CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: 021012

PHARMACOLOGY REVIEW(S)

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

Division of Medical Imaging and Radiopharmaceutical Drug Products
HFD-160.

Reviewer: **David E. Bailey, Ph.D.** MAY 20 1999

Review: **#2**

NDA Number: **21-012**

Submissions Reviewed:

Designation	Letter Date	Stamp Date	# Volumes	Contents
BZ	21-JAN-1999	22-JAN-1999	5	Resubmission
BM	05-FEB-1999	08-FEB-1999	3	Resubmission
BL	12-FEB-1999	16-FEB-1999	1	Labeling

Type of Submission: Resubmission of NDA

Information to Sponsor: Yes ( ) No (XX)

Completion Date: Draft Date: May 20, 1999
Final Date: 5/20/99

Sponsor: Diatide, Inc
9 Delta Drive
Londonderry, NH 03053
603-437-8970

Representative: Mr. J. Kris Piper
Senior Director Regulatory Affairs

Manufacturer:

Depreotide trifluoroacetate [redacted]

Drug Name: Kit for Preparation of Technicium Tc99m Depreotide

Chemical Name: Cyclo(-L-homocysteinyl-N-methyl-L-phenylalanyl-L-tyrosyl-D-tryptophyl-L-lysyl-L-valyl), (1→1')-sulfide with 3-[(mercaptoacetyl)amino]-L-alanyl-L-lysyl-L-cysteinyl-L-lysine amide, trifluoroacetate salt

CAS Registry Number: 161982-62-3

Molecular Weight: 1356.7

Relevant Other IND [redacted]
DMF [redacted]

Drug Class: Radiopharmaceutical Imaging Agent

Indication: Diagnostic agent for scintigraphic imaging of malignant tumors in the lung.

Clinical Formulation:

P829 Peptide Trifluoroacetate	**50 μg**
Tin(II) Chloride Dihydrate	**50 μg**
Sodium Glucoheptonate Dihydrate	**5 mg**
Disodium Edetate, Dihydrate	**100 μg**
Sterile Water for Injection	**qs to 2.0 mL**

Clinical Route of Administraion: Intravenous

Clinical Dose: 1 μg peptide/kg body weight

Introduction/Drug History:

The original submission of this NDA was approvable for nonclinical pharmacology and toxicology except for dosing solution data for six studies. In the approvable letter the division requested formulation records to document the content of reconstituted technetium Tc 99m P827 in the dosing solutions for the six pivotal GLP nonclinical studies of question. In the current submission, the Sponsor has provided the formulation records as requested, and the review of those records will be the substance of this review.

REVIEW:

Formulations records were reviewed for the following studies:

R4.50 A single-dose two-day and two-week toxicity study of P829 adminsitered by intravenous injection to rabbits.

R4.52 Acute intravenous toxicity study (in mice).

R4.53 A fourteen-day repeated dose toxicity study of P829 in Sprague-Dawley rats.

R4.54 A two-week study of P829 administered by intravenous injection to rats.

R4.56 A fourteen-day repeated dose toxicity study of P829 in New Zealand White rabbits.

R4.57 A two-week toxicity study of P829 administered by intravenous injection to rabbits.

THIS REVIEWER'S COMMENT:

The Sponsor submitted copies of the formulations records for the GLP studies listed above. Each document was carefully reviewed for presence and consistency of the following entries: Study number, dose group, date, lot number of test material, concentration, formulations calculations, technician signature and date, verifier signature and date, QA data audit signature and date, and calculation check and date.

In every case, formulations records support the accurate preparation and administration of the test material in the studies listed above.

APPEARS THIS WAY
ON ORIGINAL

SUMMARY:

The Sponsor has provided the nonclinical pharmacology and toxicology information which was requested in the approvable letter, and the Sponsor's response is adequate.

CONCLUSION:

For nonclinical pharmacology and toxicology this NDA is recommended for approval, with the suggested changes to the labeling.

REVIEWER'S SIGNATURE:

/S/

David E. Bailey, Ph.D.

May 20, 1999

Date

TEAM LEADER CONCURRENCE:

/S/

Nakissa Sadrieh, Ph.D.

5/20/99

Date

APPEARS THIS WAY ON ORIGINAL

CC:

ORIGINAL, NDA#21-012
HFD-160
HFD-160/PHARM/BAILEYD/SADRIEHN
HFD-160/CSO/FERREC
HFD-160/MO/LOEWKES
HFD-160/CHEM/HARIPINHALLIR

Appendix: Labeling Review.
Draft Date: May 20, 1999
Final Date: 5/20/99
Addendum: None

1 Page Redacted

DRAFT LABELING

HFD-160/Meyers

01. REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

Division of Medical Imaging and Radiopharmaceutical Drug Products
HFD-160.

Reviewers: **David E. Bailey, Ph.D.**
Adebayo Laniyonu, Ph. D.

Review # 1

02. Electronic File Number:

03. NDA Number: **21-012**

04. Serial Number: **000**

Date Received: June 16, 1998

Type of Submission: N, Original Submission

05. Information to Sponsor: Yes (X) No ( )

06. Completion Date: Draft Date: September 5, 1998
Revision Date: October 2, 1998
Final Date: November 16, 1998

07. Sponsor: Diatide, Inc
9 Delta Drive
Londonderry, NH 03053
603-437-8970

Representative: Mr. J. Kris Piper
Senior Director Regulatory Affairs

08. Manufacturer (drug substance/drug product):

Depreotide trifluoroacetate: [redacted]

09. Drug Name: Kit for Preparation of Technetium Tc99m Depreotide

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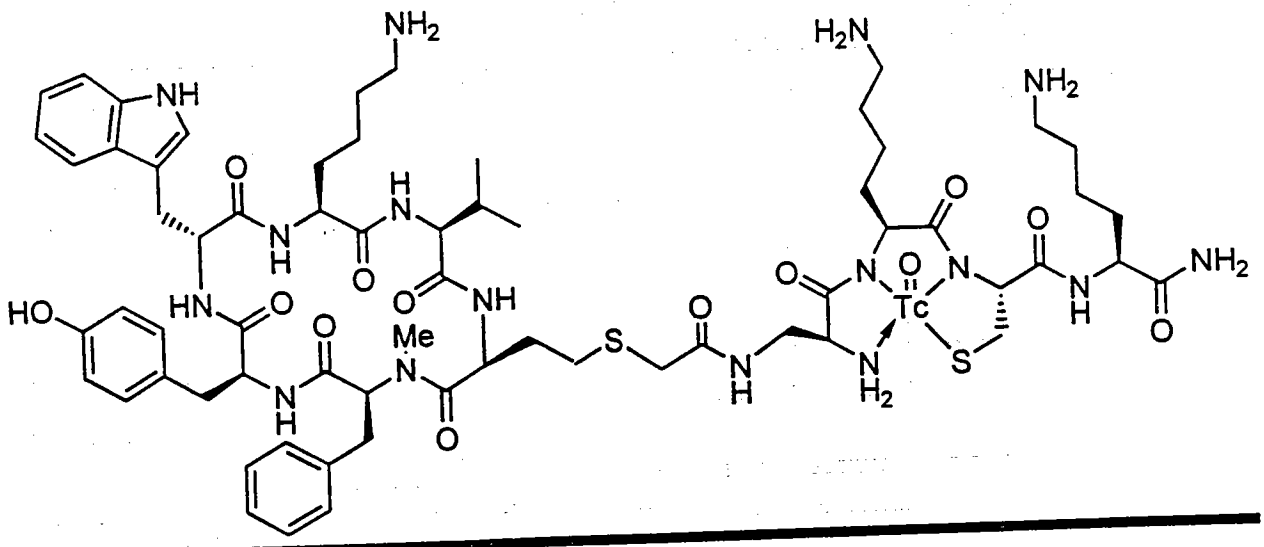
10. Chemical Name:
Cyclo(-L-homocysteinyl-N-methyl-L-phenylalanyl-L-tyrosyl-D-tryptophyl-L-lysyl-L-valyl), (1→1')-sulfide with 3-[(mercaptoacetyl)amino]-L-alanyl-L-lysyl-L-cysteinyl-L-lysine amide, trifluoroacetate salt

11. CAS Registry Number: 161982-62-3

12. Structure: **(Taken From Sponsor's Submission)**

TcO - P829 Complex



13. Molecular Weight: 1356.7

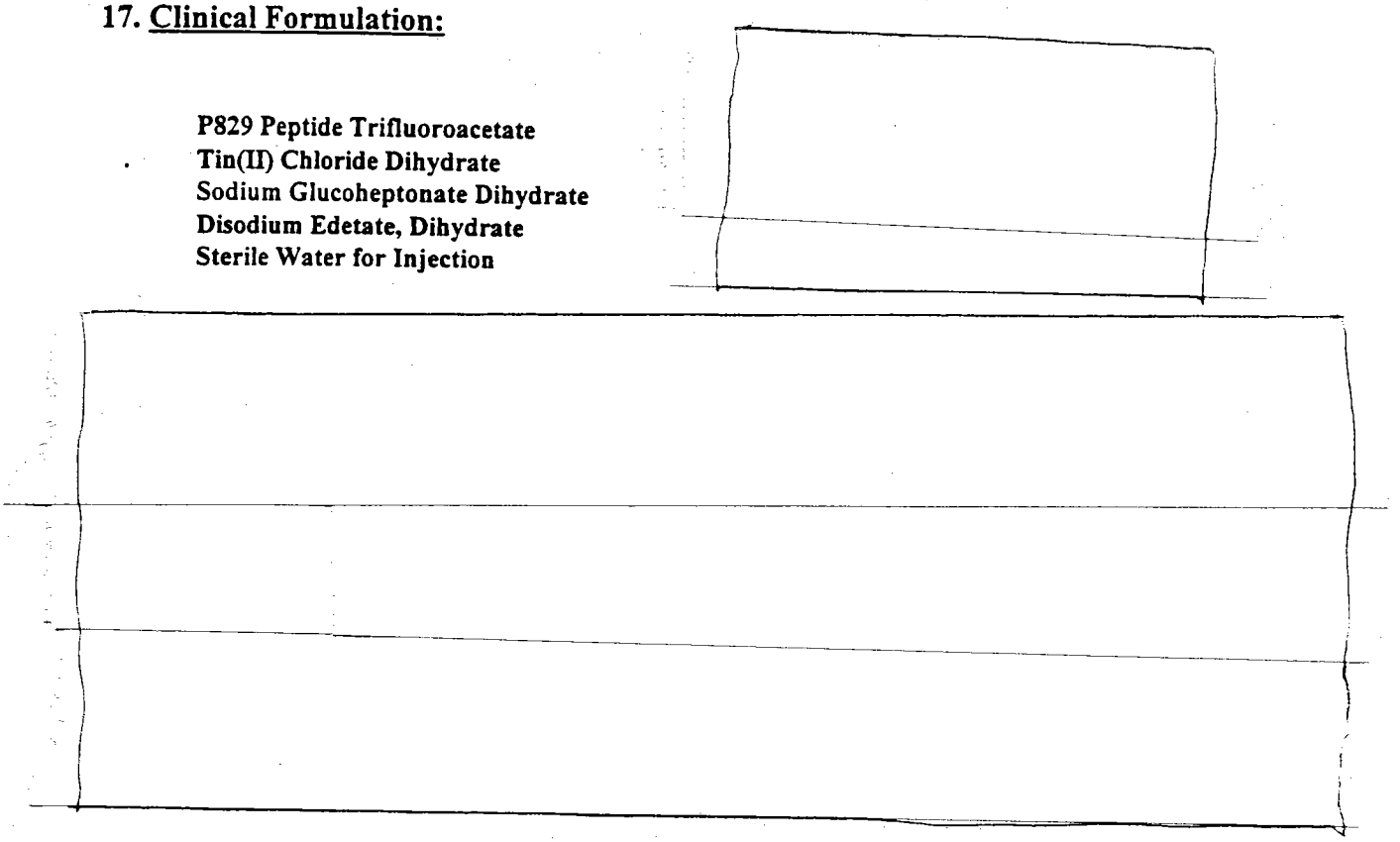
14. Relevant Other IND:
DMF:

15. Drug Class: Radiopharmaceutical Imaging Agent

16. Indication: Diagnostic agent indicated as a scintigraphic aid in the detection of lung tumors bearing somatostatin receptors.

17. Clinical Formulation:

P829 Peptide Trifluoroacetate
Tin(II) Chloride Dihydrate
Sodium Glucoheptonate Dihydrate
Disodium Edetate, Dihydrate
Sterile Water for Injection



18. Clinical Route of Administration: Intravenous

19. Clinical Dose: 1 μg peptide/kg body weight

20. Studies Reviewed Within This Submission: (Alpha and numeric designations of the studies are those used by the Sponsor in this submission)

Pharmacology Studies

PUBLICATION: Preclinical evaluation of technetium-99m-labeled somatostatin receptor binding peptides.

R4.6. The binding of technetium Tc 99m P829 to human tumor cell lines.

R4.68 Somatostatin receptor subtype specificity and in vivo binding properties of a novel tumor tracer, ^{99m}Tc-P829.

R4.41 The inhibition of ^{125}I-somatostatin binding to tumor membrane somatostatin receptors by P829 and P875.

R 4.73. The binding affinity of technetium Tc 99m P829 with radiochemical [redacted] for somatostatin receptors in AR42J tumor membranes.

R4.79. The binding of the syn and anti isomers of technetium Tc 99 P829 to somatostatin receptors in AR42J tumor membranes.

R4.81. The biodistribution of technetium Tc 99m P829 with radiochemical [redacted] in the AR42J tumor xenograft model.

R4.80. The biodistribution of the syn and anti isomers of technetium Tc 99m P829 in the AR42J tumor xenograft model.

R4.82. Tumor uptake of technetium Tc 99m P829 injection and the syn and anti isomers in Lewis rats with CA20948 pancreatic tumors.

R4.28. The binding of technetium Tc 99m P829 to somatostatin receptors in human tumor membranes.

R4.83. The binding of technetium Tc 99m P829 to somatostatin receptors in human microvascular endothelial cell membranes.

R4.67. Effect of P829 and somatostatin on arginine-induced glucagon release in male rats.

Pharmacokinetic Studies:

R4.38. Distribution of technetium Tc 99m P829 between human blood components in vitro.

R4.27. Pharmacokinetics and biodistribution of technetium Tc 99m P829 injection in male and female rats.

R4.74. Pharmacokinetics and biodistribution of technetium Tc 99m P829 injection in Sprague-Dawley rats with experimental renal dysfunction.

R4.77 Biodistribution and pharmacokinetics of technetium Tc 99m P829 injection in the New Zealand White rabbit.

R4.71. Pharmacokinetics, distribution, metabolism and elimination of technetium Tc 99m P829 injection in the Rhesus monkey.

R4.69. Pharmacokinetics and biodistribution of [^{3}H-Tyr]P829 in male Sprague-Dawley rats.

R4.26. An evaluation of the metabolism of technetium Tc 99m P829 in the Sprague-Dawley rat.

R4.84 Incubation of P829 peptide in human plasma and rat plasma.

Single Dose Toxicology Studies

R4.52 Acute intravenous toxicity study (in mice).

R4.50 A single-dose two-day and two-week toxicity study of P829 administered by intravenous injection to rabbits.

Repeat Dose Toxicology Studies

R4.51 A ten-day repeated dose toxicity study of P829 in Sprague-Dawley rats.

R4.53 A fourteen-day repeated dose toxicity study of P829 in Sprague-Dawley rats.

R4.54 A two-week toxicity study of P829 administered by intravenous injection to rats.

R4.55. A ten day repeated dose toxicity study of P829 in New Zealand White rabbits.

R4.56 A fourteen day repeated dose toxicity study of P829 in New Zealand White rabbits.

R4.57 A two-week toxicity study of P829 administered by intravenous injection to rabbits.

Genotoxicity Studies

R4.63 Mutagenicity test with peptide P829 in the Salmonella - Escherichia coli/mammalian-microsome reverse mutation assay preincubation method with a confirmatory assay.

R4.64 Mutagenicity test with kit for the preparation of technetium Tc 99m in the Salmonella - Escherichia coli/mammalian-microsome reverse mutation assay preincubation method with a confirmatory assay.

R4.65 Mutagenicity test on "kit for the preparation of Technicium Tc 99M depreotide" (mock labeled) in the L5178Y TK $^{+/-}$ mouse lymphoma forward mutation assay with a confirmatory assay.

R4.66 Mutagenicity test with kit for the preparation of technetium Tc 99m in an in vivo mouse micronucleus assay.

Special Toxicology Studies

R4.23 An evaluation of the compatibility of technetium Tc 99m P829 injection prepared with decayed generator eluate with human blood or serum.

R4.59 Systemic antigenicity study in the guinea pig.

R4.58 Perivascular irritation test.

21. Studies Not Reviewed Within This Submission: None

22. Disclaimer - Use of Sponsor's Material:

Any direct use of Sponsor's material in this review is identified by italics.

23. Introduction/Drug History:

P829 is a synthetic 10 amino acid peptide comprised of a linear tetrapeptide attached to the side-chain of one of the amino acid residues of a cyclic hexapeptide. The cyclic hexapeptide domain contains the pharmacophore tyrosine-D-tryptophan-lysine-valine that binds to the somatostatin receptors. Somatostatin receptors have been identified in the central nervous system, the pituitary, pancreas, and the gastrointestinal tract. These receptors are known to be hyperexpressed by well-differentiated tumors and their metastases. Such expression of somatostatin receptor has been used as basis for diagnosis and therapy. The sponsor surmises that the high affinity binding of the technetium Tc 99m P829 will allow for the scintigraphic imaging of tumors in the lung. Technetium Tc 99m P829 is present in two isomeric forms; syn and anti, at a ratio of syn/anti = 0.074. Both isomers are believed to bind to somatostatin receptors, with the anti isomer demonstrating higher binding affinity.

In the studies summarized in this review, unless otherwise noted, the test article is Kit for the Preparation of Technetium Tc 99m P829 reconstituted with radioactive or decayed generator eluate, incubated in a boiling water bath for 10 minutes to form Technetium Tc 99m P829 Injection. The Techentium Tc 99m P829 Injection prepared in such a manner is referred to as Tc 99m P829 Injection. The alpha-numeric identification of the peptide component, P829, is synonymous with the USAN name depreotide.

24. Previous Clinical Experience: None

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ON ORIGINAL

25. PHARMACOLOGY STUDIES:

PUBLICATION: Preclinical Evaluation of Technetium-99m-Labeled Somatostatin Receptor Binding Peptides. Vallabhajosula, S., Moyer, B.R., Lister-James, J., McBride,B.J., Lipszyc, H., Lee, H., Bastidas, D., and Dean, R., *J. Nucl. Med.* 37(6):1016-1022. (1996)

Design: The in vitro receptor binding affinity, in vivo tumor uptake and biodistribution of Technetium Tc 99m P829 are described in this study. In vitro somatostatin receptor binding affinities of P829 and its oxorhenium complex (ReO-P829) were determined in an in vitro inhibition assay using AR42J rat pancreatic tumor cell membranes with ^{125}I-[Tyr^3]-somatotostatin-14 as the probe. In vivo single and dual tracer studies of Technetium Tc 99m P829 and ^{111}In-[DPTA]octreotide were conducted using Lewis rats bearing CA20948 rat pancreatic tumor implants.

Results: The in vitro inhibition constants (K_i) obtained for P829 peptide and ReO-P829 are 10 and 0.32 nM, respectively. The ReO-P829 has an SSTR binding affinity that is an order of magnitude higher than that of the peptide. In contrast, DPTA-octreotide and the In-DPTA-octreotide have equivalent K_i values of 1.6 nm and 1.2, nm respectively. ^{99m}Tc-P829 uptake in the in vivo tumor model was greater than ^{111}In-[DPTA]octreotide, with values of 4.8 and 2.8 %ID/g, respectively. The tumor:blood and tumor:muscle ratios were comparable between the Technetium Tc 99m P829 (tumor:blood = 21; tumor:muscle = 68) and ^{111}In-[DPTA] octreotide (tumor:blood = 22; tumor:muscle = 64). Gamma camera imaging of CA20948 tumor bearing rats at 90 minutes after injection clearly demonstrated tumor uptake. Tumor uptake was influenced by the specific activity of the radiolabeled P829, where co-administration of cold P829 dramatically decreased uptake by the tumor and pancreas in the CA20948-tumor bearing rat. Gamma camera images of Technetium Tc 99m P829 in the rabbit demonstrated substantial uptake of 30% in the kidneys, 20% accumulation in the urinary bladder and less than 5% in the gastrointestinal tract.

Sponsor's Conclusion: ^{99m}Tc-P829 has demonstrated high SSTR-binding affinity and high, receptor-specific and saturable in vivo tumor uptake.

Reviewer's Comment: Agree. There are several important findings in this comprehensive study: Both Tc 99m P829 and In-DPTA octreotide exhibited comparable SSTR binding affinities indicating that Tc 99m P829 like labeled octreotide, could be used to investigate somatostatin receptor expression. However, whereas, both DPTA-octreotide and ^{111}In-[DPTA]octreotide have equivalent affinities, ReO P829 has demonstrated a greater binding affinity than P829 peptide. From a clinical perspective, the differences in affinity raise the possibility that co-injected P829 peptide will compete poorly with the ^{99m}Tc-P829 complexes for the somatostatin receptors thus allowing for the use of readily achievable specific activity. Uptake was greater in the tumor than in blood or muscle. The tumor uptake of ^{99m}Tc-P829 and P829 was saturable and similar although the sponsor claimed that uptake was greater for ^{99m}Tc-P829. Gamma camera images showing

localization of tumor and uptake by some internal organs such as the kidneys, bladder and gastrointestinal tract is indicative of pre-clinical efficacy and the fact that both the kidneys and bladder are the major organs for biodistribution.

R 4.6. The Binding of Technetium Tc 99m P829 to Human Tumor Cell Lines. Laboratory, Diatide, Inc. Report Date, January 14, 1998. Lot # 9609B01 (Final Formulation). Report in Volume 1.11, pp 86-108.

Design: Various human tumor cell lines were surveyed for specific, high affinity Technetium Tc 99m P829 binding sites. Immortalized cell lines representing various classes of tumors were chosen, including; breast cancer, T-cell, Burkitt's lymphoma, Hodgkin's lymphoma, small cell lung cancer (SCLC) , non-small cell lung cancer (NSCLC), melanoma, pancreatic and colon cancer. Tumor cell lines were obtained from American Type Culture Collection, Rockville, MD, and were cultured in the appropriate media. Membrane preparations were prepared by standard procedures. Nonspecific binding was determined in the presence of 1μM somatostatin and 1μM P829 peptide. Saturation isotherms were evaluated at 6 concentrations of Techentium Tc 99m P829, ranging from 10 pM to 2 nM. In instances were the receptor concentration is below 20 fmol/mg protein (MDA-MB-157, T-47D, ZR-75-1), an assumed Kd value of 0.6 nM was used to estimate Bmax.

Results: Of the 20 cell lines assayed, 9 were found to have high affinity binding sites for Technetium Tc 99m P829. Technetium Tc 99m P829 binds with high affinity to somatostatin receptors on membranes of cell lines derived from human breast cancer (MDA-MB-157, Bmax=81 fmol/mg protein; T-47D, 14 fmol/mg; ZR-75-1, 13 fmol/mg), SCLC (NCI-H69, 888 fmol/mg, Kd = 0.9 nM), NSCLC (ChaGo K-1, 21 fmol/mg), Burkitt's lymphoma (EB-1, 31 fmol/mg, Kd = 0.5 nM); Hodgkin's lymphoma (HS-445, 200 fmol/mg, Kd = 2.0 nM), colon cancer (Colo-320, 10 fmol/mg, Kd = 3.5 nM) and pancreatic cancer (MIA PaCa2, 9 fmol/mg).

Sponsor's Conclusions: The results indicate that Technetium Tc 99m P829 binds with high affinity to cell lines derived from human breast, SCLC, NSCLC, lymphoma, colon and pancreatic cancers. Technetium Tc 99m P829 may target classes of cancer for which immortalized cell lines are not predictive.

Reviewer's Comment: I agree with the sponsor that technetium Tc 99m P829 binds with high affinity to somatostatin receptors on some **(not all)** cell lines derived from SCLC, human breast, Burkitt's and Hodgkins lymphoma. In this study technetium Tc 99m P829 bound with greater affinity (888 fmol/mg) to the small cell lung cancer cell line (NCI-H69) than to any of the other cell lines. However, low receptor concentration (< 20 fmol/mg protein) in some of the cell lines studied (human breast cell lines and pancreas) made unequivocal conclusions difficult since Bmax was estimated from a single concentration of ligand using an assumed K_d . T-cell lymphoma H-9, human breast (MCF-7), colon (HT-29 and DLD-1), NSCLC (SW 900, Calu-3),

SCLC (NCI-H146) and all the melanoma cell lines examined did not reveal the presence of somatostatin receptor. Whether the lack of expression of somatostatin receptor by some of the cell lines resulted from true absence of somatostatin receptors, or to decreases in the expression of the receptors during passage or to the culture media conditions used for the experiments could not be determined from the study.

R4.68 Somatostatin Receptor Subtype Specificity and in vivo binding properties of a novel tumor tracer, ^{99m}Tc-P829. Irene Virgolini, Department of Nuclear Medicine, University of Vienna, Austria. Accepted for Publication in Cancer Research. Report in Volume 1.11, pp 109-153.

Design: This study was designed to characterize the binding properties, including SSTR subtype specificity, of Technetium Tc 99m P829 in primary human tumors including carcinoids, breast cancers, intestinal adenocarcinomas, pheochromocytomas, small cell and non-small cell lung cancer, and melanomas) and COS7 cell transfected with SSTR subtypes 1, 2, 3, 4 and 5. Primary tumor specimens were obtained at surgery and membrane fractions were prepared by established methods. Tumor cell lines were obtained from American Type Culture Collection, Rockville, MD, Nigata University, Nigata, Japan, and Mayo Clinic, Rochester, MN. Plasmids containing the receptor cDNAs for the human somatostatin receptors were transfected into COS7 cells. Both saturation and displacement studies were conducted to evaluate the expression of Tc 99m P829 receptors on tumor cells. All saturation studies were performed under steady state conditions. In the saturation experiments, the intact cells or the membrane fractions were incubated with increasing concentrations of Tc 99m P829 Injection in the absence and the presence of unlabeled P829 peptide, SST-14 or ReO-P829. In the displacement experiments, the intact cells or the membrane fractions were incubated with Tc 99m P829 Injection in the absence and in the presence of increasing concentrations of the unlabeled ligands; P829, ReO-P829, Tyr^3-OCT, SST-14, and vasoactive intestinal peptide (VIP). Specific binding was determined as the difference between total and nonspecific binding. Binding data were analyzed according to Scatchard.

In vivo Tc 99m P829 scintigraphy imaging was performed in 8 breast tumor and 6 melanoma patients. Some of these patients were also imaged for ^{111}In-OCT. Whole body and planer scanning as well as SPECT studies were performed within 24 hours after the injection of Tc 99m P829.

Results: Tc 99m P829 demonstrated saturable binding to somatostatin receptors subtypes 2, 3, and 5 in transfected COS7 cells. Unlabeled P829/[ReO]-P829, Tyr^3-octreotide and somatostatin-14 were able to displace Tc 99m P829 from these somatostatin receptor types 2, 3, and 5. Positive binding results were obtained for a number of cell lines breast-cancer cell line T47D, BT20, ZR75-1; colonic-adeno carcinoma cell line HT 29; melanoma cell line 518A2; basophil cell line KU812; mast cell line HMC-1 and epidermoid carcinoma cell line A431. MCF-7 breast cancer cell line did not demonstrate Tc 99m P829 binding sites. The specifically bound Tc 99m

P829 was readily displaceable by unlabeled P829, ReO-P829, SST-14, Tyr^3-OCT and VIP for most cell lines. Tc 99m P829 was bound on tumor membrane preparations in a saturable manner, indicative of specific binding sites. Scatchard analysis revealed high affinity binding sites. Binding was displaceable by somatostatin-14, Tyr^3-octreotide, P829 ReO-P829, and VIP.

Patients examined for in vivo binding of Tc 99m P829 to primary tumors or tumor metastases, scintigraphy revealed in vivo binding in 8/8 breast tumor, and 6/6 melanoma patients.

Sponsor's Conclusions: The results show that Technetium Tc 99m P829 binds with high affinity to many types of primary and immortal tumor cells. Furthermore, the results identify the SSTR2, SSTR3 (VIP-acceptor) and SSTR5 as the target receptors. Since these receptors are frequently expressed at high levels on primary tumor cells, Technetium Tc 99m P829 appears to be a novel peptide tracer for tumor imaging.

Reviewer's Comment: Agree. This study identifies the human somatostatin receptors hSSTR2, hSSTR3 and hSSTR5 as the technetium Tc 99m P829 binding sites. A variety of tumors expressed significantly higher SST/VIP binding sites than peripheral blood cells or tissues. The binding of Tc 99m P829 was displaceable by VIP presumably via the hSSTR3 receptor. This raises the possibility that injected Tc 99m P829 might demonstrate activity at VIP sites in vivo. It is therefore necessary to investigate in preclinical safety studies potential eVIP-mediated effects such as direct effects on pulmonary vasculature resulting in pulmonary hypertension, and effects on water and electrolyte balance that may produce diarrhea. Clinical studies indicated that Tc 99m P829 can reveal primary or metastatic tumor sites in breast tumor and melanoma patients.

R4.41 The Inhibition of ^{125}I-Somatostatin Binding to Tumor Membrane Somatostatin Receptors by P829 and P875. Laboratory, Diatide, Inc. Report Date, March 30, 1998. Lot # 516264 (Original Formulation). Report in Volume 1.11, pp 154-164.

Design: This study was designed to determine the affinity of P829 and P875, the oxorhenium complex of P829 (ReO-P829) for somatostatin receptors expressed in tumor plasma membranes. The concentration of each peptide that inhibited the binding of ^{125}I-somatostatin to AR42J tumor and NCI-H69 tumor cell membranes by 50% (IC_{50}) was determined. The binding assay (250 μL) consisted of tumor membranes (50 μg or 200 μg of membrane protein for the AR42J and NCI-H69 assays, respectively), 0.1 μCi of ^{125}I-somatostatin and unlabeled peptide in assay buffer. Total and nonspecific binding of ^{125}I-Somatostatin to the plasma membranes were determined in the absence and presence of somatostatin (10 μM). The inhibition of ^{125}I-somatostatin binding to plasma membranes was determined at final concentrations from 100 to 0.1 nM unlabeled peptide in three separate experiments. A control experiment was conducted with unlabeled somatostatin-14 using the AR42J tumor membranes. Binding to the tumor and tumor cell membranes was determined in the absence and presence of a saturating concentration of 10 μM somatostatin to define 100% inhibition. The specific binding and percent inhibition were calculated at each concentration of unlabeled ReO-P829 and P829 peptides. The data were plotted as the percent

inhibition against the concentration of unlabeled ReO-P829 and P829 peptides. The data points were fitted using a Stineman function to obtain smooth curves using the [redacted] software package. The IC_{50} was interpolated for each of three separate experiments for both P829 and ReO-P829 peptides.

Results: The mean ± SEM IC_{50} values were 1.1 ± 0.38 and 0.95 ± 0.24 nM for ReO-P829 in the NCI-H69 human tumor cell and AR42J rat tumor membranes, respectively. The mean value for P829 peptide was 7.4 ± 2.6 nM in the AR42J rat tumor membranes. In a single experiment using NCI-H69 tumor cell membranes, the IC_{50} for P829 was similar to that observed with the AR42J membranes being 6.1 nM. The control IC_{50} value in the AR42J tumor membranes was 11.7 nM for somatostatin-14.

Sponsor's Conclusions: The IC_{50} values were not significantly different in the human and rat models being 1.1 nM for the human NCI-H69 tumor and 0.95 nM for the AR42J rat pancreatic tumor. Thus, the AR42J rat pancreatic tumor is a reasonable animal model for pre-clinical somatostatin receptor binding and in vivo targeting studies.

Reviewer's Comment: Agree. The important finding is that technetium Tc 99m P829 binds with high affinity to the somatostatin receptors in membranes from the human NCI H69 SCLC cell line. The affinity for somatostatin receptors in these membranes was 6.1 nM. Values for the AR42J rat pancreatic tumor membranes were similar, suggesting that this is an appropriate animal model for receptor binding studies.

R 4.73. The Binding Affinity of Technetium Tc 99m P829 With Radiochemical Purities of 85% and 94% for Somatostatin Receptors in AR42J Tumor Membranes. Laboratory, Diatide, Inc. Report Dated March 19, 1998. Lot # 9509B01 and #9709B02 (Final Formulation) Report in Volume 1.11 pp 165-182.

Design: This study was designed to show that the affinity of low 85% radiochemical purity (RCP) Technetium Tc 99m P829 preparation for the SSTR was equivalent to the high, 94% RCP preparation. The binding assay consisted of tumor membranes, Technetium Tc 99m P829 with and without excess somatostatin-14 in assay buffer. The specific binding of Technetium Tc 99m P829 to AR42J membranes was determined at final concentrations of 100, 30, 10, 3, 1 and 0.3 nM of P829 in three separate experiments. The specific binding was calculated.

Results: The individual K_d and Bmax values for the binding of Technetium Tc 99m P829 with 85% RCP and 94% RCP were similar. The dissociation constants of Technetium Tc 99m P829 for the somatostatin receptor expressed in the AR42J rat pancreatic tumor membrane preparations using kits with 85% and 95% radiochemical purity were determined to be 12.3 nM and 10.3 nM, with somatostatin receptor density of 5298 and 5788 fmol/mg protein, respectively.

Sponsor's Conclusions: The 85% RCP Technetium Tc 99m P829 preparation had an affinity

for the SSTR equal to the 94% RCP preparation. In addition, there was no difference between the Bmax values of the two groups. These data demonstrate that the 85% RCP preparation was equivalent to the 94% RCP Technetium Tc 99m P829, in terms of SSTR affinity.

Reviewer's Comment: Agree. This study indicates that the 85% RCP P829 is as effective in somatostatin receptor binding as the higher purity 94% RCP material.

R4.79. The (In Vitro) Binding of the syn and anti Isomers of Technetium Tc 99 P829 to Somatostatin Receptors in AR42J Tumor Membranes. Laboratory, Diatide, Inc., Report Dated April 7, 1998. Lot # 9709B02 (Final Formulation). Report in Volume 1.11 pp183-202.

Design: This study was designed to determine the in vitro binding capacity of the syn and anti isomers of Technetium Tc 99 P829 for SSTR expressed by AR42J tumor membranes. The two isomers of Technetium Tc 99m P829 present in the reconstituted Kit for the Preparation of Technetium Tc 99m P829 are designated syn and anti. They are present in an approximate ratio of syn/anti = 0.074 in the Technetium Tc 99m P829 Injection, in other words, the preparation contains 93.2% of the anti isomer. The binding assay consisted of tumor membranes, 0.3 μCi/tube Technetium Tc 99m P829 [redacted]. The inhibition of specific binding of Technetium Tc 99m P829 to AR42J membranes was determined at final concentrations of 100, 30, 3, 1, 0.3, 0.1 and 0.03 nM of Tc 99 P829 in three separate experiments. The inhibition of binding to the AR42J cell membranes was determined in the absence and presence of a saturating concentration of somatostatin to determine nonspecific binding for each inhibition curve. The specific binding and percent inhibition were calculated.

Results: The IC_{50} value of the syn isomer was not affected when either the syn or anti isomer of the [redacted] Technetium Tc 99m P829 was used as tracer. The average IC_{50} value for the syn isomer was 0.89 ± 0.15 nM. The anti isomer of Technetium Tc 99 P829 was 6-fold more potent at displacing Tc 99m P829 from SSTR expressed in AR42J membranes than the syn isomer.

Sponsor's Conclusion: Both the syn and anti isomers bind to the SSTR expressed in the AR42J tumor with high (picomolar) affinity. The anti isomer, which is the principal isomer in the Technetium Tc 99m P829 Injection, had the highest affinity for the SSTR. Both isomers would be expected to avidly target tumors expressing SSTR in vivo.

Reviewer's Comment: Agree. In vitro binding of the two isomers is similar in the AR42J tumor model. However, since the anti isomer constitutes 93.2% of Tc 99m P829 mixture, affinity of the anti isomer is the most critical. This study indicates that although there is somatostatin receptor affinity of the 2 isomers, the value for the anti isomer is 6-fold greater, which would be expected to result in greater efficacy.

R4.81. The Biodistribution of Technetium Tc 99m P829 With Radiochemical Purities of 85% and 94% in the AR42J Tumor Xenograft Model. Laboratory, Diatide, Inc. Report Dated April 10, 1998. Lot # 9709B02 (Final Formulation). Report in Volume 1.11 pp 203-214.

Design: This study was designed to show that the 85% and 94% radiochemical purity Technetium Tc 99m P829 preparations had equivalent tumor, muscle, and blood distribution in two tumor models: the AR42J rat pancreatic and the NCI-H69 human lung tumor xenografts. Mice were injected intravascularly with 10 to 50 μCi of Technetium Tc 99m P829. The RCP 85% and94% RCP preparations were each injected into 6 mice in the same tumor model within 30 minutes after the quality control analysis by HPLC was run. Mice were sacrificed 90 minutes post-injection by decapitation and the trunk blood was collected. Tumors and samples of muscle from both hind legs were collected, weighed and counted for radioactivity.

Results: The biodistribution of the Technetium Tc 99m P829 with 85% RCP and 94% RCP are shown in the following tables for the AR42J tumor model and the NCI-H69 tumor model. There was no significant difference between the 85% and 94% RCP preparations of the blood, muscle or tumor samples in either tumor model. The average uptake for the AR42J tumor model was 5.71± 0.48 % ID/g. The tumor-to-muscle and tumor-to-blood ratios were 12.8 and 12.0 respectively. The average uptake in the NCI-H69 tumor was lower than the AR42J tumor model, 0.97 compared to 5.71 % ID/g. The tumor-to-muscle and tumor-to-blood ratios were 2.4 and 2.1, respectively. Technetium Tc 99m targeted both types of tumors as evidenced by tumor-to-blood and tumor-to-muscle ratios in both models. No difference between the 85% and 94% RCP kits was seen in any parameter in either tumor model. The mean uptake in the NCI-H69 tumor model was lower than that seen in the AR42J tumor model. The difference in the % ID/g correlated with the different SSTR site densities in the two models.

Sponsor's Conclusions: These data demonstrate that the 85% and 94% radiochemical purity Technetium Tc 99m P829 are equally efficacious in vivo at targeting SSTR in the NCI-H69 human lung tumor model and the AR42J rat pancreatic tumor model.

Reviewer's Comment: Agree. This study is similar to study R4.73 reviewed earlier, except in this study xenograft models of human lung tumor and rat pancreatic tumor were used. In this study, as found earlier, the 85% RCP and 94% RCP preparations were equally as effective in somatostatin receptor binding. Moreover, there was a correlation between in vivo tumor uptake of Technetium Tc 99m P829 and, the in vitro study results showing that membrane preparation from NCI-H69 tumor expresses a lower density of somatostatin receptor compared with AR42J tumor membrane.

R4.80. The (In Vivo) Biodistribution of the syn and anti Isomers of Technetium Tc 99m P829 in The AR42J Tumor Xenograft Model. Laboratory, Diatide, Inc., Report Dated April 13, 1998. Lot # 9709B02 (Final Formulation). Report in Volume 1.11 pp 215-228.

Design: This study was designed to evaluate the targeting of the AR42J tumor observed in vivo by the syn isomer, anti isomer and Technetium Tc 99 P829 Injection, and determine the tumor uptake of the Tc 99m-labeled peptide in the presence of excess octreotide. Mice bearing the AR42J rat pancreatic tumor xenograft were used. The syn and anti isomers are present in an approximate ratio of syn/anti = 0.074 in the reconstituted Technetium Tc 99m P829. Each Technetium Tc 99m radiolabeled isomer was injected intravenously at a dose of 50 μCi/mouse in a total volume of 100 μL. Octreotide was administered subcutaneously (200 μg/200 μL saline) to 6 animals, 30 minutes prior to the injection of either Technetium Tc 99m P829 Injection, the Tc 99m-labeled syn or the Tc 99m-labeled anti isomer. Octreotide-treated animals were co-injected with 100 μg/100 μL of octreotide and either Technetium Tc 99m P829 Injection, the syn or the anti isomer at time zero. Control animals received 200 μL of saline 30 minutes prior to the injection of radiolabeled peptide. Mice were sacrificed 90 minutes post-injection by decapitation and the trunk blood was collected. Tumors and samples of muscle from both hind legs were collected, weighed and counted for radioactivity.

Results: The biodistribution of the Tc 99m P829 syn and anti isomers 90 minutes post-injection were determined. The tumor uptake of the anti isomer (6.53 % ID/g) was significantly greater than that of the syn isomer (3.38 % ID/g), and both targeted the tumors compared to blood and muscle values. There was no difference between the %ID/g of blood, or muscle with the syn isomer compared to the anti isomer. Octreotide binds to the SSTR and was used to demonstrate specificity of the Technetium Tc 99m P829 for the tumor SSTR in vivo. The tumor uptake of the control (3.38 % ID/g), was 2.1-fold higher than the octreotide-treated group (1.58%). There was no difference in the % ID/g for blood or muscle between the control and octreotide-treated groups. The biodistribution of the anti isomer of Technetium Tc 99m P829 in the absence (control) and presence of excess octreotide was also observed. The control tumor uptake was 3-fold higher than the octreotide-treated group, despite a concomitant 9.5-fold increase in the blood levels of the radioactivity in the presence of excess octreotide in the control group. The biodistribution of Technetium Tc 99m P829 Injection in the absence (control) and presence of excess octreotide was determined. The Technetium Tc 99m P829 contains both the syn and anti isomers in an approximate ratio of syn/anti = 0.074. The tumor uptake of the control was 5 fold higher than the octreotide-treated group. There was no difference in %ID/g for blood or muscle between the control and octreotide-treated groups.

Sponsor's Conclusions: These data demonstrate that both the syn and anti isomers were efficacious for tumor targeting by specifically binding to the SSTR upregulated in the AR42J rat pancreatic tumor. The anti isomer had a higher tumor uptake (6.53 % ID/g) in these studies compared to the syn isomer (3.38 % ID/g, $p < 0.0001$). However, both isomers targeted the tumors as evidenced by tumor-to-muscle ratios of 10.2 and 16.4, and tumor-to-blood ratios of 7.0 and 18.8, for the syn and anti isomers,

respectively. Excess octreotide was able to significantly block tumor uptake of the syn and anti isomers and Technetium Tc 99m P829 Injection indicating that the targeting of the AR42J tumors by both isomers was specific for the SSTR. These data demonstrate that mechanism by which the syn and anti isomers localize in SSTR positive tumors is due to their specific high affinity interaction with SSTR.

Reviewer's Comment: Agree. This study is similar to the earlier study #R4.79 where the affinity of the syn and anti isomers were determined. In this study with the rat pancreatic xenograft tumor model, somatostatin receptor binding of the anti isomer was 2-fold greater than for the syn isomer. Pretreatment with octreotide greatly reduced uptake of the anti isomer, the syn isomer and Tc 99m P829.

R4.82. Tumor Uptake of Technetium Tc 99m P829 Injection and the syn and anti Isomers in Lewis Rats With CA20948 Pancreatic Tumors. Laboratory, Diatide, Inc., Report Dated April 8, 1998. Lot # 9709B02 (Final Formulation). Report in Volume 1.11 pp 229-247.

Design: This study was designed to examine the tumor uptake of Technetium Tc 99m P829 Injection in male Lewis rats possessing CA20948 rat pancreatic tumors, a somatostatin-receptor (SSTR) expressing tumor line. The two isomeric forms of Tc 99m P829 Injection isolated by reversed-phase high-performance liquid chromatography, the syn and anti isomers, were also examined separately for their respective tumor uptake in the model. In each treatment group, half of the animals were administered octreotide at 3.5 mg octreotide /kg subcutaneously at 30 minutes prior to injection of either Tc 99m P829 Injection or the Tc 99m P829 syn or anti isomers to competitively block tumor uptake of the radiolabeled peptide. Tc 99m P829 Injection, Tc 99m P829 syn and Tc 99m P829 anti were administered intravenously at a dose of 1 μg P829 peptide/kg. Tc 99m P829 Injection had a specific activity of 1 mCi/μg of total P829 peptide. Tc 99m P829 syn was 6 μCi/μg and Tc 99m P829 anti was 20 μCi/μg. The rats were sacrificed 90 minutes after administration and percent injected dose per gram was determined for blood, tumor and muscle.

Results: The tumor uptake of the syn and anti isomers was equivalent in this tumor model, yet uptake of the Technetium Tc 99m P829 Injection preparation was greater. Differences were noted in tumor uptake between control and octreotide-pretreated animals. Tumor uptake decreased 74% for Tc 99m P829 Injection with octreotide pretreatment. Tc 99m P829 syn and Tc 99m P829 anti had substantial decreases in tumor uptake with octreotide preadministration decreasing uptake 50% and 26%, respectively. The tumor-to-blood ratios declined 74% for Tc 99m P829 Injection, 57% for Tc 99m P829 syn and 43% for Tc 99m P829 anti with octreotide pretreatment. Tumor-to-muscle ratios decreased 59% for Tc 99m P829 Injection, 37% for Tc 99m P829 syn and 38% for Tc 99m P829 anti. There were no differences between normal and octreotide-pretreated rats with respect to terminal blood or muscle for Tc 99m P829 Injection, Tc 99m P829 syn or Tc 99m P829 anti. The blood-to-muscle ratio increased 69% for Tc 99m P829 Injection, 45% for Tc 99m P829 syn but was unchanged for Tc 99m P829 anti with octreotide pretreatment.

Sponsor's Conclusion: Uptake of Tc 99m P829 Injection was demonstrated in the SSTR-expressing CA20948 rat pancreatic tumor model. Tc 99m P829 Injection provides a greater tumor uptake relative to either of the isomeric forms. Tc 99m P829 Injection tumor uptake was significantly blocked by pretreatment with octreotide. Tc 99m P829 syn tumor uptake appears to be more readily blocked by octreotide than Tc 99 P829 anti uptake.

Reviewer's Comment: Agree. This study uses the Lewis rat with the CA20948 pancreatic tumor which is a somatostatin receptor expressing line. Findings with this model are similar to findings that were reported with other somatostatin receptor binding tumor models. Technetium Tc 99m P829 binds with high affinity to somatostatin receptors in the Lewis rat model. Both the anti and syn isomers also bind to the receptors, but uptake was less for the isomers than with the complete formulation. Binding of both isomers and technetium Tc 99m P829 was blocked by pretreatment with octreotide. Tumor:blood and tumor:muscle ratios were 5.8 and 21.7 respectively for technetium Tc 99m P829.

R4.28. The Binding of Technetium Tc 99m P829 to Somatostatin Receptors in Human Tumor Membranes. Laboratory, Diatide, Inc., Report Dated May 6, 1998. Lot # 9709B02 (Final Formulation). Report in Volume 1.11 pp248-261.

Design: This study was designed to measure the specific binding of Technetium Tc 99m P829 to somatostatin receptors expressed in human tumors that were surgically removed after imaging. The binding assay consisted of tumor membranes, Technetium Tc 99m P829 with and without excess 500nM somatostatin-14 in assay buffer. The specific binding of Technetium Tc 99m P829 to human tumor membranes was determined at final concentrations of 30, 10, 3, 1, 0.3 and 0.1 nM of P829 in three separate experiments. Tumor membranes were also assayed for specific binding of ^{125}I-Somatostatin. The assay conditions were identical to those described for the Technetium Tc 99m P829 assay except ^{125}I-Somatostatin was added in the absence and presence of 500 nM somatostatin-14, somatostatin-28 or ReO-P829.

Results: Three types of non-small cell lung carcinomas were assayed: squamous cell (n = 7); adenocarcinomas (n = 5); and large cell carcinoma (n = 1). All samples were somatostatin receptor positive as demonstrated by binding of ^{125}I-somatostatin which was displaced by somatostatin-14 and/or somatostatin-28 (500 nM). P829-specific SSTR binding was observed using either Technetium Tc 99m P829 (displaced by somatostatin-14), or by ^{125}I-somatostatin (displaced by ReO-P829). The average Bmax and K_d values for the squamous cell carcinomas were 44.3 ± 29.3 fmol/mg protein and 2.1 nM, respectively. In one squamous cell sample there was also evidence of a second class of SSTR recognized by Technetium Tc 99m with a site density of 280 fmol/mg and a K_d > 10 nM. Two classes of binding sites for the adenocarcinomas were also suggested with average Bmax values of 337 ± 241 and 6.5 ± 1.4 fmol/mg for the >10nM and the 1.3 ± 1.4 nM K_d sites, respectively. The large cell carcinoma had a site density of

at least 184 fmol/mg protein with a K_d of > 10 nM. The breast cancer included in this study was also positive for Technetium Tc 99m P829 binding with Bmax values of 45 and 339 fmol/mg protein and K_d values of 2.4 and > 10 nM, respectively. A section of normal lung tissue had no detectable binding of Technetium Tc 99m P829 (displaced by somatostatin-14) and showed that only a fraction of the ^{125}I-SST binding sites were displaced by ReO-P829.

Sponsor's Conclusions: These results support a receptor-peptide interaction as the mechanism by which Tc 99m P829 can detect and localize receptor-expressing tumors in the lung.

Reviewer's Comment: Agree. As the Sponsor concludes, this study supports a receptor -peptide interaction as a mechanism of somatostatin receptor binding by technetium Tc 99m P829. However, it is noted that only 15 tissues were imaged. Of these 15 tissues with positive technetium Tc 99m imaging, only 11 were positive for technetium Tc 99m P829 somatostatin receptor binding. Four tissues were either not detectable for somatostatin receptor binding of technetium Tc 99m P829 or were not done. While the technical challenges poised by this type of experiment including inadequate sample material is noted, nevertheless one is concerned that some tissues were positive for Somatostatin-14 and/or Somatostatin-28 receptor binding but were negative for technetium Tc 99m P829. If true, the implication is that there is potential for imaging with technetium Tc 99m P829 to miss some somatostatin producing tumors.

R4.83. The Binding of Technetium Tc 99m P829 To Somatostatin Receptors in Human Microvascular Endothelial Cell Membranes. Laboratory, Diatide, Inc., Report Dated April 14, 1998. Lot # 9609B02 (Final Formulation). Report in Volume 1.11 pp 262-271.

Design: This study was designed to document the specific binding of Technetium Tc 99m P829 to SSTR expressed by human microvascular endothelial cells to explain the observed generalized biodistribution of Technetium Tc 99m P829 to soft tissue in man. The binding assay consisted of endothelial cell membranes, 1 nM Technetium Tc 99m P829 in assay buffer. The inhibition of specific binding of Technetium Tc 99m P829 was determined in the absence and presence of 500 nM somatostatin-14.

Results: Less than 200 μg of membrane protein was obtained from either the dermal or the lung microvascular endothelial cell culture. The experimental scope was limited to a single point determination of Technetium Tc 99m P829 binding in the absence and presence of 500 nM somatostatin-14. Specific binding of Technetium Tc 99m P829 was observed for both the lung and dermal microvascular endothelial cells at 1 nM Technetium Tc 99m P829. The site density was 97.6 and 88.2 fmol/mg protein in the lung and dermal microvascular endothelial cells, respectively. With a K_d of 1 nM the Bmax of the microvascular endothelial cells is 195.2 and 176.4 fmol/mg respectively.