C.; Fellows, G.J. *Br. J. Urol.,* **1986**, *58*, 287.

[8] Knapp, F.F. Jr. *Cancer Biotherap. Radiopharm.* **1998**, *13*(5), 337.

[9] Iznaga-Escobar, N. *Appl. Radiat. Isotopes,* **2001**, *54*(3), 399.

[10] Sykes, T.R.; Somayaji, V.V.; Bier, S.; Woo, T.K.; Kwok, C.S.; Snieckus, V.; Noujaim, A.A. *Appl. Radiat. Isotopes,* **1997**, *48*(7), 899.

[11] Dadachova, E.; Mirzadeh, S. *Nucl. Med. Biol.,* **1997**, *24*(6), 605.

[12] Winnard, P. Jr.; Virzi, E.; Fogarasi, M.; Rusckowski, M.; Hnatowich, D.J. *Q. J. Nucl. Med.,* **1996**, *40*(2),151.

[13] Griffiths, G.L.; Goldenberg, D.M.; Diril, H.; Hansen, H.J. *Cancer,* **1994**, *73*(3 Suppl.), 761.

[14] Arteaga de Murphy, C.; Ferro-Flores, G.; Pedraza-Lopez, M.; Melendez-Alafort, L.; Croft, B.Y.; Ramirez, F.M.; Padilla, J. *Appl. Radiat. Isotopes,* **2001**, *54*(3), 435.

[15] Lin, W.Y.; Hsieh, J.F.; Lin, C.P.; Hsieh, B.T.; Ting, G.; Wang, S.J.; Knapp, F.F. Jr. *Nucl. Med. Biol.,* **1999**, *26*(4), 455.

[16] Melendez-Alafort, L.; Ferro-Flores, G.; Arteaga-Murphy C.; Pedraza-Lopez, M.; Gonzalez-Zavala, M.A.; Tendilla, J.I.; Garcia-Salinas, L. *Int. J. Pharm.,* **1999**, *182*(2),165.

[17] Prakash, S.; Went, M.J.; Blower, P.J. *Nucl. Med. Biol.,* **1996**, *23*(4), 543.

[18] Safavy, A.; Khazaeli, M.B.; Qin, H.; Buchsbaum, D.J. *Cancer,* **1997**, *80*(12 Suppl.), 2354.

[19] Du, J.; Marquez, M.; Hiltunen, J.; Nilsson, S. *Appl. Radiat. Isotopes,* **2000**, *53*(3), 443.

[20] Du, J.; Marquez, M.; Hiltunen, J.; Nilsson, S.; Holmberg, A.R. *Appl. Radiat. Isotopes,* **2001**, *55*(2), 181.

[21] Demaimay, F.; Roucoux, A.; Dazord, L.; Noiret, N.; Moisan, A.; Patin, H. *Nucl. Med. Biol.,* **1999**, *26*(2), 225.

[22] Demaimay, F.; Dazord, L.; Roucoux, A.; Noiret, N.; Patin, H.; Moisan, A. *Nucl. Med. Biol.,* **1997**, *24*(8),701.

[23] Hosono, M.N.; Hosono, M.; Mishra, A.K.; Faivre-Chauvet, A.; Gautherot, E.; Barbet, J.; Knapp, F.F. Jr.; Chatal, J.F. *Ann. Nucl. Med*., **2000**, *14*(3), 173.

[24] Seitz, U.; Neumaier, B.; Glatting, G.; Kotzerke, J.; Bunjes, D.; Reske, S.N. *Eur. J. Nucl. Med.,* **1999**, *26*(10), 1265.

[25] Hosono, M.N.; Hosono, M.; Zamora, P.O.; Guhlke, S.; Haberberger, T.; Bender, H.; Knapp, F.F.; Biersack, H.J. *Ann. Nucl. Med*., **1998**, *12*(2), 83.

[26] Blower, P.J.; Lam, A.S.; O'Doherty, M.J.; Kettle, A.G.; Coakley, A.J.; Knapp, F.F. Jr. *Eur. J. Nucl. Med.,* **1998**, *25*(6), 613.

[27] Chen, J.Q.; Strand, S.E.; Tennvall, J.; Lindgren, L.; Hindorf, C. *J. Nucl. Med.,* **1997**, *38*(12), 1934.

[28] Wang, S.J.; Lin, W.Y.; Chen, M.N.; Hsieh, B.T.; Shen, L.H.; Tsai, Z.T.; Ting, G.; Knapp, F.F. Jr. *Eur. J. Nucl. Med.,* **1996**, *23*(1), 13.

[29] Li, S.; Liu, J.; Zhang, H.; Tian, M.; Wang, J.; Zheng, X. *Clin. Nucl. Med.,* **2001**, *26*(11), 919.

[30] Palmedo, H.; Guhlke, S.; Bender, H.; Sartor, J.; Schoeneich, G.; Risse, J.; Grunwald, F.; Knapp, F.F. Jr.; Biersack, H.J. *Eur. J. Nucl. Med.,* **2000**, *27*(2), 123.

[31] Maxon, H.R. 3rd; Schroder, L.E.; Washburn, L.C.; Thomas, S.R.; Samaratunga, R.C.; Biniakiewicz, D.; Moulton, J.S.; Cummings, D.; Ehrhardt, G.J.; Morris, V. *J. Nucl. Med.,* **1998**, *39*(4), 659.

[32] Roka, R.; Sera, T.; Pajor, L.; Thurzo, L.; Lang, J.; Csernay, L.; Pavics, L. *Orvosi Hetilap.,* **2000**, *141*(19), 1019.

[33] Liepe, K.; Hliscs, R.; Kropp, J.; Gruning, T.; Runge, R.; Koch, R.; Knapp, F.F. Jr.; Franke, W.G. *Cancer Biotherap. Radiopharm.,* **2000**, *15*(3), 261.

[34] Liepe, K.; Franke, W.G.; Kropp, J.; Koch, R.; Runge, R.; Hliscs, R. *Nuclear-Medizin,* **2000**, *39*(6), 146.

[35] Blower, P.J.; Kettle, A.G.; O'Doherty, M.J.; Coakley, A.J.; Knapp, F.F. Jr. *Eur. J. Nucl. Med.*, **2000**, *27*(9), 1405.

[36] Wang, S.J.; Lin, W.Y.; Chen, M.N.; Chen, J.T.; Ho, W.L.; Hsieh, B.T.; Huang, H.; Shen, L.H.; Ting, G.; Knapp, F.F. Jr. *Nucl. Med. Biol.,* **2001**, *28*(6), 727.

[37] Wang, S.J.; Lin, W.Y.; Hsieh, B.T.; Shen, L.H.; Tsai, Z.T.; Ting, G.; Knapp, F.F. Jr. *Eur. J. Nucl. Med.,* **1995**, *22*(6), 505.

[38] Jeong, J.M.; Lee, Y.J.; Kim, Y.J.; Chang, Y.S.; Lee, D.S.; Chung, J.K.; Song, Y.W.; Lee, M.C. *Appl. Radiat. Isotopes,* **2000**, *52*(4), 851.

[39] Bunjes, D.; Buchmann, I.; Duncker, C.; Seitz, U.; Kotzerke, J.; Wiesneth, M.; Dohr, D.; Stefanic, M.; Buck, A.; Harsdorf, S.V.; Glatting, G.; Grimminger, W.; Karakas, T.; Munzert, G.; Dohner, H.; Bergmann, L.; Reske, S.N. *Blood,* **2001**, *98*(3), 565.

[40] Dadachova, E.; Bouzahzah, B.; Zuckier, L.S.; Pestell, R.G. *Nucl. Med. Biol.,* **2002**, *29*(1), 13.

[41] Zamora, P.O.; Bender, H.; Gulhke, S.; Marek, M.J.; Knapp, F.F. Jr. Rhodes, B.A.; Biersack, H.J. *Anticancer Research,* **1997**, *17*(3B), 1803.

[42] Novak-Hofer, I.; Schubiger, P.A. *Eur. J. Nucl. Med.,* **2002**, *29*(6), 821.

[43] DeNardo, G.L.; Kukis, D.L.; Shen, S.; DeNardo, S.J. *J. Nucl. Med.,* **1996**, *37*(2), 302.

[44] Shen, S.; DeNardo, G.L.; DeNardo, S.J.; Salako, Q.; Morris, G.; Banks, D.; Yuan, A.; DeNardo, D.A. *J. Nucl. Med.*, **1996**, *37*(1), 146.

[45] Bhalgat, M.K.; Roberts, J.C.; Mercer-Smith, J.A.; Knotts, B.D.; Vessella, R.L.; Lavallee, D.K. *Nucl. Med. Biol.,* **1997**, *24*(2), 179.

[46] Moran, J.K.; Greiner, D.P.; Meares, C.F. *Bioconj. Chem.,* **1995**, *6*(3), 296.

[47] Kukis, D.L.; Diril, H.; Greiner, D.P.; DeNardo, S.J.; DeNardo, G.L.; Salako, Q.A.; Meares, C.F. *Cancer,* **1994**, *73*(3 Suppl.), 779.

[48] Meares, C.F.; McCall, M.J.; Reardan, D.T.; Goodwin, D.A.; Diamanti, C.I.; McTigue, M. *Anal. Biochem.,* **1984**, *142*, 68.

[49] Moi, M.K.; Meares, C.F.; McCall, M.J.; Cole, W.C.; DeNardo, S.J. *Anal. Biochem.,* **1985**, *148*, 249.

[50] Novak-Hofer, I.; Zimmermann, K.; Schubiger, P.A. *Cancer Biotherap. Radiopharm.,* **2001**, *16*(6).

[51] O'Donnell, R.T.; DeNardo, G.L.; Kukis, D.L.; Lamborn, K.R.; Shen, S.; Yuan, A.; Goldstein, D.S.; Mirick, G.R.; DeNardo, S.J. *Clin. Cancer Res.,* **1999**, *5*(10 Suppl.), 3330s.

[52] Mirick, G.R.; O'Donnell, R.T.; DeNardo, S.J.; Shen, S.; Meares, C.F.; DeNardo, G.L. *Nucl. Med. Biol.,* **1999**, *26*(7), 841.

[53] Novak-Hofer, I.; Zimmermann, K.; Maecke, H.R.; Amstutz, H.P.; Carrel, F.; Schubiger, P.A. *J. Nucl. Med.,* **1997**, *38*(4), 536.

[54] DeNardo, G.L.; Kukis, D.L.; Shen, S.; DeNardo, D.A.; Meares, C.F.; DeNardo, S.J. *Clin. Cancer Res.,* **1999**, *5*(3), 533.

[55] Novak-Hofer, I.; Amstutz, H.P.; Macke, H.R.; Schwarzbach, R.; Zimmermann, K.; Morgenthaler, J.J.; Schubiger, P.A. *Cancer Research,* **1995**, *55*(1), 46.

[56] DeNardo, G.L.; Kukis, D.L.; DeNardo, S.J.; Shen, S.; Mausner, L.F.; O'Donnell, R.T.; Lamborn, K.R.; Meyers, F.J.; Srivastava, S.C.; Miers, L.A. *Cancer,* **1997**, *80*(12 Suppl.), 2576.

[57] DeNardo, G.L.; DeNardo, S.J.; Kukis, D.L.; O'Donnell, R.T.; Shen, S.; Goldstein, D.S.; Kroger, L.A.; Salako, Q.; DeNardo, D.A.; Mirick, G.R.; Mausner, L.F.; Srivastava, S.C.; Meares, C.F. *Anticancer Research,* **1998**, *18*(4B), 2779.

[58] Shen, S.; DeNardo, G.L.; Jones, T.D.; Wilder, R.B.; O'Donnell, R.T.; DeNardo, S.J. *J. Nucl. Med.,* **1998**, *39*(7), 1223.
[59] DeNardo, G.L.; Kukis, D.L.; Shen, S.; Mausner, L.F.; Meares, C.F.; Srivastava, S.C.; Miers, L.A.; DeNardo, S.J. *Clin. Cancer Res.,* **1997**, *3*(1), 71.
[60] Delaloye, A.B.; Delaloye, B.; Buchegger, F.; Vogel, C.A.; Gillet, M.; Mach, J.P.; Smith, A.; Schubiger, P.A. *J. Nucl. Med.,* **1997**, *38*(6), 847.
[61] O'Donnell, R.T.; DeNardo, G.L.; Kukis, D.L.; Lamborn, K.R.; Shen, S.; Yuan, A.; Goldstein, D.S.; Carr, C.E.; Mirick, G.R.; DeNardo, S.J. *J. Nucl. Med.,* **1999**, *40*(12), 2014.
[62] DeNardo, G.L.; DeNardo, .S.J.; O'Donnell, R.T.; Kroger, L.A.; Kukis, D.L.; Meares, C.F.; Goldstein, D.S.; Shen S. *Clinical Lymphoma,* **2000**, *1*(2), 118.
[63] Kroger, L.A.; DeNardo, G.L.; Gumerlock, P.H.; Xiong, C.Y.; Winthrop, M.D.; Shi, X.B.; Mack, P.C.; Leshchinsky, T.; DeNardo, S.J. *Cancer Biotherap. Radiopharm.,* **2001**, *16*(3), 213.
[64] Simms, M.S.; Hughes, O.D.; Limb, M.; Price, M.R.; Bishop, M.C. *BJU International,* **1999**, *84*(3), 350.
[65] Simms, M.S.; Murray, A.; Denton, G.; Scholfield, D.P.; Price, M.R.; Perkins, A.C.; Bishop, M.C. *Urol. Res.,* **2001**, *29*(1), 13.
[66] Simms, M.S.; Perkins, A.C.; Price, M.R.; Scholfield, D.P.; Bishop, M.C. *BJU International,* **2001**, *88*(7), 686.
[67] Murray, A.; Simms, M.S.; Scholfield, D,P.; Vincent, R.M.; Denton, G.; Bishop, M.C.; Price, M.R.; Perkins, A.C. *J. Nucl. Med.,* **2001**, *42*(5), 726.
[68] Hughes, O.D.; Bishop, M.C.; Perkins, A.C.; Wastie, M.L.; Denton, G.; Price, M.R.; Frier, M.; Denley, H.; Rutherford, R.; Schubiger, P.A. *J. Clin. Oncology,* **2000**, *18*(2), 363.
[69] Hughes, O.D.; Bishop, M.C.; Perkins, A.C.; Frier, M.; Price, M.R.; Denton, G.; Smith, A.; Rutherford, R.; Schubiger, P.A. *Eur. J. Nucl. Med.,* **1997**, *24*(4), 439.