

Imaging and Therapy of Tumors Induced to Express Somatostatin Receptor by Gene Transfer Using Radiolabeled Peptides and Single Chain Antibody Constructs

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The fields of radioimmunodetection and radioimmunotherapy began with an initial paradigm that a targeting molecule (eg, antibody) carrying a radioisotope had the potential of selectively imaging and delivering a therapeutic dose of radiation to tumor sites. A second paradigm was developed in which injection of the targeting molecule was separated from injection of a short-lived radioisotope-labeled ligand (so-called "pretargeting strategy"). This strategy has improved radioisotope delivery to tumors in animal models, enhanced radioimmune imaging in man, and therapeutic trials are in an early phase. We proposed a third paradigm to achieve radioisotopic localization at tumor sites by inducing tumor cells to synthesize a membrane expressed receptor with a high affinity for infused radiolabeled ligands. The use of gene transfer technology to induce expression of high affinity membrane receptors can enhance the specificity of radioligand localization, while the use of radioisotopes with the ability to deliver radiation damage across several cell diameters will compensate for less than perfect transduction efficiency. This approach was termed "Genetic Radioisotope Targeting

THE USE of radiolabeled monoclonal antibodies (mAb) to "target" radioactivity to tumor sites is a promising strategy, as shown in a number of animal models and in human trials.¹⁻⁷ Radioimmunotherapy (RIT) is typically administered systemically. However, it also has been delivered intraperitoneally (IP) or intralesionally. Favorable results have been observed in the treatment of lymphomas, in tumors confined within the peritoneal cavity (ovarian), and in malignant gliomas through direct intratumoral injection.⁸⁻²⁰ Another strategy has used radiolabeled peptides (eg, Octreoscan, Mallinckrodt, Hazelwood, MO, or

Strategy." Using this strategy, induction of high levels of gastrin releasing peptide receptor or human somatostatin receptor subtype 2 expression and selective tumor uptake of radiolabeled peptides was achieved. The advantages of the genetic transduction approach are (1) constitutive expression of a tumor-associated antigen/receptor is not required; (2) tumor cells are altered to express a new target receptor or increased quantities of an existing receptor at levels that may significantly improve tumor targeting of radiolabeled ligands compared with normal tissues; (3) gene transfer can be achieved by intratumoral or regional injection of gene vectors; (4) it is feasible to target adenovirus vectors to receptors overexpressed on tumor cells by modifying adenoviral tropism (binding) so that the virus will be targeted specifically to the desired tumor; and (5) it is possible to coexpress the receptor gene and a therapeutic gene, such as cytosine deaminase, for molecular prodrug therapy to produce an enhanced therapeutic effect.

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Neotect, Berlex Laboratories, Inc, Wayne, NJ) to target human somatostatin receptor subtype 2 (hSSTR2) positive tumor cells for imaging in patients.²¹⁻²⁴ The peptides can be labeled with radioisotopes for therapeutic applications, which have been performed in preclinical animal models and in patients with cancer.²⁵⁻³² Despite the success of these investigations, improved delivery systems and/or targeting strategies are needed for RIT to have an even greater effect in the treatment of cancer.

LIMITATIONS OF CURRENT TARGETED RADIODETECTION AND RADIOTHERAPY STRATEGIES

The success of radioimmunodetection (RID) and RIT has been limited by the low percentage uptake of injected radioactivity in tumors due to low target antigen expression and slow penetration of the large (160 kDa) intact mAb, the normal tissue uptake of the radiolabeled mAb, the bone marrow toxicity due to the long blood circulation time of the radiolabeled mAb in blood, radioresistance of the tumor, and the development of human anti-mouse antibody responses.^{2,33} Strategies to overcome these limitations have included the use of

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mAb fragments, single chain antibodies (scFv) and minibodies or diabodies,³⁴⁻³⁷ radiolabeled peptides,^{21,22,25,27,38} locoregional administration of the radiolabeled ligand,³⁹⁻⁴¹ the use of cleavable chelating agents,^{42,43} biological response modifiers such as interferon or gene transfer methods to upregulate tumor-associated antigen/receptor expression,⁴⁴⁻⁵¹ irradiation of the tumor to increase vascular permeability,² the use of radiosensitizing agents,⁵²⁻⁵⁴ and antiangiogenic therapy.⁵⁵⁻⁵⁷

Strategies to Overcome Targeted Radiotherapy Limitations

There are several strategies designed to overcome the limitations of RID and RIT. Several desirable features can be envisioned for optimum receptor-ligand systems for tumor detection and therapy. Expression of an endogenous receptor exclusively on malignant cells within the normal tissue parenchyma would provide a certain degree of specificity and safety. If cross-reactivity of the radioligand with corresponding human receptors is known, it would be most desirable if the human receptor-positive cells were expendable or were isolated from the treatment area. Thus, nontumor localization of radioligand would not have significant deleterious clinical sequelae. Our group showed that an adenoviral (Ad) vector encoding the gene for carcinoembryonic antigen (CEA) could infect human glioma cells and induce the expression of CEA *in vitro* and *in vivo*, as evidenced by an increase in the binding and localization of a radiolabeled anti-CEA antibody when compared with uninfected cells.⁴⁸ Another desirable feature of candidate ligand-receptor systems would be the potential for a high affinity ligand-receptor interaction. Our recent focus has been on the development of recombinant vectors that transfer receptor-encoding genes with high binding affinities to radiolabeled peptides to tumor cells. Receptors that we investigated for targeting with radiolabeled peptides include hSSTr2 and the gastrin releasing peptide receptor (GRPr). Our group has published on the use of Ad vectors encoding the genes for hSSTr2,⁵⁸⁻⁶¹ GRPr,^{49,50} and epidermal growth factor receptor^{62,63} in the genetic radioisotope targeting approach. A potential advantage of genetic transduction of a receptor is that the level of expression may be higher than what are generally otherwise low tumor concentrations of such receptors.³⁸

Radiolabeled Peptides Targeting Somatostatin Receptors in Cancer Detection and Therapy

We identified one lead system that seemed to offer the desired features for use in the proposed context. The target of most imaging and therapy studies with radiolabeled peptides has been the hSSTr2, which is expressed on a number of human tumors, including neuroendocrine, ovarian, renal, breast, lung, and meningiomas.⁶⁴⁻⁶⁸ The somatostatin receptor group includes gene products encoded by 5 separate somatostatin receptor genes.⁶⁹ The subtype 2 exists in 2 forms due to alternate splicing of hSSTr2 messenger ribonucleic acid, which produces 2 products. The subtype 2A receptor (herein referenced as hSSTr2) is slightly longer (369 amino acids), while the shorter subtype 2B differs only in regard to a truncation on the C-terminal tail (356 amino acids). The receptors have varying tissue levels in the brain, gastrointestinal tract, pancreas, kidney, and spleen.⁷⁰⁻⁷² It is for this reason that it might be helpful to produce ligands reactive with a mutated form of hSSTr2 to achieve more selective binding to transfected tumors without inducing toxicity to normal tissues that naturally express hSSTr2. All 5 receptors show high affinity binding to natural somatostatin peptide, either somatostatin-14 or somatostatin-28. Octreotide, P829, and P2045 are synthetic somatostatin analogues that preferentially bind with high affinity to somatostatin receptor subtypes 2, 3, and 5 of human, mouse, or rat origin.⁷⁰⁻⁷⁴ Somatostatin and its analogues effectively inhibit the proliferation of various types of cancer cells as a result of binding to hSSTr2.⁷⁵⁻⁷⁷

Octreotide is an 8 amino-acid peptide that has a high affinity for hSSTr2 and is stable towards *in vivo* degradation relative to the endogenous 14 amino-acid somatostatin-14 peptide. Octreotide and other somatostatin analogues have been modified with bifunctional chelating agents, for complexing radioactive metals, and by changing the amino acid sequence to increase their hSSTr2 binding affinity and optimize their normal organ clearance. Somatostatin analogues have been labeled with ¹¹¹In, ⁹⁰Y, ⁶⁴Cu, and ¹⁸⁸Re for therapeutic applications. Smith-Jones and coworkers showed that a single 0.48 mCi IP injection of a ⁹⁰Y-labeled somatostatin analogue in nude mice bearing subcutaneous (s.c.) AR42J rat pancreatic tumors resulted in a significant reduction in tumor

growth.⁷⁸ Stolz and colleagues showed that a single dose of ⁹⁰Y-DOTA-D-Phe¹-Tyr³-octreotide (⁹⁰Y-SMT 487) led to reductions of 60% and 50% of the initial tumor volume in nude mice bearing AR42J and NCI-H69 human small cell lung cancer tumors, respectively.⁷⁹ Complete remissions were observed in rats bearing (s.c.) CA20948 rat pancreatic tumors when a single 2.0 mCi dose of ⁹⁰Y-DOTA-Tyr³-octreotide was administered.²⁷ The ⁹⁰Y-DOTA-lanreotide that binds to hSSTr produced a therapeutic response in a patient with metastatic gastrinoma.⁸⁰ The ⁹⁰Y-SMT 487 was administered to 20 patients with malignant tumors (17 carcinoids, 1 breast cancer, 1 medullary thyroid cancer, 1 meningioma) in a phase I trial.⁸¹ Complete and partial responses were obtained in 25% of patients along with 55% showing stable disease lasting at least 3 months. The ⁹⁰Y-SMT 487 has been tested in patients with neuroendocrine tumors.^{27,81,82}

Zamora and coworkers labeled the somatostatin analogue RC-160 with ¹⁸⁸Re and administered 7 doses of 0.2 mCi over a 14-day period intralesionally to nude mice bearing PC-3 human prostate cancer tumors.²⁵ They reported that animals receiving ¹⁸⁸Re-RC-160 had 60% survival compared with no survivors when control animals were injected with saline. Anderson and colleagues showed tumor growth inhibition of s.c. CA20948 tumors in Lewis rats using either single or multiple intravenous (IV) doses of ⁶⁴Cu-TETA-octreotide.²⁶ Thus, several radiolabeled somatostatin analogues have shown potential as radiotherapeutic agents in animal tumor models and in humans. However, in most of the published studies, there has been limited tumor uptake and retention of the radiolabeled peptides (peak uptake <10% injected dose [ID]/g), presumably due to the rapid clearance from the blood. This has resulted in the use of rather high quantities of radionuclides in preclinical studies with multiple administrations. Moreover, large radionuclide doses (0.4 to 1 Ci) have been administered to patients.⁸³⁻⁸⁷

Tumor Killing is Enhanced by Increasing Radiosensitivity Through Molecular Prodrug Therapy

The molecular prodrug gene therapy approach involves insertion and expression of an enzyme in a target cell that converts a nontoxic prodrug to a toxic drug.^{88,89} The enzyme cytosine deaminase

(CD) is a nonmammalian enzyme that normally catalyzes the formation of uracil by the deamination of cytosine. When 5-fluorocytosine (5-FC) is the substrate, this enzyme will produce 5-fluorouracil (5-FU), a potent cancer chemotherapeutic and radiosensitizing agent.⁹⁰ The genes for bacterial and yeast CD have been cloned.⁹¹⁻⁹⁴ Because mammalian cells do not normally express the CD gene, 5-FC is nontoxic to these cells, even at high concentrations. The 5-FC has been used as an antifungal drug because of its relative nontoxicity in humans. The CD gene has been used in gene therapy strategies to mediate intracellular conversion of 5-FC to 5-FU, and has been effective in animal tumor models.^{95,96} This therapeutic strategy has the advantage of intracellular production of high concentrations of radiosensitizing drug as an alternative to systemic administration, therefore potentially limiting systemic toxicities.⁹⁷ Direct injection of 5-FU itself into a solid tumor would not be effective because it would be washed out immediately. Converted 5-FU passively diffuses across the cell membrane from CD-positive cells to nontransduced cells.^{98,99} This bystander effect compensates for the inability of current vector systems to transduce all but a small fraction of cells in a given tumor.^{89,100}

Recent studies by our group and others involving combination of radiation therapy with molecular prodrug therapy have shown that CD-based prodrug therapy sensitizes tumor cells to radiation in vitro and in vivo.¹⁰¹⁻¹⁰³ Human colon and head and neck cancer cells that were stably transduced to express the CD gene were radiosensitized by the addition of 5-FC in vitro and in vivo.^{93,94,104,105} The use of Ad vectors to encode CD and convert 5-FC to 5-FU to achieve cell killing has been reported by our group^{102,103,106,107} and others.^{96,99,101,108} We initially used an Ad vector encoding CD under the control of the cytomegalovirus promoter (AdCMVCD) in combination with 5-FC and radiation treatment to show enhanced cytotoxicity against human colon, pancreatic, glioblastoma, and cholangiocarcinoma cells in vitro and in vivo.^{101-103,109} Recent results in a lung cancer animal model are described later. The results of a CD/5-FC molecular prodrug therapy phase I trial in patients with breast cancer have been published.¹¹⁰ There was evidence of reduction of tumor volume in 4 of 12 patients.

Phage Display Technology: In Vitro Generation of Recombinant Antibodies

Within the last decade, a novel approach has facilitated the in vitro production of recombinant antibodies directed against a variety of targets. The key technology in this approach is the surface expression of antigen-binding fragments of mAb on filamentous bacteriophage (Phage Display).¹¹¹ Recombinant antibodies can be expressed in *Escherichia coli* as a single polypeptide consisting of 2 antigen binding domains, V_H and V_L , joined by a flexible peptide linker (termed scFv – single chain antibody variable fragment). The further development of recombinant antibody technology has led to creation of libraries of antibody genes obtained from immunized or nonimmunized donors. These antibody genes are expressed as scFvs on the surface of bacteriophage. This phage display approach allows selection of clones with highly specific antigen binding out of a vast number of primary clones in the library. Using this principle, extremely large antibody gene libraries can be screened.

Recombinant antibodies also can be isolated from hybridoma cell lines. The genetic information for the recombinant antibody V_H and V_L structural domains is amplified from hybridoma cells using the polymerase chain reaction with antibody gene-specific primers. The necessity of creating phage display libraries when recombinant mAb are generated from hybridomas is dictated by their sequence heterogeneity.¹¹²

Tumor Targeting With Radiolabeled Minibodies and Diabodies

The engineering of antibodies can be used to produce recombinant fragments with properties optimized for in vivo applications. Intact, murine mAb are immunogenic in humans and display poor pharmacokinetics for RID. ScFvs (constructed from hybridoma cells or isolated via phage display, as described previously) are themselves poor reagents for targeting radionuclides to tumors due to their small size and that they only contain a single binding site. Nonetheless, scFvs provide an excellent building block for intermediate size engineered fragments, such as minibodies, in which the scFv have been fused to the human IgG1 hinge and CH3 regions to provide a dimerization domain¹¹³ and diabodies, where scFv self-assemble into non-covalent dimers containing 2 functional antigen

binding sites.¹¹⁴ These fragments show antigen binding comparable to intact bivalent antibodies, and may show improved tumor penetration and faster normal tissue clearance. They include the scFv (27 kDa), diabody (a noncovalent dimer of scFv, 55 kDa), and the minibody (a dimer of scFv-hinge-CH3, 80 kDa).

Biodistribution and tumor targeting studies of radioiodinated or radiometal-labeled anti-CEA minibodies and diabodies in athymic mice bearing s.c. LS174T human colon cancer xenografts showed that these fragments localize to CEA-positive tumors with fast clearance from blood and normal tissues (β half-life 3 to 5 hours) after IV injection.^{113,115,116} Maximum uptake levels of 10% to 15% ID/g for radioiodinated diabody at 1 to 2 hours after injection, or 20% to 25% ID/g for minibody at 6 to 12 hours after injection occurred following IV administration to mice. The blood pharmacokinetics of ¹¹¹In- and ⁶⁴Cu-labeled minibodies was similar, and high uptake in LS174T tumors occurred. However, radiometal-chelate conjugated minibody showed uptake in the liver, and the anti-CEA diabody localized in the kidneys. Nevertheless, the rapid blood clearance of both of these antibody fragments resulted in high tumor-to-normal tissue ratios for other tissues. Results in the CEA system have been confirmed in other tumor-associated antigen systems, including TAG-72, Her2/neu, placental alkaline phosphatase, and fibronectin ED-B domain.¹¹⁷ Furthermore, antibody fragments, such as diabodies or scFv, show improved tumor penetration.^{34,118} Anti-CEA minibodies labeled with ⁶⁴Cu have been used for imaging by micro-positron emission tomography (PET),¹¹⁷ and potentially provide a vehicle for the delivery of therapeutic radionuclides either as a single agent or in genetic radioisotope and molecular prodrug therapy approaches as described herein.

COMBINATION OF GENE THERAPY AND TARGETED RADIOTHERAPY ADDRESSES THE KEY SHORTCOMINGS IN GENE THERAPY (LESS THAN COMPLETE TRANSDUCTION) AND TARGETED RADIOTHERAPY (INADEQUATE DELIVERY/LOCALIZATION OF RADIOLIGANDS)

The ability of recombinant Ad vectors to accomplish efficient gene transfer to tumor cells in vivo has led to the use of this vector approach in several,

clinical cancer gene therapy trials.¹¹⁹ A number of gene therapy approaches use direct in situ transduction of tumor for the purpose of achieving an anticancer effect. In these various strategies, the limited transduction frequency achievable with currently available vectors mitigates against efficacy. Thus, strategies to amplify the biologic effects of genetic transduction events would potentially allow the enhanced therapeutic effect of these gene therapy methods. By linking tumor transduction to the induced binding of radiolabeled ligands, it is possible that this effect may be achieved because cells in proximity to bound ligand may be killed as a result of exposure to the local radiation field. It should be understood that with this approach, we are attempting to increase specifically the number of receptors on tumor cells that normally express a receptor or to induce specifically expression on tumor cells that do not ordinarily express the receptor by genetic transduction, with the result being increased targeting of the radiolabeled ligand to the tumor site. It is our hypothesis that one can deliver a larger fraction of the administered dose of the radiolabeled ligand to the tumor cells selectively through increased receptor expression at the tumor site.

When used with radiation therapy, uniform systemic incorporation of the genetic construct into tumor cells is not necessary. It should be emphasized that the advantage of the proposed strategy is that transduction of 20% to 40% of tumor cells may be all that is necessary for radiolabeled ligands to produce tumor responses, given the ability of β -emitters to deliver radiation across several cell diameters in primary tumor sites and metastases. Current strategies only transduce 5% to 10% of the tumor cells. The chief stumbling block in the use of radiolabeled peptides has been the low dose of radiation that can be delivered to the tumor due to rapid catabolism. The use of high affinity radiolabeled minibodies and diabodies will likely increase the delivered radiation dose to tumor due to better tumor uptake and retention. There should be enhanced cell killing as a result of gene transfer being combined with radiation therapy.

We have developed an approach to increase expression of targetable cell surface receptors in tumor cells using a gene transfer strategy. Using this strategy, we have accomplished induction of high levels of receptors for radioligand targeting, as described later. The advantages of the genetic

transduction approach are (1) constitutive expression of a tumor-associated receptor is not required, and (2) tumor cells are altered to express a new target receptor at levels that may significantly improve tumor to normal tissue targeting of radiolabeled ligands. This method thus represents a new paradigm by which augmented therapeutic efficacy can be achieved through enhanced radiolabeled ligand localization to tumors transduced in situ to express unique and novel receptors. The use of modern molecular biology techniques to increase the expression of tumor-associated receptors for radiolabeled ligands is a novel approach to the treatment of cancer. The importance of this approach lies in that although each modality alone has limitations, the combination of gene transfer and radioligand therapy would be synergistic in effect.

INDUCTION OF RECEPTOR IN VITRO

We produced an Ad encoding the gene for hSSTr2 under the control of the cytomegalovirus promoter (AdCMVhSSTr2), and showed in vitro binding of ¹²⁵I-somatostatin and ¹¹¹In-DTPA-D-Phe¹-octreotide to cell membrane preparations of SK-OV-3.ip1 human ovarian cancer cells and A-427 human nonsmall cell lung cancer cells infected with AdCMVhSSTr2.⁶⁰ The cells were infected with various amounts of AdCMVhSSTr2, and binding assays on membrane preparations from these cells showed high expression of hSSTr2 (Fig 1).¹²⁰ In addition, reverse transcriptase polymerase chain reaction analysis showed the presence of hSSTr2 messenger ribonucleic acid in A-427 and SK-OV-3.ip1 cells infected with AdCMVhSSTr2. Scatchard analysis of A-427 cells infected with 10 plaque forming units (pfu) AdCMVhSSTr2 and binding of ^{99m}Tc-P829 (Neotect) showed a B_{max} of 19,000 fmol/mg and the affinity of ^{99m}Tc-P829 to be 7 nM.

INDUCTION OF RECEPTORS IN VIVO

To evaluate the ability to induce receptor expression in vivo, AdCMVhSSTr2 was injected IP to induce hSSTr2 expression on SK-OV-3.ip1 tumors 5 days after tumor cell injection in the peritoneum in nude mice. Two days later, tumor localization of ¹¹¹In-DTPA-D-Phe¹-octreotide 4 hours after IP injection was equal to 60.4% ID/g of the radiolabeled peptide.⁶⁰ However, the uptake in tumor decreased to 18.6% ID/g at 24 hours after

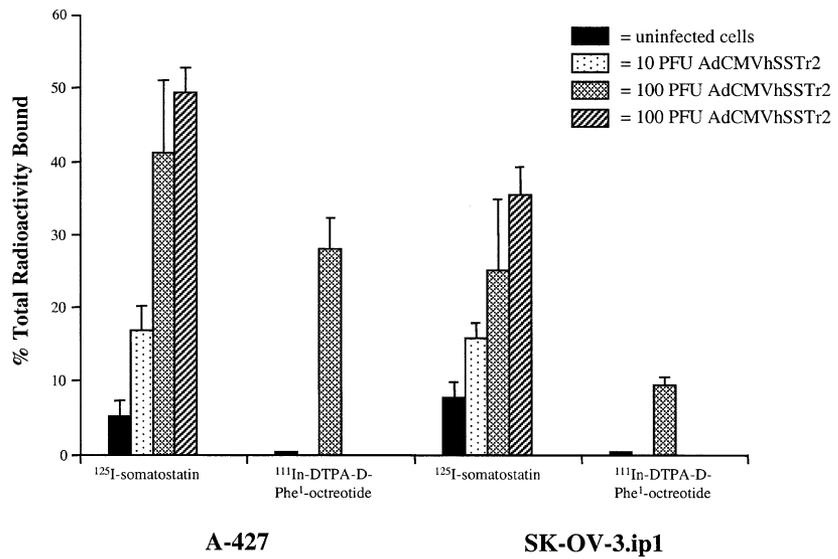


Fig 1. Binding of ¹²⁵I-Tyr¹-somatostatin and ¹¹¹In-DTPA-D-Phe¹-octreotide to A-427 and SK-OV-3.ip1 membrane preparations.

injection (Fig 2). The tumor localization was significantly lower (1.6% ID/g) when a control Ad (AdCMVGRPr) was injected. These studies also showed that the tumor uptake of ¹¹¹In-DTPA-D-Phe¹-octreotide was similar 1, 2, or 4 days after AdCMVhSSTR2 injection and that 2 injections of AdCMVhSSTR2 did not improve the tumor localization of ¹¹¹In-DTPA-D-Phe¹-octreotide. Thus, these studies showed that tumor uptake of ¹¹¹In-DTPA-D-Phe¹-octreotide could be achieved after infection of the ovarian tumor in vivo with AdCMVhSSTR2.

Other studies have investigated the localization

of ¹¹¹In-DTPA-D-Phe¹-octreotide to s.c. A-427 non-small cell lung tumors injected intratumorally (i.t.) with AdCMVhSSTR2.¹²¹ The gamma camera region of interest analysis showed the tumor uptake of ¹¹¹In-DTPA-D-Phe¹-octreotide to be 2.8% ID/g 48 hours after a single intratumoral AdCMVhSSTR2 injection and 3.1% ID/g at 96 hours. Uptake of ¹¹¹In-DTPA-D-Phe¹-octreotide in control Ad-injected tumors was <0.3% ID/g at both times.

Gamma camera imaging was used to detect hSSTR2 expression in s.c. A-427 tumors infected with AdCMVhSSTR2 using a ^{99m}Tc- or ¹⁸⁸Re-

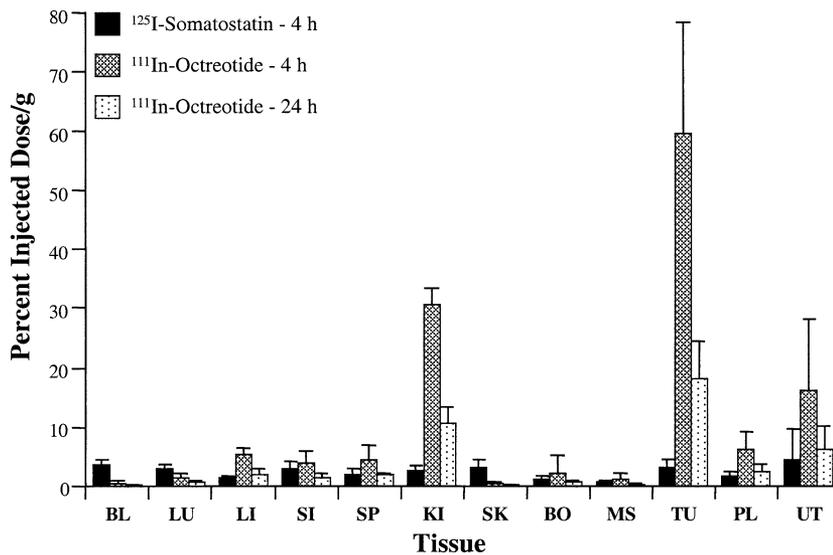


Fig 2. Biodistribution of ¹²⁵I-somatostatin and ¹¹¹In-DTPA-D-Phe¹-octreotide in mice bearing intraperitoneal (IP) SK-OV-3.ip1 tumors injected with AdCMVhSSTR2. BL, blood; LU, lung; LI, liver; SI, small intestine; SP, spleen; KI, kidney; SK, skin; BO, bone; MS, muscle; TU, tumor; PL, peritoneal lining; UT, uterus. Reprinted with permission from the American Association for Cancer Research, Inc.

**Imaging the expression
of 2 genes following Ad
transfer to A-427 tumors**

Images below are 5 h
after *i.v.* injection of 2
radiotracers

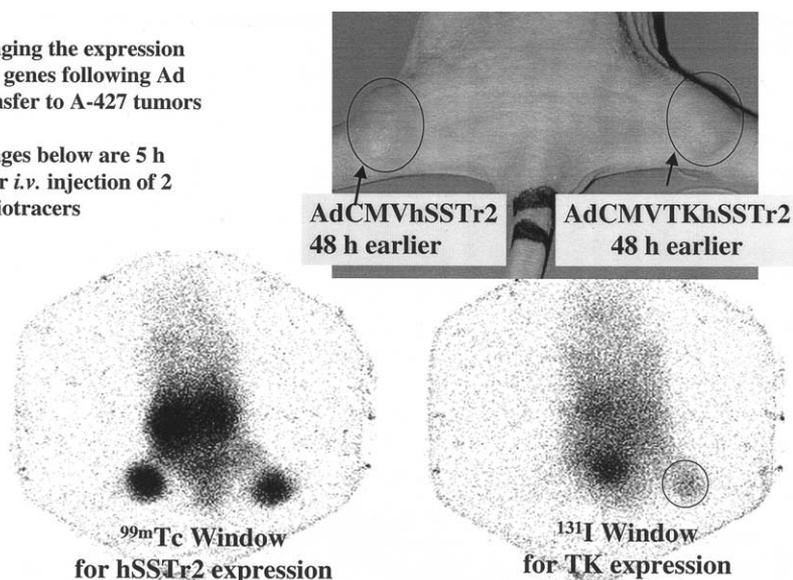


Fig 3. In vivo simultaneous imaging for human somatostatin receptor subtype 2 (hSSTr2) and thymidine kinase (TK) expression. Photograph of the mouse shows tumor locations and adenoviral (Ad) doses. The expression of hSSTr2 was depicted with imaging tumor accumulation of ^{99m}Tc -labeled P2045 (bottom left), while TK expression was depicted with imaging tumor accumulation of ^{131}I -labeled FIAU (bottom right). The images were obtained 5 hours after intravenous (IV) injection of the radiotracers. Reprinted with permission from the Radiological Society of North America, Inc.

labeled somatostatin analogue.¹²² The somatostatin analogue, P829, was radiolabeled with ^{99m}Tc at high specific activity and was shown to bind with high affinity to hSSTr2-positive tumors by external scintigraphic imaging. The ^{99m}Tc -P829 and ^{188}Re -P829 bound with high affinity (6 to 7 nM) to membrane preparations from A-427 cells infected with AdCMVhSSTr2, and were internalized similarly by AdCMVhSSTr2-infected A-427 cells.¹²² Mice bearing s.c. A-427 tumors injected i.t. with AdCMVhSSTr2 showed uptake of IV-injected ^{99m}Tc -P829 and ^{188}Re -P829 detected by gamma camera imaging, while uptake was not observed when the tumors were infected with a control Ad. This result was confirmed by counting the tumors in a gamma counter, which showed 3.8% and 2.9% ID/g of ^{99m}Tc -P829 and ^{188}Re -P829 in the AdCMVhSSTr2 injected tumors, respectively, compared with <0.4% ID/g in the tumors infected with the control Ad. Independent confirmation of hSSTr2 expression was shown by immunohistochemical analysis.

A novel ^{99m}Tc -labeled peptide (P2045) recently described by Diatide, Inc.¹²³ binds with high affinity to hSSTr2 and has favorable *in vivo* biodistribution. This peptide was evaluated in mice bearing SK-OV-3.ip1 tumors in the peritoneum. Tumor uptake of ^{99m}Tc -P2045 at 48 hours after IV injection averaged $2.2 \pm 0.3\%$ ID/g for mice injected IP with AdCMVhSSTr2 (1×10^9 pfu), as compared with $0.18 \pm 0.002\%$ ID/g in control mice not receiving Ad injection ($P < 0.05$) or in tumors

from mice injected IP with an Ad encoding the green fluorescent protein, which averaged $0.26 \pm 0.17\%$ ID/g.¹²⁴ We also evaluated P2045 in mice bearing s.c. A-427 tumors injected i.t. with AdCMVhSSTr2 or with a control Ad. The ^{99m}Tc -P2045 was injected IV 2 or 4 days after AdCMVhSSTr2 injection, and the animals were imaged using a gamma camera equipped with a pinhole collimator 3.5 to 4.5 hours later. The images showed similar uptake of ^{99m}Tc -P2045 in the tumors injected with AdCMVhSSTr2, but background uptake in tumors injected with control Ad. The biodistribution results in the mice 4 days after AdCMVhSSTr2 injection and 4 hours after ^{99m}Tc -P2045 injection showed 7.8% ID/g in the positive tumor. No other tissue had higher uptake than the AdCMVhSSTr2-injected tumor.¹²⁰

Further studies were reported using a bicistronic Ad vector encoding hSSTr2 and thymidine kinase (TK) in the same mouse tumor model.¹²⁵ The tumors were injected i.t. with the bicistronic vector (AdCMVhSSTr2TK), and the animals were imaged for hSSTr2 expression with ^{99m}Tc -P2045 and TK with ^{131}I -FIAU (Fig 3). The biodistribution results showed the uptake of ^{99m}Tc -labeled P2045 and ^{131}I -labeled FIAU for AdCMVhSSTr2TK-injected tumors ($n = 8$) was 11.1% and 1.6% ID/g, respectively. AdCMVhSSTr2-injected tumors ($n = 4$) accumulated 10.2% ID/g of the ^{99m}Tc -labeled P2045 and 0.3% of the ^{131}I -labeled FIAU. AdCMVTK-injected tumors ($n = 4$) had 0.2% ID/g for

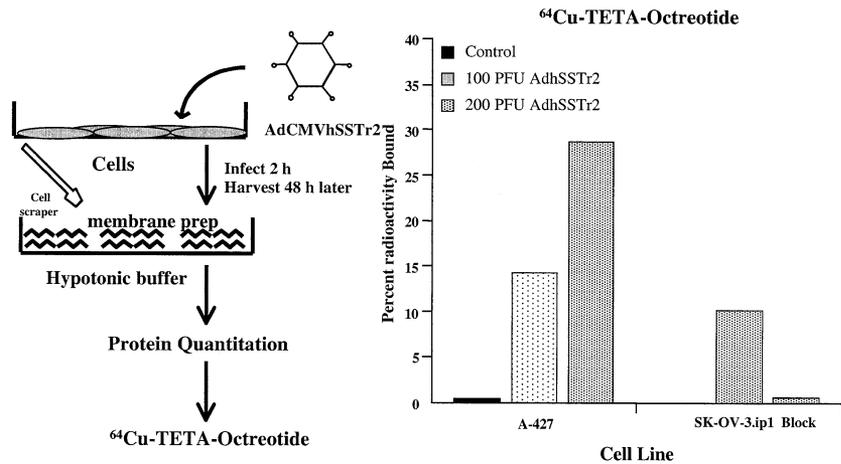


Fig 4. Induction of human somatostatin receptor subtype 2 (hSSTR2) and ⁶⁴Cu-TETA-octreotide binding in vitro.

the ^{99m}Tc-labeled P2045 and 3.7% for ¹³¹I-labeled FIAU.

It was shown that ⁶⁴Cu-TETA-octreotide bound to cell membrane preparations of A-427 and SK-OV-3.ip1 cells infected with AdCMVhSSTR2 (Fig 4). Tumor localization and pharmacokinetics of ⁶⁴Cu-TETA-octreotide was investigated in mice bearing IP SK-OV-3.ip1 human ovarian tumors induced to express hSSTR2 with AdCMVhSSTR2. Mice bearing IP SK-OV-3.ip1 tumors infected with 1 × 10⁹ pfu AdCMVhSSTR2 5 days after tumor cell inoculation followed by IP injection of ⁶⁴Cu-TETA-octreotide 2 days later had median tumor uptake of 25.1% ID/g at 4 hours after ⁶⁴Cu-TETA-octreotide administration (Fig 5). The uptake at 4 hours was significantly higher than when the control Ad (AdCMVLacZ) was given (1.6% ID/g). The tumor uptake of ⁶⁴Cu-TETA-octreotide decreased to 7.2% ID/g at 18 hours after injection.

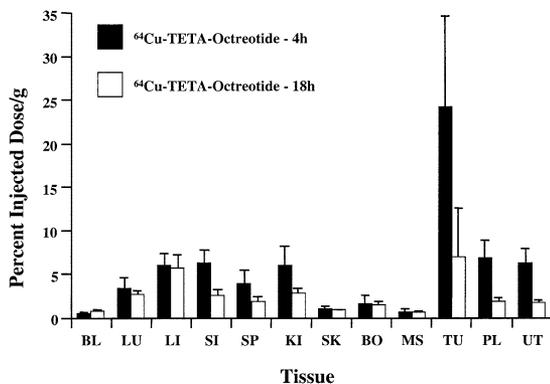


Fig 5. Biodistribution of ⁶⁴Cu-TETA-octreotide in mice bearing intraperitoneal (IP) SK-OV-03.ip1 tumors injected with AdCMVhSSTR2.

THERAPY STUDIES WITH THE SINGLE GENE VECTOR AdCMVhSSTR2

In a therapy study, we evaluated a single administration of 1.4 or 2.0 mCi of ⁶⁴Cu-TETA-octreotide 2 days after AdCMVhSSTR2 injection in mice bearing IP SK-OV-3.ip1 tumors. Also, mice received 2 doses of ⁶⁴Cu-TETA-octreotide. In this group of animals, 1.4 mCi of ⁶⁴Cu-TETA-octreotide was administered 2 days after AdCMVhSSTR2, followed by an additional dose of AdCMVhSSTR2 13 days after the first, and administration of 700 μCi of ⁶⁴Cu-TETA-octreotide 2 days later. Untreated animals had a median survival of 34 days, while median survival following a single 1.4 mCi dose was 36 days, and a single 2.0 mCi dose was 14 days. The mice that received 2 doses of ⁶⁴Cu-TETA-octreotide (1.4 plus 0.7 mCi) had a median survival of 62 days (Fig 6). Overall, the combination of gene therapy and ⁶⁴Cu-TETA-octreotide resulted in significantly (*P* < 0.05) longer survival of the mice. These results establish

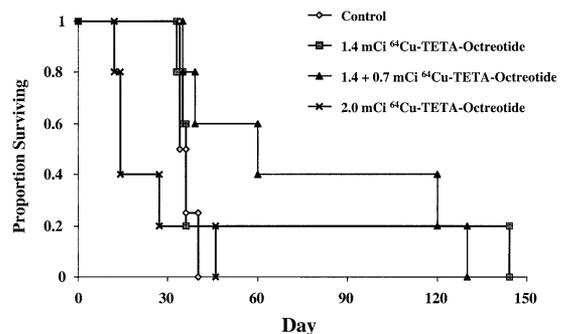


Fig 6. Survival of nude mice bearing intraperitoneal (IP) SK-OV-3.ip1 tumors treated with ⁶⁴Cu-TETA-octreotide.

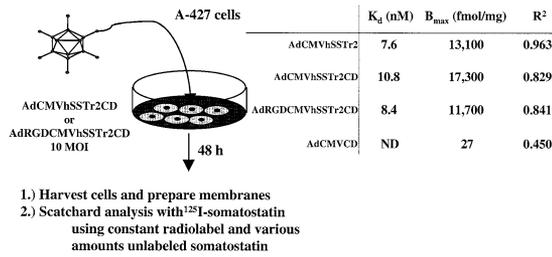


Fig 7. Evaluation of AdSSTR2CD bicistronic vectors for expression of human somatostatin receptor subtype 2 (hSSTR2).

the key feasibilities of inducing hSSTR2 expression in ovarian tumors and achieving therapy with a radiolabeled somatostatin analogue. Our hypothesis is that a radiolabeled ligand with a longer retention time would deliver a higher radiation absorbed dose and result in higher therapeutic efficacy.

Another somatostatin analogue that is being used for therapy is ^{90}Y -SMT 487.^{27,81} Nude mice were inoculated s.c. with 2×10^6 A-427 cells. Twenty-four days later the mice were administered 1×10^9 pfu AdCMVhSSTR2 i.t. (day 0), and the first measurement of the tumor size (surface area equal to length x width) was made with vernier calipers. Mice received an IV injection of either 400 or 500 μCi ^{90}Y -SMT 487 on days 2 and 4. The mice then received an additional i.t. injection of AdCMVhSSTR2 on day 7, followed by 2 more 400 or 500 μCi doses of ^{90}Y -SMT 487 on days 9 and 11. Control tumor-bearing mice either did not receive treatment or received 4, 500 μCi doses of ^{90}Y -SMT 487 on days 2, 4, 9, and 11 without AdCMVhSSTR2 injections.¹²¹ Mice that received

2 i.t. injections of AdCMVhSSTR2 and 4 doses of 400 or 500 μCi ^{90}Y -SMT 487 had median tumor quadrupling times of 40 and 44 days, respectively. The log-rank test revealed a statistically significant difference in time to tumor, quadrupling between the AdCMVhSSTR2 + ^{90}Y -SMT 487 treatment groups and the control groups ($P < 0.02$). The median tumor quadrupling times of the no treatment group and the no virus + 4 doses of 500 μCi ^{90}Y -SMT 487 group were 16 and 25 days, respectively.

THERAPY WITH THE BICISTRONIC VECTOR AdCMVhSSTR2CD

We have constructed and evaluated bicistronic Ad vectors encoding for hSSTR2 and the CD enzyme.¹²⁶ The rationale for the construction of these vectors is 2-fold. First, hSSTR2 can be used for noninvasive imaging to determine the expression of the therapeutic gene (CD) in vivo.¹²⁵ Second, hSSTR2 can be used for therapy as discussed previously, and the combination of this with CD mediated therapy through conversion of 5-FC to 5-FU may result in an additive or synergistic therapeutic effect. The A-427 cells infected with bicistronic vectors AdCMVhSSTR2CD or AdCMVhSSTR2CDRGD with the arginine-glycine-aspartic acid (RGD) peptide genetically engineered in the fiber knob to retarget Ad binding to integrins on the cell surface had equivalent hSSTR2 expression as the single gene vector AdCMVhSSTR2 (Fig 7). In addition, the AdCMVhSSTR2CD and AdCMVhSSTR2CDRGD vectors produced similar CD enzyme activity levels as the single gene vector AdCMVCD (Fig 8). Thus, both genes

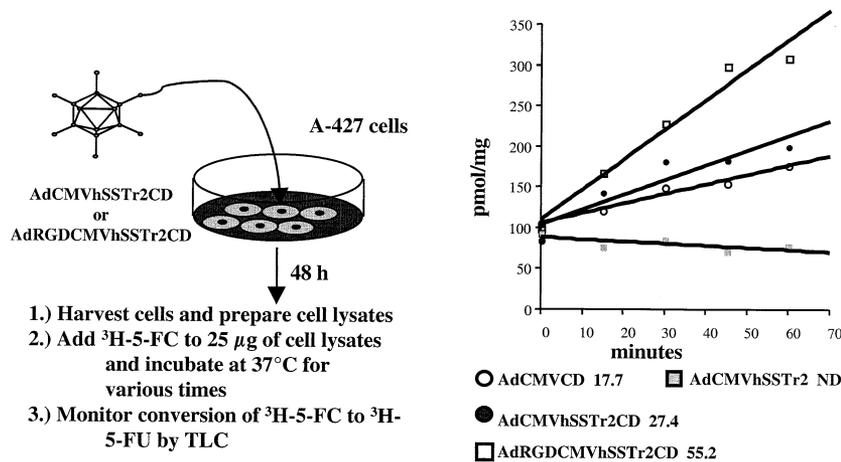


Fig 8. Evaluation of AdSSTR2CD bicistronic vectors for cytosine deaminase (CD) activity.

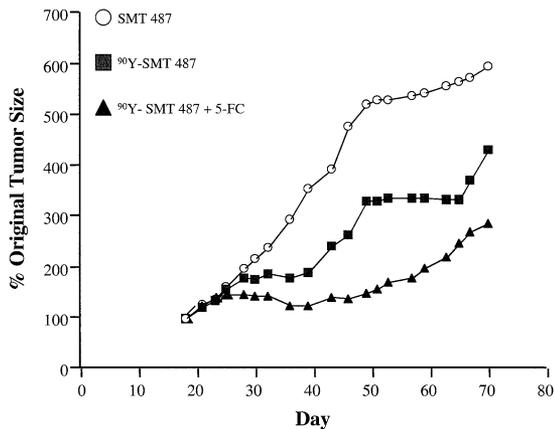


Fig 9. Therapy results with AdCMVSSTr2CD, 5-FC, and ⁹⁰Y-SMT 487 in athymic nude mice bearing subcutaneous (s.c.) A-427 xenografts.

were active in the bicistronic vectors. Therefore, therapy studies were initiated with AdCMVhSSTr2CD and ⁹⁰Y-SMT 487 in combination with 5-FC.

The AdCMVhSSTr2CD was injected i.t. into A-427 tumors at 1×10^9 pfu on days 20 and 27. The ⁹⁰Y-SMT 487 was administered IV on days 22, 24, 29, and 31 at 500 μ Ci per injection. The 5-FC was administered IP at 400 mg/kg twice a day for 5 days beginning on day 21, followed by another 5-day cycle beginning on day 28. Tumor inhibition results showed that ⁹⁰Y-SMT 487 and ⁹⁰Y-SMT 487 in combination with 5-FC inhibited tumor growth (Fig 9). Importantly, the combination treatment had a higher tumor growth inhibition than the ⁹⁰Y-SMT 487 treatment alone. In addition, the levels of toxicity (weight loss) were modest (Fig 10).

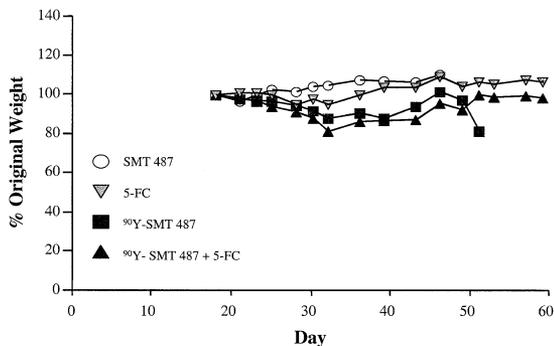


Fig 10. Toxicity with AdCMVSSTr2CD, 5-FC, and ⁹⁰Y-SMT 487 in athymic nude mice bearing subcutaneous (s.c.) A-427 xenografts.

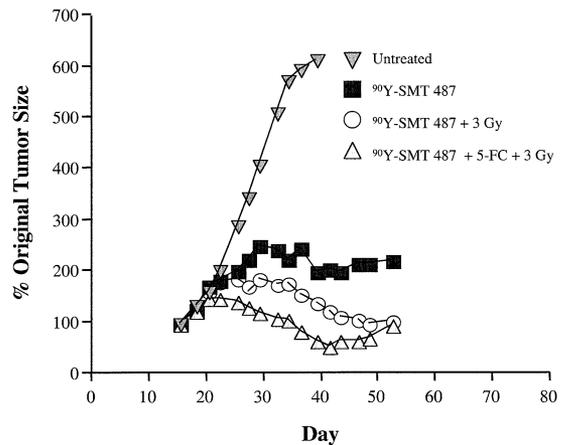


Fig 11. Therapy results with AdCMVSSTr2CD, 5-FC, ⁶⁰Co, and ⁹⁰Y-SMT 487 in athymic nude mice bearing subcutaneous (s.c.) A-427 xenografts.

The next therapy study consisted of intratumoral injections of AdCMVhSSTr2CD into A-427 tumors at 1×10^9 pfu on days 18, 25, 32, and 39. The ⁹⁰Y-SMT 487 was administered IV on days 20, 22, 27, 29, 34, 36, 41, and 43 at 500 μ Ci per injection. The 5-FC was administered IP at 400 mg/kg twice a day for 5 days beginning on day 19, followed by 3 more 5-day cycles beginning on days 26, 33, and 40. The ⁶⁰Co was given as a single 3 Gy dose on days 21, 28, 35, and 42. Tumor inhibition results were extremely encouraging because they show that the combination of ⁹⁰Y-SMT 487 + 5-FC + 3 Gy resulted in tumor regressions (Fig 11 and Table 1). All combination therapies had at least 2 complete regressions with most being recurrence-free. The triple therapy groups had the highest mean tumor growth suppression of all treatment groups, but these differences were not statistically significant ($P = 0.116$). The problem, however, was that the intense therapeutic regimen was not well tolerated (Table 2). A summary of the weight change data is shown in Table 2. From before and after weight (paired *t*-test) comparisons, the controls had a significant weight gain ($P < 0.001$), while ⁹⁰Y-SMT 487 (400 μ Ci x8) and ⁹⁰Y-SMT 487 (500 μ Ci x4) + 5-FC + 3 Gy had no significant weight changes ($P = 0.065$ and 0.216, respectively), and all other treatments groups had significant mean weight losses (all $P \leq 0.01$). We plan to investigate lower doses of 5-FC and ⁹⁰Y-SMT 487 in future studies.

Table 1. Tumor Doubling Times and Complete Regressions in AdCMVhSSTR2CD Experiments

Treatment	no.	Tumor Doubling Time (mean/median)	Complete Regressions	
			Total (n)	No Relapse (n)
Untreated controls	15	16/10	1	1
⁹⁰ Y-SMT 487 (500 μ Ci \times 8)	8	19/9	0	0
⁹⁰ Y-SMT 487 (400 μ Ci \times 8)	8	25/12	0	0
⁹⁰ Y-SMT 487 (500 μ Ci \times 8) + 3 Gy	8	29/25	3	2
⁹⁰ Y-SMT 487 (400 μ Ci \times 8) + 3 Gy	8	32/19	2	1
⁹⁰ Y-SMT 487 (500 μ Ci \times 4) + 5-FC + 3 Gy	8	47/19	3	3
⁹⁰ Y-SMT 487 (400 μ Ci \times 8) + 5-FC + 3 Gy	8	53/32	3	3

CONCLUSION

These studies show that genetic induction of hSSTR2 results in tumor localization of radiolabeled peptides at a level sufficient to produce therapeutic effects. Efforts continue to optimize this novel approach to cancer gene therapy by molecular chemotherapy and radiation therapy.

Table 2. Animal Weight Changes in AdCMVhSSTR2CD Experiments

Treatment	no.	Percent Change	Percentage with >20% loss
Untreated controls	15	117	0
⁹⁰ Y-SMT 487 (500 μ Ci \times 8)	8	78	63
⁹⁰ Y-SMT 487 (400 μ Ci \times 8)	8	85	38
⁹⁰ Y-SMT 487 (500 μ Ci \times 8) + 3 Gy	8	84	38
⁹⁰ Y-SMT 487 (400 μ Ci \times 8) + 3 Gy	8	78	50
⁹⁰ Y-SMT 487 (500 μ Ci \times 4) + 5-FC + 3 Gy	8	91	50
⁹⁰ Y-SMT 487 (400 μ Ci \times 8) + 5-FC + 3 Gy	8	79	50

The development of new ligands (eg, minibody and diabodies) against hSSTR2 and mutant forms of hSSTR2 offer the potential for higher and more specific tumor uptake and, thus, improved sensitivity of detection and higher therapeutic efficacy. Other approaches that are under active investigation include gene transfer of the type-2 dopamine receptor expressed on the tumor cell surface detected by PET using a radiolabeled antagonist, or herpes simplex virus TK gene transfer detected by gamma camera or PET imaging with radiolabeled substrates trapped intracellularly after phosphorylation by the kinase.¹²⁷⁻¹²⁹ Gene transfer of the sodium iodide symporter has also been used for imaging and therapy. Clinical studies are needed to determine the most promising approach.

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