Published in final edited form as:

PET Clin. 2009 January ; 4(1): 49–67. doi:10.1016/j.cpet.2009.04.013.

Copper-64 Radiopharmaceuticals for Oncologic Imaging

Jason P. Holland, DPhil^a, Riccardo Ferdani, PhD^b, Carolyn J. Anderson, PhD^b, and Jason S. Lewis, PhD^{a,*}

^aDepartment of Radiology, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10065, USA

^bMallinckrodt Institute of Radiology, Washington University School of Medicine, 510 South Kingshighway, Boulevard, Campus Box 8225, St Louis, MO 63110, USA

Keywords

Copper 64; Radiopharmaceuticals; Peptides; Antibodies; PET

Over the last century, advances in imaging technology have revolutionized clinical practice and the importance of medical imaging is apparent. More recently, the use of nuclear medicine, which includes aspects of diagnostic imaging and radiotherapy, has become one of the primary tools available to clinicians and research scientists in the fight against cancer.¹ In combination with the use of radiolabeled pharmaceuticals, single photon computerized tomography (SPECT), and positron emission tomography (PET) provide detailed information about the physiology of, for example, a solid tumor. This information is complementary with the more familiar anatomic imaging techniques, which include conventional radiography (X rays), CT scans, MR imaging, and ultrasound. The principal advantage of nuclear imaging lies in its ability to identify the location of cancerous or otherwise diseased tissue, and simultaneously allows conclusions to be made about its physiologic state. The complementarity between anatomic CT or MRI imaging and the functional detail available from SPECT and PET have driven new developments in hybrid imaging modalities. For example, simultaneous PET/CT images are routinely used in oncology. In an elegant study, Quon and colleagues² obtained three-dimensional images by using hybrid PET/CT in combination with the well-established metabolic marker, [¹⁸F]-2-fluoro-2-deoxyglucose, [¹⁸F]-FDG, as a presurgical tool to identify the location of cancer lesions in the lungs of a patient at John Hopkins University Hospital (Baltimore, USA). Although images recorded by using [¹⁸F]-FDG and other ¹⁸F radiolabeled compounds account for the majority of PET scans, there is currently significant interest in the development of new PET radiopharmaceuticals based on the coordination of metallic radionuclides including ⁶⁸Ga, ⁸⁹Zr, and ⁶⁴Cu.^{3–7}

Copper has a range of radionuclides with varying decay characteristics which offer the potential for use in imaging and radiotherapy.⁶ The production, decay, and relevant chelation chemistry of copper has been the subject of several excellent reviews.^{6,8–10} This review

*Corresponding author. lewisj2@mskcc.org (J.S. Lewis).

article will focus on the use of ^{64}Cu as a radionuclide with the potential to be used for PET imaging applications. Copper-64 has a radioactive half-life of 12.7 hours and decays to either ^{64}Ni by positron emission ($\beta^+ = 17.9\%$, $E_{\text{max}} = 660 \text{ keV}$, $E_{\text{average}} = 288 \text{ keV}$) or electron capture ($\text{EC} = 43.1\%$, $E = 1675 \text{ and } 1346 \text{ keV}$), or ^{64}Zn by β^- -decay ($\beta^- = 39.0\%$, $E = 190.2 \text{ keV}$) as shown in Fig. 1.¹⁰ These decay modes mean that ^{64}Cu has the potential to be used in diagnostic PET imaging and radiotherapeutic applications. Resolution measurements using the “Derenzo” phantom indicate that PET images acquired using ^{64}Cu are comparable in quality to those obtained using ^{18}F as the nuclide.¹¹ The longer half-life allows ^{64}Cu to be produced at regional or national cyclotron facilities and distributed to local nuclear medicine departments with minimal loss in activity. In addition, the longer half-life is compatible with the timescales required for optimal bio-distribution of monoclonal antibodies (mAbs) and oligopeptides for use in radioimmunotherapy (RIT). This article will describe the synthesis and evaluation of a variety of ^{64}Cu radiolabeled complexes, oligopeptides, and mAb conjugates which have been, or are close to being, translated to the clinic.

The most common production method for ^{64}Cu uses the $^{64}\text{Ni}(p,n)^{64}\text{Cu}$ reaction,^{12–16} which involves the irradiation of enriched ^{64}Ni which has been electroplated on a gold^{12–14,17,18} or rhodium platform.¹⁹ McCarthy and colleagues¹² have described the efficient production of high specific-activity ^{64}Cu by using a small biomedical cyclotron and a ^{64}Ni enriched ($>95\%$) target. The $^{64}\text{Ni}(p,n)^{64}\text{Cu}$ transmutation reaction is high yielding ($2.3\text{--}5.0 \text{ mCi h}^{-1}$) and after purification by using an ion exchange column, high specific activity samples of $[\text{Cu}^{64}]\text{-CuCl}_2(\text{aq})$ were obtained ($95\text{--}310 \text{ mCi } \mu\text{g}^{-1}$). Obata and colleagues,¹⁴ reported yields of 0.6 to greater than $3.0 \text{ mCi}/\mu\text{Ah}$, averaging $1.983 \text{ mCi}/\mu\text{Ah}$ with a radionuclidic purity of over 99% using a 12 MeV cyclotron. Using a tangential target on the National Institutes of Health CS-30 cyclotron, Szajek and colleagues¹⁸ reported yields of $10.5 \pm 3 \text{ mCi}/\mu\text{Ah}$, when bombarded with a 12.5 MeV proton beam, which was comparable to the theoretical yield, and in over 3 hours produced greater than 1 Ci of radioactivity. The use of ^{64}Cu has increased dramatically in the past decade²⁰ and its production has now been reported by academic sources in the United States,^{12,18} Europe,^{15,19} and Japan.¹⁴

TUMOR HYPOXIA AND $^{60/62/64}\text{Cu}$ -ATSM

It is well-established that hypoxia is an important determinant of the overall response of the tumor to conventional therapy. The presence of hypoxia can result in an increase in tumor aggressiveness, failure of local control, and activation of transcription factors that support cell survival and migration.^{21–27} The ability to locate and quantify the extent of hypoxia (Fig. 2) within solid tumors by using noninvasive nuclear imaging would facilitate early diagnosis and help clinicians select the most appropriate treatment for each individual patient.^{22,28}

In 1997, Fujibayashi and colleagues discovered that the neutral, lipophilic copper(II) complex of the N_2S_2 tetradentate ligand, diacetyl-2,3-*bis*(N^4 -methyl-3-thiosemicarbazone), commonly referred to as Cu-ATSM, showed hypoxia-selective uptake in ex vivo ischemic, perfused, isolated rat-heart models (Fig. 3).^{29,30} Copper-ATSM was later shown to be hypoxia-selective in vitro (Fig. 4) and for tumor hypoxia in vivo.^{29,31–46} Recent

experimental and computational work provided the first experimental evidence directly probing the reduction, reoxidation, and pH-mediated ligand dissociation reactions of Cu-ATSM and their relationship to hypoxia selectivity.⁴¹ Several questions remain, particularly pertaining to the processes involved in cellular uptake, but the current revised mechanism indicates that hypoxia selectivity of Cu-ATSM (Fig. 5) arises because of a delicate balance of equilibria in which the rates of reduction (most likely enzyme-mediated), reoxidation, and protonation are fast relative to the rate of pH-mediated ligand dissociation.

PATIENT STUDIES WITH ^{60/62/64}Cu-ATSM

Takahashi and colleagues⁴⁷ reported the first human studies of the uptake of Cu-ATSM in 10 subjects: four normal subjects and six with lung cancer. In this study the short-lived ⁶²Zn/⁶²Cu generator-produced radionuclide, ⁶²Cu ($t_{1/2}$ = 0.16 h, β^+ = 98%, EC = 2%), was used; however, the biodistribution will be unaffected by the change in radionuclide. Preliminary results suggested that ⁶²Cu-ATSM was cleared rapidly from the blood with only a small fraction being accumulated in normal lung tissue (0.43 ± 0.09 uptake ratio; divided by the arterial input function). In contrast, high tumor uptake was observed (3.00 ± 1.50 uptake ratio) in all subjects with lung cancer. Further human studies followed in which the efficacy of using ⁶²Cu-ATSM for identifying myocardial ischemia was measured in seven subjects with coronary artery disease.⁴⁸ [¹⁸F]-FDG uptake was also measured. Four subjects displayed clinically unstable angina and showed abnormal [¹⁸F]-FDG uptake. In these subjects, ⁶²Cu-ATSM uptake was higher, and the main conclusion was that Cu-ATSM showed promise as a PET tracer for imaging hypoxia in acute ischemia.

Dehdashti and colleagues reported the first correlative studies comparing the uptake of ⁶⁰Cu-ATSM ($t_{1/2}$ = 0.16 h, β^+ = 98%, EC = 2%)^{6,17} with response to conventional therapies in patients with non-small cell lung cancer (NSCLC),⁴⁹ and cervical cancer.⁵⁰ In the NSCLC study, response to therapy was evaluated using ⁶⁰Cu-ATSM tumor to muscle (T:M) uptake ratios. Imaging with [¹⁸F]-FDG was also conducted as part of routine clinical evaluation. Of the 14 subjects studied, eight responded to radiotherapy (five showed a complete response with three partial responders) and six showed no response. The mean ⁶⁰Cu-ATSM T:M ratio of nonresponders (3.4 ± 0.8) was found to be much larger than uptake observed in responders (1.5 ± 0.4) [$P = 0.002$]. However, no significant differences were observed in the standardized uptake values (SUV) between the tumors of responders (3.5 ± 1.0) and nonresponders (2.8 ± 1.1) [$P = 0.2$]. The threshold T:M value of 3.0 was identified as a reliable cutoff value for distinguishing responders from nonresponders. In contrast to the results with ⁶⁰Cu-ATSM, no significant differences were observed in either the mean T:M ratios or SUV for the uptake of [¹⁸F]-FDG in responders (12.7 ± 10.4) and nonresponders (10.9 ± 4.1) [$P = 0.7$]. In addition, no statistically significant correlation between ⁶⁰Cu-ATSM and [¹⁸F]-FDG uptake was observed.

Clinical studies have also assessed the ability of ⁶⁰Cu-ATSM to predict response to neoadjuvant chemoradiotherapy in patients with rectal carcinoma.⁵¹ A total of 19 subjects were enrolled in the study, and the data from 17 was analyzed. After chemoradiotherapy, which consisted of 45 Gy administered in 25 fractions to the pelvis and combined with continuous intravenous infusion of 5-fluorouracil (225 mg/m².day), 14 subjects had a

reduction in tumor size and 13 were down staged. As with the studies on patients with NSCLC, the median T:M ratio for uptake of Cu-ATSM of 2.6 was found to discriminate responders from nonresponders. T:M values greater than 2.6 indicate the presence of hypoxia in the tumors and correlate with poor prognosis for progression-free patient survival.

Since the first PET study demonstrating the ability of ^{60}Cu -ATSM to act as an indicator of response to radiotherapy in patients with cervical cancer,⁵⁰ several additional studies have been conducted.^{52–54} These reports include the first clinical comparison between the imaging characteristics of ^{60}Cu -ATSM and ^{64}Cu -ATSM (and [^{18}F]-FDG) in cancers of the uterine cervix conducted after Cu-ATSM was approved for study as an Investigational New Drug (IND 62,675) (Fig. 6).⁵⁴ The study concluded that tumor uptake of Cu-ATSM as measured in images recorded between 1 to 9 days was reproducible, irrespective of the radionuclide used. This important result showed that Cu-ATSM is a marker for chronic tumor hypoxia as opposed to acute hypoxia. Pre-therapy imaging has also confirmed previous results indicating that PET imaging of Cu-ATSM provides clinically relevant information about tumor oxygenation and is predictive of the likelihood of disease-free survival post-treatment in patients with cervical cancer.⁵³

Before radiolabeled Cu-ATSM could be used for routine clinical analysis, accurate dosimetry measurements were required. In 2005, Laforest and colleagues⁵⁵ used the Medical Internal Radionuclide Dose approach to provided estimates of human absorbed doses from $^{60/61/62/64}\text{Cu}$ -ATSM by extrapolating data acquired from biodistribution data in rat models. Calculated organ doses for ^{61}Cu , ^{62}Cu and ^{64}Cu were extrapolated from the results obtained for ^{60}Cu -ATSM dosimetry. For ^{64}Cu -ATSM, the liver was identified as the dose limiting organ with an average radiation dose of 1.443 rad/mCi. Whole body doses were predicted to be 0.096 rad/mCi and the effective dose was 0.133 rad/mCi. In the animal model, measurable activity was identified in the gastrointestinal tract, suggesting that this is the primary excretion pathway for Cu-ATSM. However, humans have slower metabolism than rodents, which may account for the fact that no activity was observed in the bladder or gastrointestinal tract of human patients. Therefore, gastrointestinal residence times for ^{64}Cu -ATSM are likely to be higher than predicted and the actual doses received by these organs will be slightly elevated. Human doses using ^{64}Cu -ATSM have also been estimated from biodistribution data in nontumor bearing hamsters.³³

^{64}Cu -ATSM AS A RADIOTHERAPEUTIC AGENT

Due to the electron capture and β -decay pathways, ^{64}Cu -ATSM has also been characterized as a potential radiotherapeutic agent.^{33,56} Hamsters bearing either 7-day or 15-day old human GW39 colon cancer tumors were treated with 10 mCi of ^{64}Cu -ATSM. After treatment, animals with 7-day old tumors showed a 6-fold increase in survival (135 days) in 50% of the population compared with controls (20 days, [$P = 0.002$]). In animals with 15-day old tumors, greater than 6 mCi caused a statistically significant increase in survival time compared with controls ($P = 0.001$). Administration of the proligand, H_2ATSM , or the nonradioactive Cu-ATSM showed no difference in survival compared with controls. Again, high liver uptake was observed with a calculated human dose of 0.693 ± 0.037 rad/mCi $\pm$

SD. In hamsters, higher uptake in both the upper and lower large intestine walls was observed with extrapolated human doses of 1.201 ± 0.383 and 1.430 ± 0.186 rad/mCi, respectively.

⁶⁴Cu-LABELED SOMATOSTATIN ANALOGS FOR TARGETING NEUROENDOCRINE TUMORS

Somatostatin is a 14-amino-acid peptide that is involved in the regulation of several hormones (growth factor, insulin, glucagon, prolactin, and so forth) in organs, such as the gastrointestinal tract, the exocrine and endocrine tissue of the pancreas, the hypothalamus, the pituitary gland, and the central nervous system. The inhibitory effect of somatostatin is mediated by somatostatin receptors (SSTr) which are surface proteins that belong to the G-protein coupled receptor family. There are at least five known SSTr subtypes (SSTr1-SSTr5),⁵⁷ and because they have been found to be abundant on the surface of several human tumors,⁵⁷⁻⁶¹ (SSTr2 in particular)^{62,63} the use of somatostatin analogs to target these biomarkers for diagnostic and therapeutic purposes has been investigated for many years.⁶⁴⁻⁶⁹ Somatostatin analogs are used instead of somatostatin itself because the latter has a very short half-life in circulation, due to rapid metabolism by ubiquitous peptidase enzymes.⁷⁰ Two of these analogs, octreotide (Sandostatin)⁷¹⁻⁷⁴ and lanreotide (Somatuline)^{73,75,76} are already used clinically, and further derivatives with potentially higher SSTr affinity and improved activity are under investigation.^{77,78}

Somatostatin analogs have been linked to bifunctional chelators which are able to complex ⁶⁴Cu, and the resulting compounds have been studied as potential radiotracers for PET imaging of SSTr-positive tumors (Fig. 7). Anderson and colleagues⁷⁹ conjugated octreotide (OC) to 1,4,8,1-tetraazacyclotetradecane-1,4,8,1-tetraacetic acid (TETA) and labeled the resulting compound with ⁶⁴Cu. This compound (⁶⁴Cu-TETA-OC) showed high affinity for SSTr,⁸⁰ and in some subjects it delineated SSTr-positive lesions that were not observed with commonly used imaging agents.^{81,82} Other somatostatin analogs were also conjugated to TETA, and the ⁶⁴Cu-labeled compound ⁶⁴Cu-TETA-Tyr3-Octreotate (⁶⁴Cu-TETA-Y3-TATE) was found to have the highest receptor targeting/affinity and good clearance properties.⁸³⁻⁸⁶

Evidence that compounds, such as ⁶⁴Cu-TETA-OC, dissociate to give free Cu²⁺ ions in vivo^{81,87,88} stimulated the search for new chelates with higher kinetic stability for conjugation to somatostatin derivatives. The Cu²⁺ complex of the 1,4-bis(carboxymethyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane proligand (CB-TE2A)⁸⁹ was found to have higher thermodynamic and kinetic stability than the ⁶⁴Cu complex of TETA in vivo.⁹⁰ Metabolism studies in normal rat liver revealed that ⁶⁴Cu-CB-TE2A resulted in significantly lower values of protein-associated ⁶⁴Cu than ⁶⁴Cu-TETA [$13 \pm 6\%$ versus $75 \pm 9\%$ at 4 h],⁹⁰ and as a consequence the corresponding octreotate (⁶⁴Cu-CB-TE2A-Y3-TATE) conjugate demonstrated improved tumor uptake and blood and liver clearance compared with ⁶⁴Cu-TETA-OC.^{91,92}

⁶⁴Cu-LABELED INTEGRIN-TARGETING PEPTIDES

Integrins are transmembrane proteins that regulate cell-cell and cell-matrix interactions. They are dimers that consist of two non-covalently bound subunits (α and β) that have an extracellular domain arranged in a characteristic way that imparts different adhesion properties to the cell.^{93–95} Integrin proteins have been found to play important roles in angiogenesis and vasculogenesis and tumor metastasis. In addition, cell adhesion mediated by integrin-protein interactions, directly impacts cell migration, survival and proliferation.⁹⁶ So far, 24 different integrins have been identified, constituted by combinations of 18 α and 8 β subunits. Alpha-v beta-3 ($\alpha_v\beta_3$) is one of the most widely studied integrin since it is up-regulated in endothelial cells involved in active angiogenesis but not in quiescent endothelial cells,⁹⁶ making it an ideal biomarker for angiogenesis and tumor imaging.⁹⁷ Tumors where $\alpha_v\beta_3$ is found to be highly expressed include glioblastomas, breast and prostate tumors, malignant melanomas, and ovarian carcinomas.^{98–101} The $\alpha_v\beta_3$ integrin binds to extracellular proteins through a specific binding pocket that recognizes the three amino acid sequence arginine-glycine-aspartic acid (Arg-Gly-Asp or RGD).^{102–106} This discovery has led to the design of many RGD-based imaging agents^{97,107–115} and several investigations involving the ⁶⁴Cu radiolabeled complexes have been reported (Fig. 8).

Chen and colleagues¹¹⁶ conjugated 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) to c(RGDyK) and labeled it with ⁶⁴Cu for cancer imaging studies but found only moderate uptake in U87MG human glioma tumors ($1.44 \pm 0.09\%$ ID/g at 4 hours post-injection) with high liver and kidney retention (2.84 ± 0.17 and $1.98 \pm 0.06\%$ ID/g at 4 hours post-injection, respectively). To improve tumor uptake and in vivo kinetics, they substituted the monomeric RGD derivative for dimeric compounds (E[c(RGDyK)₂ and E[c(RGDfK)₂) and observed improved tumor targeting. However, kidney uptake remained too high for the compounds to be considered for further clinical studies.¹¹⁷ In an attempt to modulate the kidney retention, polyethylene glycol (PEG) groups were added to the monomeric RGD peptide derivative and it was observed that ⁶⁴Cu-DOTA-c(RGDyK)-PEG had very similar uptake in brain tumors compared with ⁶⁴Cu-DOTA-c(RGDyK), but a much lower liver uptake and a faster clearance from blood and kidneys.¹¹⁸ In a lung cancer study, the PEG-ylated dimeric RGD derivative ⁶⁴Cu-DOTA-E[c(RGDyK)₂-PEG was found to be an excellent PET imaging agent despite a lower binding affinity. The superior image quality was a direct result of minimal activity accumulation in normal heart and lung tissue which allowed for ideal imaging of the primary lung tumor.¹¹⁹ By using tetrameric¹²⁰ and octameric¹²¹ RGD derivatives, binding affinity and tumor uptake in glioblastoma cells improved; however, liver and kidney uptake also increased. In a recent article, the same group reported a DOTA-conjugated RGD derivative (RGD4C) linked to tumor necrosis factor- α (TNF α). Once radiolabeled with ⁶⁴Cu this compound was taken up by several $\alpha_v\beta_3$ -positive tumors, because of integrin and TNF α receptor recognition. The anti-tumor activity observed was improved compared with that of TNF α alone, since its toxicity was localized to integrin-positive cells only.¹²²

Sprague and colleagues conjugated c(RGDyK) to a different chelator, CB-TE2A, and found that the corresponding ⁶⁴Cu complex was taken up specifically by osteoclasts,¹²³ which are up regulated in osteolytic lesions and bone metastases.¹²⁴ These investigations open the

possibility of other applications for imaging $\alpha_v\beta_3$ in diseases such as osteoarthritis or osteoporosis, and imaging osteolytic bone metastases.

McQuade and colleagues conjugated DOTA to bitistatin, a small protein which is part of the disintegrin family and contains an RGD motif (or an analogous sequence) at the apex of one of its loops. The ^{64}Cu -labeled bitistatin showed specific uptake in mammary carcinoma tumors. However, maximum tumor uptake required around 6 hours and remained 8-fold lower than the ^{125}I -bitistatin derivative. In an attempt to improve tumor uptake and biodistribution kinetics, a spacer was introduced between DOTA and the targeting peptide; however, higher tumor uptake was accompanied by higher uptake in nontarget organs.¹²⁵

In a recent patent, Cochran and colleagues¹²⁶ reported the conjugation of DOTA to a library of many miniproteins derived from knotting proteins whose 25 to 40 amino acid sequences have been enriched by an RGD loop. After screening for initial integrin binding ability, some of the chelators were labeled with ^{64}Cu . Biodistribution and small-animal PET imaging studies showed good specific uptake in U87MG tumors (glioblastoma). However, kidney uptake was consistently higher than tumor uptake over a 25-hour period.¹²⁶

^{64}Cu -LABELED MONOCLONAL ANTIBODY 1A3 FOR TARGETING COLORECTAL CANCER

One of the first agents to be labeled with ^{64}Cu that was evaluated in humans was ^{64}Cu -BAT-2IT (where BAT = 6-bromoacetamidobenzyl-1,4,8,11-tetraazacyclotetradecane-*N,N',N'',N'''*-tetraacetic acid and 2IT = 2-iminothiolane) conjugated to the monoclonal antibody (mAb) 1A3.¹²⁷ ^{64}Cu -BAT-2IT-1A3 showed high uptake in GW39 human colon carcinoma tumor-bearing hamsters.¹²⁷ In the first Phase I/II clinical study with a ^{64}Cu radiopharmaceutical, Philpott and colleagues¹²⁸ compared ^{64}Cu -BAT-2IT-1A3 and [^{18}F]-FDG in 36 subjects with suspected advanced primary or metastatic colorectal cancer. ^{64}Cu -BAT-2IT-1A3 was more specific for detecting colorectal tumors than [^{18}F]-FDG, as FDG showed false positives in subjects with inflammatory lesions (Fig. 9). The sensitivity of ^{64}Cu -BAT-2IT-1A3 was 86% per subject and 71 % per lesion, which was improved over radioimmunoscintigraphy with ^{111}In -labeled mAb 1A3 (76% per subject and 63% per lesion).^{128,129}

^{64}Cu -BAT-2IT-1A3 was also evaluated for radio-immunotherapy in the GW39 tumor-bearing hamster model and was compared with ^{67}Cu -BAT-2IT-1A3, where ^{67}Cu is a therapeutic radionuclide ($t_{1/2} = 67$ hours; 100% β^-).¹³⁰ Hamsters were injected with ^{64}Cu -, ^{67}Cu -BAT-2IT-1A3, Cu-labeled nonspecific IgG (MOPC), or saline and were sacrificed 6 to 7 months after therapy or when tumors were greater than 10 g. Of the hamsters with small tumors (mean weight 0.43 g), 87.5% were disease-free 7 months after treatment with 2 mCi (74 MBq) of ^{64}Cu -BAT-2IT-1A3 or 0.4 mCi (14.8 MBq) of ^{67}Cu -BAT-2IT-1A3. These therapeutic responses were obtained at low radiation absorbed doses, with the mean tumor doses for ^{64}Cu - and ^{67}Cu -BAT-2IT-1A3 being 586 and 1269 rad, respectively. The maximum tolerated dose of ^{64}Cu -BAT-2IT-1A3 was later determined to be 150 mCi/kg, and at this dose 62.5% of hamsters with larger GW39 tumors (~600 mg) were

still alive 4 months post-treatment.¹³¹ This study demonstrated the therapeutic capabilities of ⁶⁴Cu.

⁶⁴Cu-LABELED ANTIBODIES FOR TARGETING EPIDERMAL GROWTH FACTOR

The epidermal growth factor (EGF) family of membrane receptors (EGFR) is one of the most relevant targets in the tyrosine kinase family. EGFR expression is increased in many human tumors, such as breast cancer, squamous-cell carcinoma of the head and neck, and prostate cancer.¹³² Activation of EGFR contributes to several tumorigenic mechanisms, and in many tumors, EGFR expression may act as a prognostic indicator, predicting patient survival or the presence of diseases in advanced stages.¹³² At present, mAbs, which block the binding of EGF to the extracellular ligand-binding domain of the receptor, have shown promise from a therapeutic standpoint. Cetuximab (C225, Erbitux) was the first mAb targeted against the EGFR approved by the Food and Drug Administration for the treatment of patients with EGFR-expressing, metastatic colorectal carcinoma. Cetuximab binds competitively to the extracellular domain of EGFR with an affinity comparable to the natural ligand ($K_D = 1.0$ nM), inhibiting the binding of the activating ligand to the receptor.^{133,134}

Cai and colleagues¹³⁵ reported the evaluation of ⁶⁴Cu-DOTA-cetuximab in several tumor-bearing mouse models. By using Western blots, they showed that a positive correlation existed between the expression of EGFR and uptake of ⁶⁴Cu-DOTA-cetuximab in several different EGFR-expressing tumor-bearing mouse models. At Washington University, ⁶⁴Cu-DOTA-cetuximab was synthesized for the small-animal PET imaging of EGFR expression.¹³⁶ For the cell binding affinity evaluation, high EGFR-expressing A431 and low EGFR-expressing MDA-MB-435 cells were used. An equilibrium dissociation constant (K_D) of 0.28 nM was obtained with the A431 cells. The K_D and maximum receptor density (B_{max}) were in agreement with the reported literature values of unlabeled cetuximab with A431 cells.¹³⁶ In vivo evaluation of ⁶⁴Cu-DOTA-cetuximab was performed in A431 and MDA-MB-435 tumor-bearing mice. Biodistribution and small-animal PET data showed a higher uptake in the EGFR positive A431 tumor than in the EGFR negative MDA-MB-435 tumor. Metabolism experiments were also performed to determine the extent of ⁶⁴Cu transchelation to blood, liver, and tumor proteins in A431 tumor-bearing mice. The results showed minimal metabolism of ⁶⁴Cu-DOTA-cetuximab in the blood out to 24 hours post-injection. Liver metabolism studies demonstrated that transchelation of ⁶⁴Cu to three proteins occurred. By using size-exclusion chromatography, two of these proteins were identified as superoxide dismutase and metallothionein. The third metabolite was believed to be a protein aggregate.

Over expression of EGFR has been found in over 70% of carcinomas of the cervix. The potential of ⁶⁴Cu-DOTA-cetuximab to measure EGFR concentration was evaluated by PET imaging in cervical-cancer tumors.¹³⁷ In this study, ⁶⁴Cu-DOTA-cetuximab was used to correlate EGFR densities on the surface of five different cervical cancer lines with the EGFR messenger RNA (mRNA) expression. Based on the cellular data, small-animal PET imaging was performed on tumor-bearing mice using the highest EGFR-expressing cervical cancer

cell line, CaSki. For the in vitro analysis, five cervical-cancer cell lines were selected after a screen of 23 human cervical-cancer lines based on their level of EGFR gene expression by gene expression microarray analysis. The five cell lines had different ranges of EGFR expression with the following order: CaSki (high), ME-180 and DcTc2 4510 (mid-range), HeLa (low), and C-33A (negative). The cell-surface EGFR expression was evaluated by conducting saturation binding assays at 4 °C, and the results paralleled the levels of EGFR expression determined by microarray analysis. In vivo biodistribution and small-animal PET studies with ^{64}Cu -DOTA-cetuximab in CaSki tumor-bearing nude mice showed high tumor uptake at 24 hours after injection (13.2% ID/g), with significant retention of radioactivity in blood and liver (Fig. 10). Overall, this study demonstrated that ^{64}Cu -DOTA-cetuximab is a useful marker of EGFR expression levels, and a potential PET agent for determining patient-specific therapies and therapeutic monitoring.

^{64}Cu RADIOPHARMACEUTICALS IN THE PIPELINE

Thiosemicarbazonato Complexes

The neutral, lipophilic, radiolabeled copper complex of the proligand pyruvaldehyde-bis(4-*N*-methyl-3-thiosemicarbazone), $^{62/64}\text{Cu}$ -PTSM, has been investigated as a potential PET tracer for imaging blood perfusion,³⁸ and also for treating tumor growth (^{64}Cu -PTSM) at wound sites following laparoscopic surgery (Fig. 11).¹³⁸ In 1993, Fujibayashi and colleagues¹³⁹ also studied the ^{62}Cu -PTSM complex as a potential brain-imaging agent. Unlike Cu-ATSM, which is more difficult to reduce because of the additional methyl substituent on the ligand backbone (one-electron reduction potential of $E_{1/2} = -0.646$ V versus the saturated calomel reference electrode, SCE), Cu-PTSM is readily reduced ($E_{1/2}(\text{SCE}) = -0.565$ V) in normoxic and hypoxic cells.⁴¹ Reduction followed by facile protonation,⁴⁰ leads to intracellular release of the copper ion, which leads to potential use of labile copper bis(thiosemicarbazonato) complexes, such as Cu-PTSM in the treatment of Alzheimer's Disease.^{140–142}

Recently, a synthetic methodology for rapid and facile functionalization and ^{64}Cu radiolabeling of bis(thiosemicarbazonato) complexes was presented.¹⁴³ The new bifunctional chelate, $\text{H}_2\text{ATSM/A}$, has a reactive hydrazinic amine group, which has been successfully conjugated to a range of biologically active molecules including saccharides, amino acids, alkyl, and aromatic substituents, including derivatized 4-nitroimidazoles (see Fig. 11).^{143,144} Properties, such as lipophilicity, reduction potentials, and $\text{p}K_a$, can be controlled by functionalization. In vitro and in vivo PET imaging in BALB/c mice bearing EMT6 tumors demonstrated that these new functionalized complexes remain hypoxia selective and have improved biodistribution and clearance characteristics.

In addition to bis(thiosemicarbazonato) complexes, mono-thiosemicarbazonato complexes of copper have also been investigated because of their potential use as PET imaging and therapeutic agents targeting topoisomerase-II α (Topo-II α).¹⁴⁵ Topo-II α is a 170 kDa protein which decatenates supercoiled DNA during replication by generating transient double-strand DNA breaks while retaining genetic integrity. The mechanism by which copper mono-thiosemicarbazonato complexes, such as the copper(II) complexes of EPH144 and EPH270 (see Fig. 11), inhibit the action of Topo-II α is uncertain. However, it has been postulated that

binding and stabilization of the intermediate Topo-II α -DNA cleavable complex may prevent replication and induce cell apoptosis.

⁶⁴Cu-labeled Nanoparticles

Nanotechnology is an applied science that creates and studies molecules or aggregates that have an overall size in the 1 to 1000 nm range (<1 μ m). In the last few years nanodevices and nanoparticles have been used in biomedical studies investigating new and improved diagnosis and therapy agents. Oncology is one of the disciplines that has benefited the most from nanotechnology. Several nanoparticles are used in diagnostic assays for cancer, as contrast agents for MR imaging, as drug delivery agents, as tumor visualization agents during surgery, and even as therapeutic agents.^{146–148} Several types of nanoparticle platforms have been evaluated for imaging applications, including iron oxide nanoparticles,^{149–154} gold nanoparticles,^{155–160} liposomes,^{161–163} emulsions,^{164–166} dendrimers,^{167–172} and nanotubes (Fig. 12).^{173–175} Nanoparticles conjugated with bifunctional chelators and targeting ligands are particularly useful for PET imaging purposes because their higher surface area per volume allows a higher number of targeting residues and radionuclides per particle, which in turn translates into higher affinity and higher specific activity, respectively.¹⁷⁶

Cai and colleagues¹⁷⁷ conjugated c(RGDyK) and DOTA to quantum dots (QD) obtaining a 20 nm nanoparticle having approximately 28 DOTA and 90 RGD residues on its surface. They observed selective targeting of the vasculature of $\alpha_v\beta_3$ positive tumors, such as U87MG human glioblastoma with minimal extravasation, which would be necessary for high tumor uptake. This selective targeting led to a lower than expected tumor uptake, with most of the ⁶⁴Cu-DOTA-QD-RGD being taken up by liver, spleen, and bone marrow. The authors concluded that smaller particles would probably have improved tumor-targeting properties because of easier extravasation and lower reticulo-endothelial system uptake.¹⁷⁷ Lee and colleagues¹⁷⁸ reported 5 nm iron oxide nano-particles coated with polyaspartic acid functionalized with an estimated 35 RGD peptides and 30 DOTA macrocycles per particle. PET studies gave high contrast images of the tumor; however, liver uptake was still high. This behavior may be explained by the fact that while the core diameter of the particles was 5 nm, their hydrodynamic size was much larger (45 nm) so the same problems observed with the QD nanoparticles persisted.¹⁷⁸

Pressly and colleagues¹⁷⁹ prepared well-defined amphiphilic copolymers with a predetermined number of reactive functionalities, PEG chains of variable length and low polydispersity. Upon collapsing in water, these polymers formed three-dimensional, three-layered nanoparticles with a hydrophobic inner core surrounded by a hydrophilic shell where the functional groups are located, and finally a PEG outer shell. The thickness of each layer, the number of reactive sites, and the dimension of the particle are determined by the composition of the initial linear polymer. When DOTA molecules were conjugated to these nanoparticles, ⁶⁴Cu labeling was achieved and biodistribution studies were conducted. Not surprisingly, particles with longer PEG chain lengths had longer circulation in blood and lower liver uptake.^{179,180}

Rossin and colleagues conjugated fluorescein isothiocyanate-labeled polystyrene latex beads (100 nm diameter) with DOTA and an anti-intracellular adhesion molecule antibody for use as an imaging agent for the lung. High lung uptake of the ^{64}Cu -labeled particle was observed; however, it was accompanied by high liver uptake and partial copper transchelation.¹⁸¹

Sun and colleagues prepared shell-crosslinked nanoparticles (SCK) by crosslinking to different degrees micelles formed by amphiphilic block copolymers. When TETA was incorporated onto the final SCK the yield was low and the labeling efficiency was unsatisfactory.¹⁸² This problem was solved by preincorporating the copper chelator (DOTA in this case) into the copolymer before the nanoparticles were formed.¹⁸³ Tuning of the pharmacokinetics of these particles was performed by introducing different numbers and different lengths of PEG chains.¹⁸³ The extent of crosslinking and the dimensions of the linker between nanoparticle and copper chelator were found to have a dramatic impact on the specific activity of the radiolabeled particle.¹⁸⁴

Bartlett and colleagues synthesized nanoparticles based on cyclodextrin polycations and DOTA-siRNA. Their study revealed that targeted (transferrin) and nontargeted particles had similar biodistribution and tumor localization; however, only the targeted ones were internalized by the tumor.¹⁸⁵

Nahrendorf and colleagues¹⁸⁶ reported a dextranated and diethylenetriaminepentaacetic-functionalized iron oxide nanoparticle (20 nm diameter) that was labeled with ^{64}Cu and successfully used to image macrophages in inflammatory atherosclerosis.

SUMMARY

Copper-64-based radiopharmaceuticals are being explored as agents for the delineation of disease in humans. By exploitation of the chemistry of Cu(II) and the decay characteristics of ^{64}Cu , agents based on small molecules, peptides, and larger biomolecules, such as antibodies, are in development for clinical translation. Those agents already translated for use in humans have shown a high degree of success in their respective applications. A diverse array of highly specific molecular ^{64}Cu -radiopharmaceutical imaging probes will inevitably lead to improved patient-specific treatments and therefore overall survival.

REFERENCES

1. Rudin M, Weissleder R. Molecular imaging in drug discovery and development. *Nat Rev Drug Discov.* 2003; 2(2):123–131. [PubMed: 12563303]
2. Quon A, Napel S, Beaulieu CF, et al. “Flying through” and “flying around” a PET/CT scan: Pilot study and development of 3D integrated ^{18}F -FDG PET/CT for virtual bronchoscopy and colonoscopy. *J Nucl Med.* 2006; 47(7):1081–1087. [PubMed: 16818940]
3. Anderson CJ, Welch MJ. Radiometal-Labeled Agents (Non-Technetium) for Diagnostic Imaging. *Chem Rev.* 1999; 99(9):2219–2234. [PubMed: 11749480]
4. Blower P. Towards molecular imaging and treatment of disease with radionuclides: the role of inorganic chemistry. *Dalton Trans.* 2006; (14):1705–1711. [PubMed: 16568178]
5. Blower PJ. Small coordination complexes as radio-pharmaceuticals for cancer targeting. *Trans Met Chem.* 1998; 23:109–112.

6. Blower PJ, Lewis JS, Zweit J. Copper radionuclides and radiopharmaceuticals in nuclear medicine. *Nucl Med Biol.* 1996; 23(8):957–980. [PubMed: 9004284]
7. Reichert DE, Lewis J, Anderson CJ. Metal complexes as diagnostic tools. *Coord Chem Rev.* 1999; 184:3–66.
8. Anderson, CJ.; Green, MA.; Fujibayashi, Y. Chemistry of copper radionuclides and radiopharmaceutical products. In: Welch, MJ.; Redvanly, CS., editors. *Handbook of Radiopharmaceuticals.* J. Wiley, Inc.; 2003. p. 401-422.
9. Novak-Hofer I, Schubiger PA. Copper-67 as a therapeutic nuclide for radioimmunotherapy. *Eur J Nucl Med Mol Imaging.* 2002; 29(6):821–830. [PubMed: 12029558]
10. Smith SV. Molecular imaging with copper-64. *J Inorg Biochem.* 2004; 98(11):1874–1901. [PubMed: 15522415]
11. Lewis JS, Herrero P, Sharp TL, et al. Delineation of hypoxia in canine myocardium using PET and copper(II)-diacetyl-bis(N(4)-methylthiosemicarbazone). *J Nucl Med.* 2002; 43(11):1557–1569. [PubMed: 12411560]
12. McCarthy DW, Shefer RE, Klinkowstein RE, et al. Efficient production of high specific activity ^{64}Cu using a biomedical cyclotron. *Nucl Med Biol.* 1997; 24(1):35–43. [PubMed: 9080473]
13. Szelecsenyi F, Blessing G, Qaim SM. Excitation functions of proton induced nuclear reaction on enriched ^{61}Ni and ^{64}Ni : possibility of production of no-carrier added ^{61}Cu and ^{64}Cu at a small cyclotron. *Appl Radiat Isot.* 1993; 44(3):575–580.
14. Obata A, Kasamatsu S, McCarthy DW, et al. Production of therapeutic quantities of ^{64}Cu using a 12 MeV cyclotron. *Nucl Med Biol.* 2003; 30:535–539. [PubMed: 12831992]
15. Hou X, Jacobsen U, Jorgensen JC. Separation of no-carrier added ^{64}Cu from a proton irradiated enriched nickel target. *Appl Radiat Isot.* 2002; 57(6):773–777. [PubMed: 12406615]
16. Zweit J, Smith AM, Downey S, et al. Excitation functions for deuteron induced reactions in natural nickel: production of no-carrier added ^{64}Cu from enriched ^{64}Ni target for positron emission tomography. *Appl Radiat Isot.* 1991; 42:193–197.
17. McCarthy DW, Bass LA, Cutler PD, et al. High purity production and potential applications of copper-60 and copper-61. *Nucl Med Biol.* 1999; 26:351–358. [PubMed: 10382836]
18. Szajek LP, Meyer W, Plascjak P, et al. Semi-remote production of ^{64}Cu [CuCl₂] and preparation of high specific activity ^{64}Cu [Cu-ATSM] for PET studies. *Radiochim Ada.* 2005; 93:239–244.
19. Zeisler SK, Pavan RA, Orzechowski J, et al. Production of ^{64}Cu on the Sherbrooke TR-PET cyclotron. *J Radioanal Nucl Chem.* 2003; 257(1):175–177.
20. Lewis JS, Welch MJ, Tang L. Workshop on the Production, application and clinical translation of ‘non-standard’ PET nuclides: a meeting report. *Q J Nucl Med Mol Imaging.* 2008; 52:101–106. [PubMed: 18043544]
21. Brown JM. The hypoxic cell: a target for selective cancer therapy-Eighteenth Bruce F Cain Memorial Award Lecture. *Cancer Res.* 1999; 59:5863–5870. [PubMed: 10606224]
22. Tatum JL, Kelloff GJ, Gillies RJ, et al. Hypoxia: importance in tumor biology, noninvasive measurement by imaging, and value of its measurement in the management of cancer therapy. *Int J Radiat Biol.* 2006; 82:699–757. [PubMed: 17118889]
23. Graeber TG, Osmanian C, Jacks T, et al. Hypoxia-mediated selection of cells with diminished apoptotic potential in solid tumors. *Nature.* 1996; 379:88–91. [PubMed: 8538748]
24. Höckel M, Schlenger K, Aral B, et al. Association between tumor hypoxia and malignant progression in advanced cancer of the uterine cervix. *Cancer Res.* 1996; 56:4509–4515. [PubMed: 8813149]
25. Shweiki D, Itin A, Soffer D, et al. Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. *Nature.* 1992; 359:843–845. [PubMed: 1279431]
26. Gray LH, Conger AD, Ebert M, et al. The concentration of oxygen dissolved in tissues at the time of irradiation as a factor in radiotherapy. *Br J Radiol.* 1953; 26:638–648. [PubMed: 13106296]
27. Gatenby RA, Smallbone K, Maini PK, et al. Cellular adaptations to hypoxia and acidosis during somatic evolution of breast cancer. *Br J Cancer.* 2007; 97(5):646–653. [PubMed: 17687336]
28. Carroll VA, Ashcroft M. Targeting the molecular basis for tumor hypoxia. *Expert Rev Mol Med.* 2005; 7(6):1–16. [PubMed: 15831177]

29. Fujibayashi Y, Taniuchi H, Yonekura Y, et al. Copper-62-ATSM: a new hypoxia imaging agent with high membrane permeability and low redox potential. *J Nucl Med.* 1997; 38(7):1155–1160. [PubMed: 9225812]
30. Fujibayashi Y, Cutler CS, Anderson CJ, et al. Comparative studies of Cu-64-ATSM and C-11-Acetate in an acute myocardial infarction model: ex vivo imaging of hypoxia in rats. *Nucl Med Biol.* 1999; 26:117. [PubMed: 10096511]
31. Dearling JLJ, Lewis JS, McCarthy DW, et al. Redox-active metal complexes for imaging hypoxic tissues: structure-activity relationships in copper(II) bis(thiosemicarbazone) complexes. *Chem Commun.* 1998; (22):2531–2532.
32. Dearling JLJ, Lewis JS, Mullen GED, et al. Copper bis(thiosemicarbazone) complexes as hypoxia imaging agents: structure-activity relationships. *J Biol Inorg Chem.* 2002; 7(3):249–259. [PubMed: 11935349]
33. Lewis JS, Laforest R, Buettner TL, et al. Copper-64-diacetyl-bis(N⁴-methylthiosemicarbazone): an agent for radiotherapy. *Proc Natl Acad Sci U S A.* 2001; 98(3):1206–1211. [PubMed: 11158618]
34. Lewis JS, McCarthy DW, McCarthy TJ, et al. Evaluation of ⁶⁴Cu-ATSM in vitro and in vivo in a hypoxic tumor model. *J Nucl Med.* 1999; 40(1):177–183. [PubMed: 9935074]
35. Lewis JS, Sharp TL, Laforest R, et al. Tumor uptake of copper-diacetyl-bis(N⁴-methylthiosemicarbazone): effect of changes in tissue oxygenation. *J Nucl Med.* 2001; 42(4):655–661. [PubMed: 11337556]
36. Maurer RI, Blower PJ, Dilworth JR, et al. Studies on the Mechanism of Hypoxic Selectivity in Copper Bis(Thiosemicarbazone) Radiopharmaceuticals. *J Med Chem.* 2002; 45(7):1420–1431. [PubMed: 11906283]
37. Vavere AL, Lewis JS. Cu-ATSM: a radiopharmaceutical for the PET imaging of hypoxia. *Dalton Trans.* 2007; (43):4893–4902. [PubMed: 17992274]
38. Wong TZ, Lacy JL, Petry NA, et al. PET of hypoxia and perfusion with ⁶²Cu-ATSM and ⁶²Cu-PTSM using a ⁶²Zn/⁶²Cu generator. *AJR Am J Roentgenol.* 2008; 190(2):427–432. [PubMed: 18212229]
39. Wood KA, Wong WL, Saunders ML. [⁶⁴Cu]diacetyl-bis(N⁴-methyl-thiosemicarbazone) - a radiotracer for tumor hypoxia. *Nucl Med Biol.* 2008; 35(4):393–400. [PubMed: 18482676]
40. Holland JP, Green JC, Dilworth JR. Probing the mechanism of hypoxia selectivity of copper bis(thiosemicarbazone) complexes: DFT calculation of redox potentials and absolute acidities in solution. *Dalton Trans.* 2006; (6):783–794. [PubMed: 16437173]
41. Holland JP, Barnard PJ, Collison D, et al. Spectroelectrochemical and computational studies on the mechanism of hypoxia selectivity of copper radiopharmaceuticals. *Chemistry A Euro J.* 2008; 14(19):5890–5907.
42. Burgman P, O'Donoghue JA, Lewis JS, et al. Cell line-dependent differences in uptake and retention of the hypoxia-selective nuclear imaging agent Cu-ATSM. *Nucl Med Biol.* 2005; 32(6):623–630. [PubMed: 16026709]
43. O'Donoghue JA, Zanzonico P, Pugachev A, et al. Assessment of regional tumor hypoxia using ¹⁸F-fluoromisonidazole and ⁶⁴Cu(II)-diacetyl-bis(N⁴-methylthiosemicarbazone) positron emission tomography: Comparative study featuring microPET imaging, P2 probe measurement, autoradiography, and fluorescent microscopy in the R3327-AT and FaDu rat tumor models. *Int J Radiat Oncol Biol Phys.* 2005; 61(5):1493–1502. [PubMed: 15817355]
44. Dence CS, Ponde DE, Welch MJ, et al. Autoradiographic and small-animal PET comparisons between ¹⁸F-FMISO, ¹⁸F-FDG, ¹⁸F-FLT and the hypoxic selective ⁶⁴Cu-ATSM in a rodent model of cancer. *Nucl Med Biol.* 2008; 35(6):713–720. [PubMed: 18678357]
45. Yuan H, Schroeder T, Bowsher JE, et al. Intertumoral differences in hypoxia selectivity of the PET imaging agent ⁶⁴Cu(II)-diacetyl-Bis(N⁴-methylthiosemicarbazone). *J Nucl Med.* 2006; 47(6):989–998. [PubMed: 16741309]
46. Tanaka T, Furukawa T, Fujieda S, et al. Double-tracer autoradiography with Cu-ATSM/FDG and immunohistochemical interpretation in four different mouse implanted tumor models. *Nucl Med Biol.* 2006; 33(6):743–750. [PubMed: 16934693]

47. Takahashi N, Fujibayashi Y, Yonekura Y, et al. Evaluation of ^{62}Cu labeled diacetyl-bis(N^4 -methylthiosemicarbazone) as a hypoxic tissue tracer in patients with lung cancer. *Ann Nucl Med*. 2000; 14(5):323–328. [PubMed: 11108159]
48. Takahashi N, Fujibayashi Y, Yonekura Y, et al. Copper-62 ATSM as a hypoxic tissue tracer in myocardial ischemia. *Ann Nucl Med*. 2001; 15(3):293–296. [PubMed: 11545205]
49. Dehdashti F, Mintun MA, Lewis JS, et al. In vivo assessment of tumor hypoxia in lung cancer with ^{60}Cu -ATSM. *Eur J Nucl Med Mol Imaging*. 2003; 30(6):844–850. [PubMed: 12692685]
50. Dehdashti F, Grigsby PW, Mintun MA, et al. Assessing tumor hypoxia in cervical cancer by positron emission tomography with ^{60}Cu -ATSM: relationship to therapeutic response—a preliminary report. *Int J Radiat Oncol Biol Phys*. 2003; 55(5):1233–1238. [PubMed: 12654432]
51. Dietz DW, Dehdashti F, Grigsby PW, et al. Tumor Hypoxia Detected by Positron Emission Tomography with ^{60}Cu -ATSM as a predictor of response and survival in patients undergoing Neoadjuvant Chemoradiotherapy for Rectal Carcinoma: a pilot study. *Dis Colon Rectum*. 2008; 51:1641–1648. [PubMed: 18682881]
52. Grigsby PW, Malyapa RS, Higashikubo R, et al. Comparison of molecular markers of hypoxia and imaging with ^{60}Cu -ATSM in cancer of the uterine cervix. *Mol Imaging Biol*. 2007; 9(5):278–283. [PubMed: 17431727]
53. Dehdashti F, Grigsby PW, Lewis JS, et al. Assessing tumor hypoxia in cervical cancer by PET with ^{60}Cu -labeled diacetyl-bis (N^4 -methylthiosemicarbazone). *J Nucl Med*. 2008; 49(2):201–205. [PubMed: 18199612]
54. Lewis JS, Laforest R, Dehdashti F, et al. An imaging comparison of ^{64}Cu -ATSM and ^{60}Cu -ATSM in cancer of the uterine cervix. *J Nucl Med*. 2008; 49(7):1177–1182. [PubMed: 18552145]
55. Laforest R, Dehdashti F, Lewis JS, et al. Dosimetry of $^{60}/^{61}/^{62}/^{64}\text{Cu}$ -ATSM: a hypoxia imaging agent for PET. *Eur J Nucl Med Mol Imaging*. 2005; 32(7):764–770. [PubMed: 15785955]
56. Obata A, Kasamatsu S, Lewis JS, et al. Basic characterization of ^{64}Cu -ATSM as a radiotherapy agent. *Nucl Med Biol*. 2005; 32(1):21–28. [PubMed: 15691658]
57. Hoyer D, Lubbert H, Bruns C. Molecular pharmacology of Somatostatin receptors. *Naunyn Schmiedeberg Arch Pharmacol*. 1994; 350(5):441–453. [PubMed: 7870182]
58. Reubi JC. In-vitro identification of vasoactive-intestinal-peptide receptors in human tumors - implications for tumor imaging. *J Nucl Med*. 1995; 36(10):1846–1853. [PubMed: 7562054]
59. Reubi JC, Kvolis L, Krenning E, et al. Distribution of Somatostatin receptors in normal and tumor-tissue. *Metab Clin Exp*. 1990; 39(9):78–81. [PubMed: 2169575]
60. Reubi JC, Schaer JC, Laissue JA, et al. Somatostatin receptors and their subtypes in human tumors and in peritumoral vessels. *Metab Clin Exp*. 1996; 45(8):39–41. [PubMed: 8769377]
61. Reubi JC, Waser B, Schaer JC, et al. Somatostatin receptor sst1-sst5 expression in normal and neoplastic human tissues using receptor autoradiography with subtype-selective ligands. *Eur J Nucl Med*. 2001; 28(7):836–846. [PubMed: 11504080]
62. Hofland LJ, Lamberts SWJ, van Hagen PM, et al. Crucial role for somatostatin receptor subtype 2 in determining the uptake of [$\text{In-111-DTPA-D-Phe(1)}$] octreotide in somatostatin receptor-positive organs. *J Nucl Med*. 2003; 44(8):1315–1321. [PubMed: 12902423]
63. Papotti M, Bongiovanni M, Volante M, et al. Expression of somatostatin receptor types 1–5 in 81 cases of gastrointestinal and pancreatic endocrine tumors - a correlative immunohistochemical and reverse-transcriptase polymerase chain reaction analysis. *Virchows Arch*. 2002; 440(5):461–475. [PubMed: 12021920]
64. Harrison Louis B, Chadha M, Hill Richard J, et al. Impact of tumor hypoxia and anemia on radiation therapy outcomes. *Oncologist*. 2002; 7(6):492–508. [PubMed: 12490737]
65. Heppeler A, Froidevaux S, Eberle AN, et al. Receptor targeting for tumor localisation and therapy with radiopeptides. *Curr Med Chem*. 2000; 7(9):971–994. [PubMed: 10911025]
66. Li, WP.; Meyer, LA.; Anderson, CJ. Contrast Agents Iii: Radiopharmaceuticals. *Diagnostics to Therapeutics*. Vol. 252. Heidelberg: SpringerBerlin; 2005. Radiopharmaceuticals for positron emission tomography imaging of somatostatin receptor positive tumors; p. 179–192.
67. Weiner RE, Thakur ML. Radiolabeled peptides in diagnosis and therapy. *Semin Nucl Med*. 2001; 31(4):296–311. [PubMed: 11710772]

68. Weiner RE, Thakur ML. Radiolabeled peptides in the diagnosis and therapy of oncological diseases. *Appl Radiat Isot.* 2002; 57(5):749–763. [PubMed: 12433051]
69. Weiner RE, Thakur ML. Radiolabeled peptides in oncology - role in diagnosis and treatment. *Bio-Drugs.* 2005; 19(3):145–163.
70. Patel YC, Wheatley T. In vivo and in vitro plasma disappearance and metabolism of somatostatin-28 and somatostatin-14 in the rat. *Endocrinology.* 1983; 112:220–225. [PubMed: 6128222]
71. Bauer W, Briner U, Doepfner W, et al. SMS 201-995: a very potent and selective octapeptide analogue of somatostatin with prolonged action. *Life Sci.* 1982; 31(11):1133–1140. [PubMed: 6128648]
72. Bevan JS. Clinical review: The antitumoral effects of somatostatin analog therapy in acromegaly. *J Clin Endocrinol Metab.* 2005; 90(3):1856–1863. [PubMed: 15613435]
73. de Herder WW, Hofland LJ, van der Lely AJ, et al. Somatostatin receptors in gastroenteropancreatic neuroendocrine tumours. *Endocr Relat Cancer.* 2003; 10(4):451–458. [PubMed: 14713257]
74. Lamberts SWJ. A Guide to the Clinical Use of the Somatostatin Analog Sms 201-995 (Sandostatin). *Acta Endocrinol.* 1987; 116:54–66.
75. Eriksson B, Janson ET, Bax NDS, et al. The use of new somatostatin analogues, lanreotide and octastatin, in neuroendocrine gastro-intestinal tumours. *Digestion.* 1996; 57:77–80. [PubMed: 8813476]
76. Murray RD, Melmed S. A critical analysis of clinically available somatostatin analog formulations for therapy of acromegaly. *J Clin Endocrinol Metab.* 2008; 93(8):2957–2968. [PubMed: 18477663]
77. Ginj M, Zhang HW, Eisenwiener KP, et al. New pan-somatostatin ligands and their chelated versions: affinity profile, agonist activity, internalization, and tumor targeting. *Clin Cancer Res.* 2008; 14(7):2019–2027. [PubMed: 18381940]
78. Lewis I, Bauer W, Albert R, et al. A novel somatostatin mimic with broad somatotropin release inhibitory factor receptor binding and superior therapeutic potential. *J Med Chem.* 2003; 46(12):2334–2344. [PubMed: 12773038]
79. Anderson CJ, Pajean TS, Edwards WB, et al. In-vitro and in-vivo evaluation of copper-64-octreotide conjugates. *J Nucl Med.* 1995; 36(12):2315–2325. [PubMed: 8523125]
80. Parry JJ, Eiblmaier M, Andrews R, et al. Characterization of somatostatin receptor subtype 2 expression in stably transfected A-427 human cancer cells. *Mol Imaging.* 2007; 6(1):56–67. [PubMed: 17311765]
81. Anderson CJ, Dehdashti F, Cutler PD, et al. Cu-64-TETA-Octreotide as a PET imaging agent for patients with neuroendocrine tumors. *J Nucl Med.* 2001; 42(2):213–221. [PubMed: 11216519]
82. Gulec SA, Baum R. Radio-guided surgery in neuroendocrine tumors. *J Surg Oncol.* 2007; 96(4):309–315. [PubMed: 17726664]
83. Anderson CJ, Jones LA, Bass LA, et al. Radiotherapy, toxicity and dosimetry of copper-64-TETA-octreotide in tumor-bearing rats. *J Nucl Med.* 1998; 39(11):1944–1951. [PubMed: 9829587]
84. Lewis JS, Lewis MR, Cutler PD, et al. Radiotherapy and dosimetry of ⁶⁴Cu-TETA-Tyr³-octreotate in a somatostatin receptor-positive, tumor-bearing rat model. *Clin Cancer Res.* 1999; 5(11):3608–3616. [PubMed: 10589778]
85. Lewis JS, Lewis MR, Srinivasan A, et al. Changes in target tissue uptake over a multiple dose radiotherapy regimen with copper-64-TETA-Y3-octreotate [abstract]. *J Nucl Med.* 1999; 40(5):224. [PubMed: 10025827]
86. Lewis JS, Lewis MR, Srinivasan A, et al. Comparison of four ⁶⁴Cu-labeled Somatostatin analogs in vitro and in a tumor-bearing rat model: evaluation of new derivatives for positron emission tomography imaging and targeted radiotherapy. *J Med Chem.* 1999; 42(8):1341–1347. [PubMed: 10212119]
87. Bass LA, Wang M, Welch MJ, et al. In vivo transchelation of copper-64 from TETA-octreotide to superoxide dismutase in rat liver. *Bioconjug Chem.* 2000; 11(4):527–532. [PubMed: 10898574]

88. Wang M, Caruano AL, Lewis MR, et al. Subcellular localization of radiolabeled somatostatin analogues: Implications for targeted radiotherapy of cancer. *Cancer Res.* 2003; 63(20):6864–6869. [PubMed: 14583484]
89. Sun X, Wuest M, Weisman GR, et al. Radiolabeling and in vivo behavior of copper-64-labeled cross-bridged cyclam ligands. *J Med Chem.* 2002; 45(2):469–477. [PubMed: 11784151]
90. Boswell CA, Sun XK, Niu WJ, et al. Comparative in vivo stability of copper-64-labeled cross-bridged and conventional tetraazamacrocyclic complexes. *J Med Chem.* 2004; 47(6):1465–1474. [PubMed: 14998334]
91. Eiblmaier M, Andrews R, Laforest R, et al. Nuclear uptake and dosimetry of ^{64}Cu -labeled chelator-somatostatin conjugates in an SSTR2-transfected human tumor cell line. *J Nucl Med.* 2007; 48(8):1390–1396. [PubMed: 17631550]
92. Sprague JE, Peng Y, Sun X, et al. Preparation and biological evaluation of copper-64-labeled Tyr3-octreotate using a cross-bridged macrocyclic chelator. *Clin Cancer Res.* 2004; 10(24):8674–8682. [PubMed: 15623652]
93. Hynes RO. Integrins - Versatility, Modulation, and Signaling in Cell-Adhesion. *Cell.* 1992; 69(1): 11–25. [PubMed: 1555235]
94. Lusinskas FW, Lawler J. Integrins as Dynamic Regulators of Vascular Function. *FASEB J.* 1994; 8(12):929–938. [PubMed: 7522194]
95. Ruoslahti E. RGD and other recognition sequences for integrins. *Annu Rev Cell Dev Biol.* 1996; 12:697–715. [PubMed: 8970741]
96. Hood JD, Cheresch DA. Role of integrins in cell invasion and migration. *Nat Rev Cancer.* 2002; 2(2):91–100. [PubMed: 12635172]
97. Beer AJ, Schwaiger M. Imaging of integrin $\alpha\text{v}\beta 3$ expression. *Cancer Metastasis Rev.* 2008; 27:631–644. [PubMed: 18523730]
98. Albelda SM, Mette SA, Elder DE, et al. Integrin distribution in malignant-melanoma - association of the Beta-3-subunit with tumor progression. *Cancer Res.* 1990; 50(20):6757–6764. [PubMed: 2208139]
99. Bello L, Francolini M, Marthyn P, et al. $\alpha\text{v}\beta 3$ and $\alpha\text{v}\beta 5$ integrin expression in glioma periphery. *Neurosurgery.* 2001; 49(2):380–389. [PubMed: 11504114]
100. Brooks PC, Stromblad S, Klemke R, et al. Anti-integrin $\alpha\text{v}\beta 3$ blocks human breast-cancer growth and Angiogenesis in human skin. *J Clin Invest.* 1995; 96(4):1815–1822. [PubMed: 7560073]
101. Jin H, Varner J. Integrins: roles in cancer development and as treatment targets. *Br J Cancer.* 2004; 90(3):561–565. [PubMed: 14760364]
102. Cai WB, Chen K, Mohamedali KA, et al. PET of vascular endothelial growth factor receptor expression. *J Nucl Med.* 2006; 47(12):2048–2056. [PubMed: 17138749]
103. Horton MA. The $\alpha\text{v}\beta 3$ integrin “vitronectin receptor”. *Int J Biochem Cell Biol.* 1997; 29(5):721–725. [PubMed: 9251239]
104. Ruoslahti E. The RGD story: a personal account. *Matrix Biol.* 2003; 22(6):459–465. [PubMed: 14667838]
105. Xiong JP, Stehle T, Diefenbach B, et al. Crystal structure of the extracellular segment of integrin $\alpha\text{V}\beta 3$. *Science.* 2001; 294(5541):339–345. [PubMed: 11546839]
106. Xiong JP, Stehle T, Zhang RG, et al. Crystal structure of the extracellular segment of integrin $\alpha\text{V}\beta 3$ in complex with an Arg-Gly-Asp ligand. *Science.* 2002; 296(5565):151–155. [PubMed: 11884718]
107. De Jong M, VanHagen PM, Breeman WA, et al. Evaluation of radiolabeled cyclic DTPARGD analog for tumor imaging and radionuclide therapy [abstract]. *J Nucl Med.* 2000; 41(5):232.
108. Janssen M, Buijs W, Boerman O, et al. Y-86 and In-111 labeled $\alpha\text{v}\beta 3$ binding peptides: a comparative dosimetric study in dogs. *J Nucl Med.* 2001; 42(5):241.
109. Janssen M, Oyen WJG, Massuger LFAG, et al. Comparison of a monomeric and dimeric radiolabeled RGD-peptide for tumor targeting. *Cancer Biother Radiopharm.* 2002; 17(6):641–646. [PubMed: 12537667]

110. Janssen ML, Oyen WJ, Dijkgraaf I, et al. Tumor targeting with radiolabeled alpha(v)beta(3) integrin binding peptides in a nude mouse model. *Cancer Res.* 2002; 62(21):6146–6151. [PubMed: 12414640]
111. Liu S, Cheung E, Ziegler MC, et al. Y-90 and Lu-177 labeling of a DOTA-conjugated vitronectin receptor antagonist useful for tumor therapy. *Bioconjug Chem.* 2001; 12(4):559–568. [PubMed: 11459461]
112. Liu S, Edwards DS, Ziegler MC, et al. Tc-99m-Labeling of a hydrazinonicotinamide-conjugated vitronectin receptor antagonist useful for imaging tumors. *Bioconjug Chem.* 2001; 12(4):624–629. [PubMed: 11459468]
113. Su Z, Liu GZ, Gupta S, et al. In vitro and in vivo evaluation of a technetium-99m-labeled cyclic RGD peptide as a specific marker of alpha(V) beta(3) integrin for tumor imaging. *Bioconjug Chem.* 2002; 13(3):561–570. [PubMed: 12009947]
114. van Hagen PM, Breeman WAP, Bernard HF, et al. Evaluation of a radiolabelled cyclic DTPA-RGD analogue for tumour imaging and radionuclide therapy. *Int J Cancer.* 2000; 90(4):186–198. [PubMed: 10993959]
115. van Hagen PM, De Jong M, Breeman WA, et al. In-111-labeled RGD analog for tumor imaging [abstract]. *J Nucl Med.* 2000; 41(5):286.
116. Chen XY, Park R, Tohme M, et al. MicroPET and autoradiographic imaging of breast cancer alpha(v)-integrin expression using F-18- and Cu-64-labeled RGD peptide. *Bioconjug Chem.* 2004; 15(1):41–49. [PubMed: 14733582]
117. Chen XY, Liu S, Hou YP, et al. MicroPET imaging of breast cancer alpha(v)-integrin expression with Cu-64-labeled dimeric RGD peptides. *Mol Imaging Biol.* 2004; 6(5):350–359. [PubMed: 15380745]
118. Chen XY, Hou YP, Tohme M, et al. Pegylated Arg-Gly-Asp peptide: Cu-64 labeling and PET imaging of brain tumor alpha(v)beta(3)-integrin expression. *J Nucl Med.* 2004; 45(10):1776–1783. [PubMed: 15471848]
119. Chen XY, Sievers E, Hou YP, et al. Integrin alpha(V)beta(3)-targeted imaging of lung cancer. *Neoplasia.* 2005; 7(3):271–279. [PubMed: 15799827]
120. Wu Y, Zhang XZ, Xiong ZM, et al. microPET imaging of glioma integrin alpha(v)beta(3) expression using Cu-64-labeled tetrameric RGD peptide. *J Nucl Med.* 2005; 46(10):1707–1718. [PubMed: 16204722]
121. Li ZB, Cai WB, Cao QZ, et al. ⁶⁴Cu-Labeled tetrameric and octameric RGD peptides for small-animal PET of tumor alpha(v)beta(3) integrin expression. *J Nucl Med.* 2007; 48(7):1162–1171. [PubMed: 17574975]
122. Wang H, Chen K, Cai WB, et al. Integrin-targeted imaging and therapy with RGD4C–TNF fusion protein. *Mol Cancer Ther.* 2008; 7(5):1044–1053. [PubMed: 18483294]
123. Sprague JE, Kitaura H, Zou W, et al. Noninvasive imaging of osteoclasts in parathyroid hormone-induced osteolysis using a ⁶⁴Cu-labeled RGD peptide. *J Nucl Med.* 2007; 48(2):311–318. [PubMed: 17268030]
124. Nakamura I, Duong LT, Rodan SB, et al. Involvement of alpha(v)beta(3) integrins in osteoclast function. *J Bone Miner Metab.* 2007; 25(6):337–344. [PubMed: 17968485]
125. McQuade P, Knight LC, Welch MJ. Evaluation of Cu-64- and I-125-radiolabeled bitistatin as potential agents for targeting alpha(v)beta(3) integrins in tumor angiogenesis. *Bioconjug Chem.* 2004; 15(5):988–996. [PubMed: 15366951]
126. Cochran JR, Kimura R, Levin AM, et al. Engineered integrin binding peptides [patent application]. PCT Int Appl WO. 2008045252. 2008
127. Anderson CJ, Connett JM, Schwarz SW, et al. Copper-64-labeled antibodies for PET imaging. *J Nucl Med.* 1992; 33(9):1685–1691. [PubMed: 1517844]
128. Philpott GW, Schwarz SW, Anderson CJ, et al. RadioimmunoPET: detection of colorectal carcinoma with positron-emitting copper-64-labeled monoclonal antibody. *J Nucl Med.* 1995; 36(10):1818–1824. [PubMed: 7562049]
129. Philpott GW, Siegel BA, Schwarz SW, et al. Immunoscintigraphy with a new indium-111-labeled monoclonal antibody (MAb 1A3) in patients with colorectal cancer. *Dis Colon Rectum.* 1994; 37(8):782–792. [PubMed: 8055723]

130. Connett JM, Anderson CJ, Guo LW, et al. Radioimmunotherapy with a ^{64}Cu -labeled monoclonal antibody: a comparison with ^{67}Cu . *Proc Natl Acad Sci USA*. 1996; 93(13):6814–6818. [PubMed: 8692901]
131. Connett JM, Buettner TL, Anderson CJ. Maximum tolerated dose and large tumor radioimmunotherapy studies of ^{64}Cu -labeled monoclonal antibody 1A3 in a colon cancer model. *Clin Cancer Res*. 1999; 5(10 Suppl):3207s–3212s. [PubMed: 10541365]
132. Laskin JJ, Sandler AB. Epidermal growth factor receptors: a promising target in solid tumors. *Cancer Treat Rev*. 2004; 30:1–17. [PubMed: 14766123]
133. Fan Z, Masui H, Altas I, et al. Blockage of epidermal growth factor receptor function by bivalent and monovalent fragments of C225 anti-epidermal growth factor receptor monoclonal antibodies. *Cancer Res*. 1993; 53:4322–4328. [PubMed: 8364927]
134. Mendelsohn J. Epidermal growth factor receptor inhibition by a monoclonal antibody as anti-cancer therapy. *Clin Cancer Res*. 1997; 3:2703–2707. [PubMed: 10068277]
135. Cai W, Chen K, He L, et al. Quantitative PET of EGFR expression in xenograft-bearing mice using (^{64}Cu)-labeled cetuximab, a chimeric anti-EGFR monoclonal antibody. *Eur J Nucl Med Mol Imaging*. 2007; 34(6):850–858. [PubMed: 17262214]
136. Li WP, Meyer LA, Capretto DA, et al. Receptor-binding, biodistribution, and metabolism studies of ^{64}Cu -DOTA-Cetuximab, a PET-imaging agent for epidermal growth-factor receptor-positive tumors. *Cancer Biother Radiopharm*. 2008; 23(2):158–171. [PubMed: 18454685]
137. Eiblmaier M, Meyer LA, Watson MA, et al. Correlating EGFR expression with receptor-binding properties and internalization of ^{64}Cu -DOTA-cetuximab in 5 cervical cancer cell lines. *J Nucl Med*. 2008; 49(9):1472–1479. [PubMed: 18703609]
138. Lewis JS, Connett JM, Garbow JR, et al. Copper-64-pyruvaldehyde-bis(N^4 -methylthiosemicarbazone) for the prevention of tumor growth at wound sites following laparoscopic surgery: monitoring therapy response with microPET and magnetic resonance imaging. *Cancer Res*. 2002; 62(2):445–449. [PubMed: 11809694]
139. Fujibayashi Y, Wada K, Taniuchi H, et al. Mitochondria-selective reduction of ^{62}Cu -pyruvaldehyde bis(N^4 -methylthiosemicarbazone) (^{62}Cu -PTSM) in the murine brain; a novel radiopharmaceutical for brain positron emission tomography (PET) imaging. *Biol Pharm Bull*. 1993; 16(2):146–149. [PubMed: 8395929]
140. Donnelly PS, Caragounis A, Du T, et al. Selective intracellular release of Copper and Zinc Ions from Bis(thiosemicarbazone) complexes reduces levels of Alzheimer disease Amyloid-beta peptide. *J Biol Chem*. 2008; 283(8):4568–4577. [PubMed: 18086681]
141. Donnelly PS, Wedd AG. Alzheimer's disease and the chemistry of copper. *Aust J Chem*. 2007; 74(7):18–20.
142. Donnelly PS, Xiao Z, Wedd AG. Copper and Alzheimer's disease. *Curr Opin Chem Biol*. 2007; 11(2):128–133. [PubMed: 17300982]
143. Holland JP, Aigbirio FI, Betts HM, et al. Functionalized Bis(thiosemicarbazone) Complexes of Zinc and Copper: synthetic platforms toward site-specific radiopharmaceuticals. *Inorg Chem*. 2007; 46(2):465–485. [PubMed: 17279826]
144. Bonnichs PD, Vavere AL, Lewis JS, et al. In vitro and in vivo evaluation of bifunctional bithiosemicarbazone ^{64}Cu -complexes for the positron emission tomography imaging of hypoxia. *J Med Chem*. 2008; 51(10):2985–2991. [PubMed: 18416544]
145. Wei L, Easmon J, Nagi RK, et al. ^{64}Cu -azabicyclo[3.2.2]nonane thiosemicarbazone complexes: radiopharmaceuticals for PET of topoisomerase II expression in tumors. *J Nucl Med*. 2006; 47(12):2034–2041. [PubMed: 17138747]
146. Banerjee HN, Verma M. Use of nanotechnology for the development of novel cancer biomarkers. *Expert Rev Mol Diagn*. 2006; 6(5):679–683. [PubMed: 17009903]
147. Banerjee HN, Verma M. Application of nanotechnology in cancer. *Technol Cancer Res Treat*. 2008; 7(2):149–154. [PubMed: 18345704]
148. Jain KK. Recent advances in nanooncology. *Tech Cancer Res Treat*. 2008; 7(1):1–13.
149. Lanza GM, Winter PM, Caruthers SD, et al. Magnetic resonance molecular imaging with nanoparticles. *J Nucl Cardiol*. 2004; 11(6):733–743. [PubMed: 15592197]

150. Neumaier CE, Baio G, Ferrini S, et al. MR and iron magnetic nanoparticles. Imaging opportunities in preclinical and translational research. *Tumori*. 2008; 94(2):226–233. [PubMed: 18564611]
151. Sosnovik DE, Nahrendorf M, Weissleder R. Magnetic nanoparticles for MR imaging: agents, techniques and cardiovascular applications. *Not Found In Database*. 2008; 103(2):122–130.
152. Sun C, Fang C, Stephen Z, et al. Tumor-targeted drug delivery and MRI contrast enhancement by chlorotoxin-conjugated iron oxide nanoparticles. *Nanomed*. 2008; 3(4):495–505.
153. Sun C, Lee JSH, Zhang MQ. Magnetic nanoparticles in MR imaging and drug delivery. *Adv Drug Deliv Rev*. 2008; 60(11):1252–1265. [PubMed: 18558452]
154. Waters EA, Wickline SA. Contrast agents for MRI. *Basic Res Cardiol*. 2008; 103(2):114–121. [PubMed: 18324367]
155. Absil E, Tessier G, Fournier D, et al. Full field imaging and spectroscopy of individual gold nanoparticles. *Eur Phys J Appl Phys*. 2008; 43(2):155–158.
156. Hirsch LR, Gobin AM, Lowery AR, et al. Metal nanoshells. *Ann Biomed Eng*. 2006; 34(1):15–22. [PubMed: 16528617]
157. Kho KW, Kah JCY, Lee CGL, et al. Applications of gold nanoparticles in the early detection of cancer. *J Mech Med Biol*. 2007; 7(1):19–35.
158. Kogan MJ, Olmedo I, Hosta L, et al. Peptides and metallic nanoparticles for biomedical applications. *Nanomedicine*. 2007; 2(3):287–306. [PubMed: 17716175]
159. Liong M, Lu J, Kovochich M, et al. Multifunctional inorganic nanoparticles for imaging, targeting, and drug delivery. *ACS Nano*. 2008; 2(5):889–896. [PubMed: 19206485]
160. Loo C, Lin A, Hirsch L, et al. Nanoshell-enabled photonics-based imaging and therapy of cancer. *Tech Cancer Res Treat*. 2004; 3(1):33–40.
161. Mulder WJM, Strijkers GJ, Habets JW, et al. MR molecular imaging and fluorescence microscopy for identification of activated tumor endothelium using a bimodal lipidic nanoparticle. *FASEB J*. 2005; 19(12):2008–2010. [PubMed: 16204353]
162. Saito R, Krauze MT, Bringas JR, et al. Gadolinium-loaded liposomes allow for real-time magnetic resonance imaging of convection-enhanced delivery in the primate brain. *Exp Neurol*. 2005; 196(2):381–389. [PubMed: 16197944]
163. Al-Jamal WT, Kostarelos K. Liposome-nanoparticle hybrids for multimodal diagnostic and therapeutic applications. *Nanomed*. 2007; 2(1):85–98.
164. Caruthers SD, Wickline SA, Lanza GM. Nanotechnological applications in medicine. *Curr Opin Biotechnol*. 2007; 18(1):26–30. [PubMed: 17254762]
165. Marsh JN, Partlow KC, Abendschein DR, et al. Molecular imaging with targeted perfluorocarbon nanoparticles: Quantification of the concentration dependence of contrast enhancement for binding to sparse cellular epitopes. *Ultrasound Med Biol*. 2007; 33(6):950–958. [PubMed: 17434667]
166. Winter PM, Cai K, Caruthers SD, et al. Emerging nanomedicine opportunities with perfluorocarbon nanoparticles. *Expert Rev Med Devices*. 2007; 4(2):137–145. [PubMed: 17359221]
167. Bielinska A, Eichman JD, Lee I, et al. Imaging {Au-0-PAMAM} gold-dendrimer nanocomposites in cells. *J Nanoparticle Res*. 2002; 4(5):395–403.
168. Gillies ER, Dy E, Frechet JMJ, et al. Biological evaluation of polyester dendrimer: poly(ethylene oxide) “Bow-Tie” hybrids with tunable molecular weight and architecture. *Mol Pharmacol*. 2005; 2(2):129–138.
169. Goodson T, Varnavski O, Wang Y. Optical properties and applications of dendrimer-metal nanocomposites. *Int Rev Phys Chem*. 2004; 23(1):109–150.
170. Khan MK, Nigavekar SS, Mine LD, et al. In vivo biodistribution of dendrimers and dendrimer nanocomposites - Implications for cancer imaging and therapy. *Tech Cancer Res Treat*. 2005; 4(6):603–613.
171. Kobayashi H, Kawamoto S, Jo SK, et al. Macromolecular MRI contrast agents with small dendrimers: Pharmacokinetic differences between sizes and cores. *Bioconjug Chem*. 2003; 14(2):388–394. [PubMed: 12643749]

172. Kobayashi H, Kawamoto S, Konishi J, et al. Macro-molecular MRI contrast agents with small dendrimer cores for functional kidney imaging. *Radiology*. 2002; 225:237.
173. Choi JH, Nguyen FT, Barone PW, et al. Multimodal biomedical imaging with asymmetric single-walled carbon nanotube/iron oxide nanoparticle complexes. *Nano Lett*. 2007; 7(4):861–867. [PubMed: 17335265]
174. Keren S, Zavaleta C, Cheng Z, et al. Noninvasive molecular imaging of small living subjects using Raman spectroscopy. *Proc Natl Acad Sci U S A*. 2008; 105(15):5844–5849. [PubMed: 18378895]
175. Yu X, Munge B, Patel V, et al. Carbon nanotube amplification strategies for highly sensitive immunodetection of cancer biomarkers. *J Am Chem Soc*. 2006; 128(34):11199–11205. [PubMed: 16925438]
176. Shokeen M, Fetting NM, Rossin R. Synthesis, in vitro and in vivo evaluation of radiolabeled nanoparticles. *Q J Nucl Med Mol Imaging*. 2008; 52(3):267–277. [PubMed: 18475250]
177. Cai W, Chen K, Li ZB, et al. Dual-function probe for PET and near-infrared fluorescence imaging of tumor vasculature. *J Nucl Med*. 2007; 48(11):1862–1870. [PubMed: 17942800]
178. Lee HY, Li Z, Chen K, et al. PET/MRI dual-modality tumor imaging using arginine-glycine-aspartic (RGD) - Conjugated radiolabeled iron oxide nano-particles. *J Nucl Med*. 2008; 49(8):1371–1379. [PubMed: 18632815]
179. Pressly ED, Rossin R, Hagooly A, et al. Structural effects on the biodistribution and positron emission tomography (PET) imaging of well-defined Cu-64-labeled nanoparticles comprised of amphiphilic block graft copolymers. *Biomacromolecules*. 2007; 8(10):3126–3134. [PubMed: 17880180]
180. Fukukawa, KI; Rossin, R.; Hagooly, A., et al. Synthesis and characterization of core-shell star copolymers for in vivo PET imaging applications. *Biomacromolecules*. 2008; 9(4):1329–1339. [PubMed: 18338840]
181. Rossin R, Muro S, Welch MJ, et al. In vivo imaging of Cu-64-labeled polymer nanoparticles targeted to the lung endothelium. *J Nucl Med*. 2008; 49(1):103–111. [PubMed: 18077519]
182. Sun XK, Rossin R, Turner JL, et al. An assessment of the effects of shell cross-linked nanoparticle size, core composition, and surface PEGylation on in vivo biodistribution. *Biomacromolecules*. 2005; 6(5):2541–2554. [PubMed: 16153091]
183. Sun G, Hagooly A, Xu J, et al. Facile, efficient approach to accomplish tunable chemistries and variable biodistributions for shell cross-linked nanoparticles. *Biomacromolecules*. 2008; 9(7):1997–2006. [PubMed: 18510359]
184. Xu JQ, Sun GR, Rossin R, et al. Labeling of polymer nanostructures for medical imaging: Importance of cross-linking extent, spacer length, and charge density. *Macromolecules*. 2007; 40(9):2971–2973. [PubMed: 18779874]
185. Bartlett DW, Su H, Hildebrandt IJ, et al. Impact of tumor-specific targeting on the biodistribution and efficacy of siRNA nanoparticles measured by multi-modality in vivo imaging. *Proc Natl Acad Sci U S A*. 2007; 104(39):15549–15554. [PubMed: 17875985]
186. Nahrendorf M, Zhang HW, Hembrador S, et al. Nanoparticle PET-CT imaging of macrophages in inflammatory atherosclerosis. *Circulation*. 2008; 117(3):379–387. [PubMed: 18158358]

Fig. 1.
The pathways for the decay of ^{64}Cu into ^{64}Ni and ^{64}Zn .

Fig. 2.
The decrease in oxygen tension with increasing distance from the blood supply.

Fig. 3.
Chemical structure of Cu-ATSM. Copper = silver, sulfur = yellow, nitrogen = blue, carbon = gray and hydrogen = white.

Fig. 4. Percentage uptake of ^{64}Cu -ATSM, ^{18}F -Fluoromisonidazole (^{18}F -FMISO) and pyruvaldehyde-bis(4-n-methyl-3-thiosemicarbazonato)copper(II) (^{64}Cu -PTSM) in EMT6 cells at varying oxygen concentrations. (From Lewis JS, McCarthy DW, McCarthy TJ, et al. Evaluation of ^{64}Cu -ATSM in vitro and in vivo in a hypoxic tumor model. J Nucl Med 1999;40:177-83; with permission.)³⁴

Fig. 5. The proposed mechanism of hypoxia-selectivity of copper(II) bis(thiosemicarbazonato) complexes.⁴¹

Fig. 6. Transaxial PET/CT images showing the CT image (top left), [^{18}F]-FDG image, ^{60}Cu -ATSM and ^{64}Cu -ATSM images recorded between 30 to 60 minutes in two subjects with known cervical cancers. (A) Images recorded for a patient who responded to conventional radiotherapy and (B) images from a nonresponder. (From Lewis JS, Laforest R, Dehdashti F, et al. An imaging comparison of ^{64}Cu -ATSM and ^{60}Cu -ATSM in cancer of the uterine cervix. J Nucl Med 2008;49:1177–82; with permission.)⁵⁴

Fig. 7.

Amino acid sequences of somatostatin analogs used in imaging with ^{64}Cu and the chelators used to complex ^{64}Cu .

Fig. 8. Structures of c(RGDxK) peptides used in imaging of $\alpha_v\beta_3$ expression in tumor angiogenesis and osteoclasts. x = D-Tyr or D-Phe.

Fig. 9.

Anterior volume-rendered reprojection PET images obtained after injection of FDG (*left*) and ^{64}Cu -BAT-2IT-1A3 (*right*) in a woman postresection of Dukes' C rectal cancer 1 year before enlarged mediastinal and periportal lymph nodes were observed. The lymph nodes were strongly positive on FDG-PET (*left panel, see arrows*), but were completely negative with ^{64}Cu -BAT-2IT-1A3 (*right panel*). Subsequent analysis of the lymph nodes demonstrated sarcoidosis. (*Data from Philpott GW, Schwarz SW, Anderson CJ, et al. RadioimmunoPET: detection of colorectal carcinoma with positron-emitting copper-64-labeled monoclonal antibody. J Nucl Med 1995;36(10): 1818–24.*)¹²⁸

Fig. 10. Small-animal PET imaging of ^{64}Cu -DOTA-cetuximab in CaSki tumor-bearing SCID mice at 24 hours after injection: coronal (A), sagittal (B), and transaxial (C) views. (From Eiblmaier M, Meyer LA, Watson MA, et al. Correlating EGFR expression with receptor-binding properties and internalization of ^{64}Cu -DOTA-cetuximab in 5 cervical cancer cell lines. *J Nucl Med* 2008;49(9): 1472–9; with permission.)¹³⁷

Fig. 11.

Chemical structures of several promising pipeline tracers based on the chelation of ^{64}Cu and other copper radionuclides.

DOTA-Quantum Dot

DOTA-Iron Oxide-RGD

PEGylated DOTA-star copolymer

PEGylated DOTA-shell Cross-Linked NPs

Fig. 12.

Structures of nanoparticles used in imaging include DOTA-conjugated quantum dots,¹⁷⁷ DOTA- and RGD peptide-conjugated iron oxide nanoparticles,¹⁷⁸ pegylated DOTA star copolymers,¹⁸⁰ and pegylated DOTA-shell cross-lined nanoparticles.¹⁸³