POTENTIAL USE OF ^{105}Rh-S2P-Cx_BBN RADIOPHARMACUETICALS (X = 5 or 8) FOR PROSTATE CANCER RADIOTHERAPY

B.D. Ballard,[1] R. Kannan,[3] K.V. Katti,[2,3,4] T. Hoffman,[5] H.P. Engelbrecht,[2] C.S. Cutler,[2,3] S.S. Jurisson.[1]

[1]Chemistry, University of Missouri, Columbia, MO, United States; [2]University of Missouri Research Reactor, University of Missouri, Columbia, MO, United States; [3]Radiology, University of Missouri, Columbia, MO, United States; [4]Physics, University of Missouri, Columbia, MO, United States; [5]H.S. Truman VA Hospital, Columbia, MO, United States.

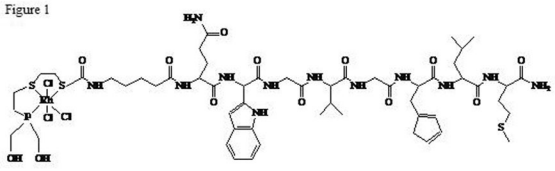
Gastrin releasing peptides (GRP) have been shown to be over expressed in small cell lung, prostate, breast, and pancreatic cancers. Of these pancreatic cancer is of great interest for targeting due to its low survival rate. It is estimated that there will be 31,800 deaths due to pancreatic cancer in the US in 2005 alone. The 14 amino acid peptide bombesin (BBN) and analogues have been shown selectively bind GRP receptors. In some cases the altered peptides have been shown to have a higher binding affinity than the native wild strain, which is isolated from the skins of the amphibian Bombina. The 9 amino acid analogue BBN(7-14) has been shown to be a powerful agonist to GRP receptors.

105Rh is a reactor-produced beta-emitting radionuclide with very appealing nuclear properties (t1/2= 35.5 h, βmax= 0.57 MeV, γ = 319 keV (19%)) for radiotherapy. Its medium beta decay energy has a 1.9 mm maximum range in H2O that will be useful for the homogeneous irradiation of medium sized tumors, while the 35.5 h half-life is sufficient for the production, shipping, labeling and in vivo localization of the labeled drug. The low abundance gamma emission is in the diagnostically useful range, allowing for dosimetry calculations. Rh-105 is readily produced in high specific activity (~10,000Ci/mmol of metal) and "no-carrier added" (NCA) levels by indirect (n,γ) activation of isotopically enriched 104Ru target. The 104Ru forms 105Ru, which beta decays to 105Rh. Alternatively, Rh-105 can be isolated from fission products once Curie quantities are required for clinical trails.

A very important advantage for the utilization of Rh-105 in radiopharmaceuticals is the high kinetic inertness of the low-spin d6 Rh(III) complexes. This property insures the in vivo stability of the complexes, a determining factor that enhances the suitability of the radiotherapeutic agent.

The dithiahydroxymethylphosphine (S2P) bifunctional chelate with different linker lengths (5 C and 8 C) between the metal chelate and the BBN(8-14) peptide were synthesized and characterized. The S2P-BBN(8-14) ligand was radiolabeled with 105Rh(III)-chloride (provided by MURR) in an aqueous ethanol solution by heating at 85oC fir 1 h. Reversed phase HPLC analysis showed the complexes formed with high radiochemical yield. Stability of the Rh-105 labeled S2P complexes was high (<25% degradation over 48 h). Animal studies (CF-1 mice) conducted with the dithiahydroxymethylphosphine 105Rh-S2P-5C-BBN(7-14) complex with the 5 carbon spacer (Figure 1) showed fast urinary clearance (77% ID in 1 hr) and low pancreatic uptake (mice express GRP receptors in the pancreas). The 8 carbon spacer S2P-8C-BBN(7-14) conjugate was synthesized to increase lipophilicity and slow down clearance. The synthesis, characterization, stability studies, IC50 values, and biodistribution data for both complexes in mice will be presented.

Figure 1

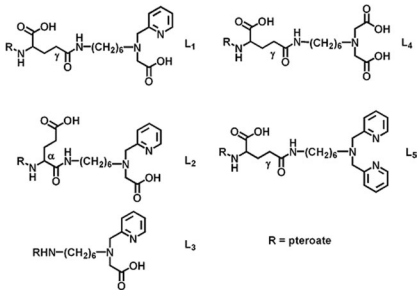


Keywords: Rhodium-105, Prostate Cancer, S2P

DEVELOPMENT OF NOVEL ORGANOMETALLIC Tc-99m AND Re-188 FOLATE AND PTEROATE DERIVATIVES FOR TARGETED RADIONUCLIDE DIAGNOSIS AND THERAPY

C. Mueller,[1] R. Schibli,[1,2] A.P. Schubiger.[1,2]
[1]Center for Radiopharmaceutical Sciences, Paul Scherrer Institute, Villigen, Switzerland; [2]Department of Chemistry and Applied Biosciences, ETH, Zurich, Switzerland.

Objectiv: The folate receptor (FR) is frequently overexpressed on tumor cells enabling targeted radionuclide diagnosis (and therapy) with pteroylglutamic acid (folic acid) derivatives. This work describes the synthesis and *in vitro/in vivo* characterization of organometallic folate and pteroate derivatives, site specifically modified with various chelating systems for tridentate complexation of the $M(CO)_3^+$-core (M = ^{99m}Tc, Re). **Methods**: The chelator-ligand systems, picolylamine monoacetic acid, iminodiacetic acid or bispyridylmethyl amine have been coupled via the γ-carboxyl- (**$L_{1,4,5}$**) or α-carboxyl group (**L_2**) of the glutamate moiety or directly (**L_3**) with pteroic acid (Figure). The derivatives and the corresponding $Re(CO)_3$-complexes (Re-**$L_{1\text{-}5}$**) were chemically characterized by means of ^{1}H-, ^{13}C-NMR, MS and IR. The $^{99m}Tc(CO)_3$-radiolabeling was carried out via the "Isolink™" method (^{99m}Tc-**$L_{1\text{-}5}$**). *In vitro*: The experiments were performed with KB cells (human cancer cell line, overexpressing the FR), cultured in folate deficient RPMI medium, as well as folate-dependent bacteria *(Enterococcus hirae)*. *In vivo*: Biodistribution studies were performed in athymic nude mice, bearing the KB-cell xenograft, fed with folate-free rodent chow. The mice were sacrificed 4h after intravenous administration of the $^{99m}Tc(CO)_3$-labeled folate or pteroate derivatives. **Results**: The organic and organometallic synthetic strategy resulted directly in uniform γ- and α-folate and pteroate conjugates in reasonable yields avoiding cumbersome purification steps. *In vitro*: The $^{99m}Tc(CO)_3$-folate and pteroate derivatives (^{99m}Tc-**$L_{1\text{-}5}$**) showed high and specific cell binding (~ 50% of total activity) and internalization (~ 16% of total activity). In presence of the folate derivatives (**$L_{1,\,2,\,4,\,5}$**) and the corresponding $Re(CO)_3$-complexes respectively, folate dependent bacteria showed significant growth. *In vivo*: Biodistribution studies showed a tumor uptake of ~ 1.9% ID/g, completely displacable with excess folic acid co-injected. Whereas all folate derivatives (^{99m}Tc-**$L_{1,\,2,\,4,\,5}$**) showed similar biodistribution data, the tumor uptake of the pteroate derivative (^{99m}Tc-**L_3**) was significantly lower (~ 0.4% ID/g).



Conclusion: The *in vitro* studies for comparison of the derivatives revealed the same binding properties regardless of the position of derivatization (^{99m}Tc-**$L_{1,2}$**) and overall charge of the complexes (^{99m}Tc-**$L_{1,4,5}$**). High specific binding to the FR was proved for all derivatives *in vitro* and *in vivo*. Moreover the derivatives revealed retained metabolic activity (vitamin), which is important in order to prevent development of resistant tumor cells. *In vivo* data showed a different biodistribution pattern for the pteroate derivative (^{99m}Tc-**L_3**) compared to the folate derivatives (^{99m}Tc-**$L_{1,2,4,5}$**). Thus we conclude that the glutamate moiety is not essential for FR-binding and vitamin activity, however it improves pharmacokinetics.

Keywords: Folate Radiopharmaceuticals, Technetium-99m-Tricarbonyl, Folate Receptor

COMPARISON OF PRETARGET AND CONVENTIONAL CC49 RADIOIMMUNOTHERAPY USING ^{149}Pm, ^{166}Ho AND ^{177}Lu

H. Mohsin,[1] G. Sivaguru,[2] F. Jia,[2] J.N. Bryan,[2] C.S. Cutler,[3] A.R. Ketring,[3] P.S. Athey,[4] J. Simon,[4] R.K. Frank,[4] L.J. Theodore,[5] D.B. Axworthy,[5] S.S. Jurisson,[1] M.R. Lewis.[2]

[1]Chemistry, University of Missouri-Columbia, Columbia, MO, United States; [2]Veterinary Medicine and Surgery, University of Missouri-Columbia, Columbia, MO, United States; [3]Research Reactor Center, University of Missouri-Columbia, Columbia, MO, United States; [4]The DOW Chemical Company, Freeport, TX, United States; [5]Altheon Pharmaceuticals, Seattle, WA, United States.

The radiolanthanides ^{149}Pm, ^{166}Ho and ^{177}Lu have nuclear properties suitable for radioimmunotherapy (RIT) of cancer. The therapeutic efficacies of radiolabeled DOTA-biotin, pretargeted by monoclonal antibody (mAb)-streptavidin fusion protein CC49 scFvSA, were compared to those of methoxy-DOTA (MeO-DOTA)-CC49 alone, using the three radiolanthanides in an animal model of human colon cancer.

Nude mice bearing LS174T colon tumors were injected sequentially with CC49 scFvSA, the blood clearing agent biotin-$GalNAc_{16}$, and ^{149}Pm-, ^{166}Ho- or ^{177}Lu-DOTA-biotin. Tumor-bearing mice were alternatively administered ^{149}Pm-, ^{166}Ho- or ^{177}Lu-MeO-DOTA-CC49. After therapy with radiolanthanide-labeled pretargeted biotin or CC49, tumor volumes were measured over a period of 6 months. Median time to tumor progression was determined by Kaplan-Meier log-rank analysis using SPSS 12.0.1 (Chicago, IL).

We previously determined that time to progression to a 1-g tumor burden (Table 1) was the statistically most relevant endpoint for this mouse model of cancer (1). Therapy with pretargeted ^{149}Pm-, ^{166}Ho- and ^{177}Lu-DOTA-biotin increased the median time to progression to a 1-g tumor to 50, 41 and 50 days post-treatment, respectively. Therapy with ^{149}Pm-, ^{166}Ho- and ^{177}Lu-MeO-DOTA-CC49 increased the median time to progression to 53, 24 and 67 days post-treatment, respectively. In contrast, saline controls showed a median time to progression of 13 days post-injection. Treatment with pretargeted ^{149}Pm-, ^{166}Ho- and ^{177}Lu-biotin or ^{149}Pm-, ^{166}Ho- and ^{177}Lu-CC49 increased tumor doubling time to 18-36 days, compared to 3 days for saline controls. Among treated mice, 23% survived >84 days post-therapy and 9% survived 6 months, but controls survived <29 days. At necropsy, long-term survivors showed evidence of extensive tumor necrosis (80-90%). Conversely, each mass consisted of 10-20% viable tumor cells exhibiting mitotic rates of 8-10 or 10-12 per 400x field. However, all long-term survivors experienced partial regression or stable disease, suggesting that, in spite of the relatively high mitotic rates, the tumor cell-loss factor substantially outweighed the degree of proliferation. No evidence of non-target tissue toxicity was found at necropsy.

Table 1. Median time to progression (days) to a 1-g tumor for LS174T-bearing mice (n = 8-10) receiving Pretarget or Conventional RIT using ^{149}Pm, ^{166}Ho and ^{177}Lu.

Radiolanthanide	Pretarget RIT	Conventional RIT
^{149}Pm	50	53
^{166}Ho	41	24
^{177}Lu	50	67

Both Pretarget and conventional RIT demonstrated considerable efficacy in an extremely aggressive animal model of cancer. Median time to progression was statistically identical for ^{149}Pm and ^{177}Lu Pretarget RIT, but ^{177}Lu afforded superior efficacy for conventional RIT ($p < 0.05$). Therefore, ^{177}Lu was selected as an optimal radiolanthanide for future evaluation of these agents in toxicity and multiple-dose therapy studies.

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Keywords: Radiolanthanides, Pretargeting, Radioimmunoherapy

FDA APPROVAL OF NEW DRUG APPLICATION (NDA) FOR FLUDEOXYGLUCOSE F18 INJECTION (FDG)

S. Vallabhajosula, P.J. Kothari, S.J. Goldsmith.
Department of Radiology, Citigroup Biomedical Imaging Center, Weill Medical College of Cornell University, New York, NY, United States.

In 1994, The Food and Drug Administration (FDA) approved the first New Drug Application (NDA) for 2-deoxy-2-[18F]fluoro-D-g1ucose (FDG) submitted by the Methodist Medical Center in Peoria, Illinois. Since then, there has been significant controversy about FDA approval for PET drugs. The FDA Modernization Act of 1997 directs FDA to regulate PET drugs. As a result, FDA has developed guidelines for appropriate procedures for the approval of PET drugs as well as current good manufacturing practice (CGMP) requirements. The United States Pharmacopeia (USP) contains standards that are of significant regulatory importance for PET drugs. Currently, FDA considers a compounded PET drug as adulterated unless it is produced in compliance with USP compounding standards and official monographs for PET drugs. Based on this information, we have formally submitted NDA for Fludeoxyglucose F18 injection (as per USP) synthesized at Citygroup Biomedical Imaging center (CBIC) of Weill Medical College of Cornell University.

FDG was synthesized using GE TracerLab MXFDG and a sterile disposable kit which was designed to perform base hydrolysis on a solid support. The kit assembly and the sampling for QC were performed in a laminar flow hood. The quality control of each batch of FDG preparation was tested based on the official monograph in the USP. Sterility and pyrogenicity tests were performed in house. Three batches were tested for stability up to 24 hours. NDA was submitted on March 10th 2004.

FDG is provided as a ready to use isotonic, sterile, pyrogen free, clear, colorless citrate buffered solution. Each mL contains between 0.37 to 3.7 GBq (10.0-100 mCi) at the end of synthesis (EOS), 4.5 mg of sodium chloride and 7.2 mg of citrate ions. The FDG solution (16 mL) is packaged in a multiple-dose glass vial and does not contain any preservative. FDG is stable up to 12 hours. Quality control tests and acceptable limits are shown in Table. In addition, control of various components including acceptance testing of components and segregation of materials used for FDG synthesis was carefully documented. The microbiological evaluation of the aseptic environment in the Laminar flow hood used in the production and QC labs was performed using Trypticase soy agar plates.

The FDA inspectors from Federal and Local offices inspected the facility in June 2004. Based on FDA recommendations, minor modifications were made to the original NDA. FDA formally approved the NDA in August 2004 for imaging studies to assess abnormal glucose metabolism in the evaluation of malignancy, coronary artery disease and left ventricular dysfunction, and foci of epileptic seizures. The guidelines developed by FDA for the CGMP requirements do specify the minimum standards that are needed for PET drug production at all types of PET centers. However, the FDMA directs that the USP standards and monographs be used for quality standards until the FDA implements final regulations for PET cGMPs.

Test	Acceptance criteria	Typical batch: FDG040803
Radionuclidic identity	T1/2 = 105 – 115 min	110.6 min
Radiochemical identity and purity	Rf of [18F]FDG ± 10% Rf of Std.	98.75%
Radinuclidic purity	99.5% gamma photons correspond to 511 KeV	511 KeV only
Assay (mCi/mL)	4 – 90 mCi/mL	40.5 mCi/mL
PH	5.0 – 7.5	6.0
Test for kryptofix 222	< 50.0 micro g/mL	< 50 micro g/mL
Test for residual solvents	Acetonitrile = <0.04%	Acetonitrile = 0.0005%
Membrane filter integrity test	Pressure of test filter > Pressure minimum acceptable	Pass
Bacterial endotoxins (LAL)	< 11.6 EU/ml	<0.05 EU/mL
Sterility test	Meets the requirements of USP	Test Started

Keywords: FDG, FDA, NDA

VALIDATION OF PET RADIO-SYNTHESIS SYSTEMS

C. Steel, D. Turton, E. Liow, P. Crosby, A. O'Brien, S. Luthra.
Hammersmith IMANET, GE Healthcare, London, United Kingdom.

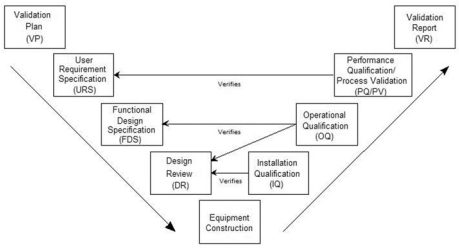
Validation of automated process equipment used for the preparation of positron labeled radiopharmaceuticals is becoming an increasingly important requirement for attaining regulatory compliance in the UK. Greater emphasis upon the preparation of PET radioligands under stringent GMP conditions is also increasing the need to provide full validation documentation for all automated production systems.

Hammersmith IMANET has generated a generic document framework for the validation of in-house constructed systems. These documents have been used to satisfy IMANET QA requirements, UK regulatory bodies and external pharma audits. The validation package has been developed to capture a typical R&D chemistry project and track progress from conception through to pre-clinical evaluation.

The validation framework is based upon a typical 'V' Validation lifecycle (Figure 1). An overview of a typical validation package is given in Figure 2. The poster will present a more detailed overview of information and tests included in each component of the documentation.

Figure 1. Basic 'V' Validation lifecycle. Figure 2. Overview of a typical validation package.

Keywords: Validation, Automation, GMP



Document Title	Purpose/Content
• Validation Master Plan	• Outline of proposed validation activities and procedures.
• User Requirement Specification	• Description of what the equipment is supposed to do including any constraints and specific regulatory requirements.
• Functional Design Specification	• Description of the equipment function covering what the system will do. Includes hardware/software design considerations, process flow/flow diagrams and service requirements.
• Design Review	• Review of detailed engineering schematics hardware/software lists prior to equipment construction. Information compiled during the design review is used to create a technical file for each system.
• **CONSTRUCTION**	• **EQUIPMENT TESTING**
• Installation Qualification	• Documented verification that the system is installed according to the required specifications. Includes testing/ verification of equipment assembly, equipment installation, service connections and hardware integration.
• Operation Qualification	• Documented verification that the system operates according to the required specifications. Includes testing/ verification of support equipment (*e.g.* chromatography system), control system/ program sequence, all hardware components (*e.g.* valves, heaters, syringe drives *etc*) and radioactive synthesis verification.
• Performance Qualification/ Process Validation	• Documentated verification that the system can control and perform the required process according to required specifications. Includes testing/ verification of compound stability, chemical and radiochemical purity and sterility.
• Validation Report	• Summary document that reviews all the stages of the validation against the Validation Plan and provides final verification that a system has been validated.