

AD _____

Award Number: DAMD17-02-1-0261

TITLE: Nuclear Imaging for Assessment of Prostate Cancer Gene Therapy

PRINCIPAL INVESTIGATOR: Dongfeng Pan, Ph.D.

CONTRACTING ORGANIZATION: University of Virginia
Charlottesville, VA 22908-1339

REPORT DATE: March 2007

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

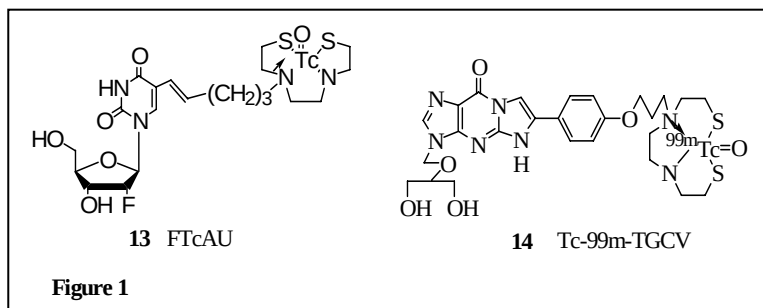
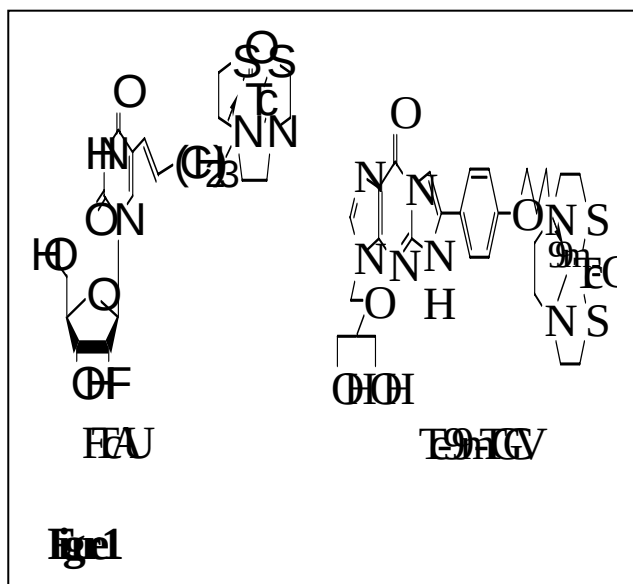
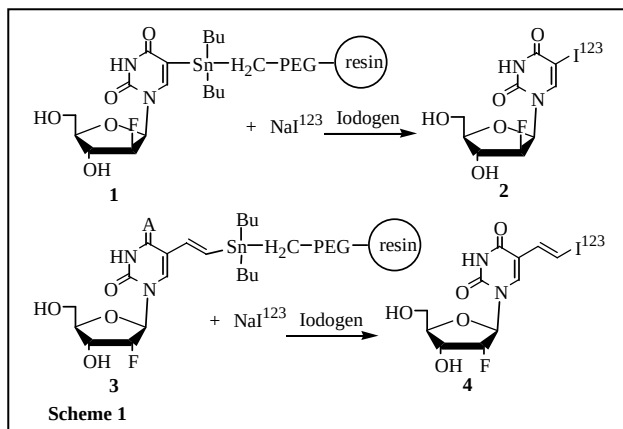
The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE 01-03-2007		2. REPORT TYPE Final		3. DATES COVERED 12 Mar 2002 – 11 Feb 2007	
4. TITLE AND SUBTITLE Nuclear Imaging for Assessment of Prostate Cancer Gene Therapy				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER DAMD17-02-1-0261	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Dongfeng Pan, Ph.D. E-mail: dp3r@virginia.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Virginia Charlottesville, VA 22908-1339				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES Original contains colored plates: ALL DTIC reproductions will be in black and white.					
14. ABSTRACT Background: Combination of the cytotoxic viral thymidine kinase (tk) and the prodrug, acyclovir (ACV) has been reported to inhibit the growth of the C4-2 tumor, a subline of LNCaP. However, it remains unsolved to non-invasively detect the in vivo distribution, expression and persistence of the toxic gene as well as to evaluate the therapeutic effect. In this project, we will develop a nuclear gene imaging approach to assist the cytotoxic gene therapy study for prostate cancer. Objective/Hypothesis: The distribution, expression, and persistence of the prostate specific Ad-PSA-tk in the C4-2 tumor xenograft model will be non-invasively and repeatedly determined in vivo by tracing the radiolabeled TK substrates with a SPECT imaging modality. Specific Aim of the first year: To synthesize a radiolabeled TK substrate, 2'-Deoxy-2'fluoro-5-{3-oxo[N,N-bis(2-mercaptoethyl)ethylenediaminato][Tc-99m] technetium(V)-1(E)-propenyl}uridine, for TK detection using a small animal gamma detector. Progress and outcome: In last report of 2003 which covers from September of 2002 to March of 2003, we reported our efforts to synthesize fragments A and B. In this report we successfully linked the radiometal chelator with fluorothymidine. We will characterize the structure of the final tracer and test the pharmacokinetics and pharmacodynamics of the tracer in next research year. Also, the Adenoviral vectors with reporter genes of tk and luciferase were constructed. The luciferase gene expression in live mouse model was non-invasively imaged and the result was posted in 2003 Annual Meeting of ASGT (American Society of Gene Therapy).					
15. SUBJECT TERMS prostate cancer, gene therapy, imaging					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	14	19b. TELEPHONE NUMBER (include area code)

Table of Contents

Introduction.....	4
Body.....	5
Key Research Accomplishments.....	13
Reportable Outcomes.....	13
Conclusions.....	13
References.....	14
Appendices.....	None

Introduction



The objective of this proposal is to develop a noninvasive imaging assay using single photon emission computed tomography (SPECT) for assessment of gene therapeutic efficacy and diagnosis of metastasis of prostate cancer. Noninvasive nuclear imaging of Herpes simplex virus type-1 thymidine kinase (HSV1-TK) expression has gained broad interests because of its potential in clinical application. The first aim of this project is to develop novel SPECT imaging probe, HSV1-TK substrates, for non-invasive assessment of gene therapeutic efficacy and diagnosis of metastasis of prostate cancer. The tracers would have merits of easy preparation and low cost.

In the original plan, we proposed a “kit” method to prepare 1-(2-deoxy-2-fluoro-β-D-ribofuranosyl)-5(E)-(2-iodovinyl)uracil (IVFRU) and 1-(2-deoxy-2-fluoro-β-D-arabino furanosyl)-5-iodo-uracil (FIAU) with solid-phase chemistry (Scheme 1). In brief, the precursors of IVFRU and FIAU will be linked to a resin bead via C-Sn bond at the site where the radioiodine would be attached (1 and 3). The oxidative radioiodination would cleave and produce only the desired products from the resin material. The excess of precursors would remain attached with the beads. The solid-phase synthetic approach would provide a simple and clean protocol to radiolabel IVFRU and FIAU with high specific activity and chemical purity.

However, we failed to produce compound **11** (Scheme 2), which is the key intermediate to synthesize both precursors **1** and **3**. Meanwhile, recent progress of

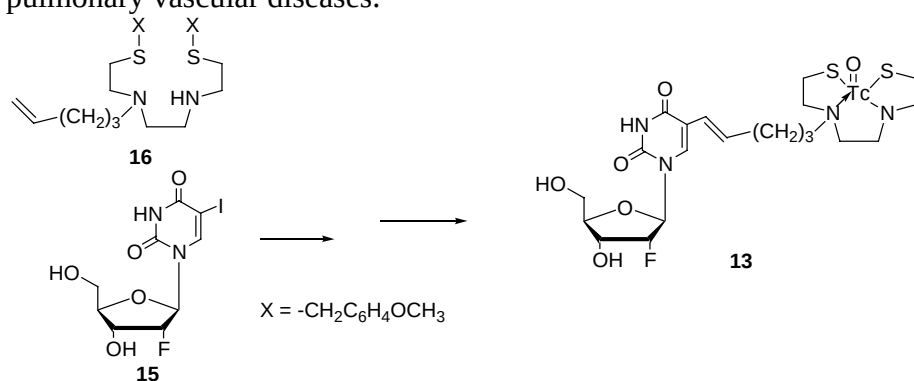
Tc-99m chemistry that Tc-99m labeled radiopharmaceuticals, such as TRODAT, being able to penetrate lipid membrane raised our interest to explore a Tc-99m labeled TK substrate for gene imaging, because of the nearly optimal nuclear properties of Tc-99m, as well as its convenient and low cost production by means of commercial generator columns made it even more appealing than radioiodine

labeled probes. As a result, we designed a series of thymidine and acyclic guanine analogs with Tc-99m conjugated. The fact that among the thymidine substrates of HSV1-TK the substitution on 5 position of thymine ring shows the most variability and flexibility[1-4] lead us to design our first Tc-99m-labeled probe using 5-substituted 2'-fluoro-2'-deoxyuridine as a template. The first compound we synthesized is 2'-Deoxy-2'-fluoro-5-{3-oxo[N,N-bis(2-mercaptoethyl)ethylenediaminato][Tc-99m] technetium(V)-1(E)-propenyl}uridine **13** (FTcAU) (Figure 1), in which a neutral metal chelating moiety is linked. Later, we explored a tricyclic analog of GCV, 3-((1,3-dihydroxypropan-2-yloxy)methyl)-6-(4-(3-((2-mercaptoethyl)(2-mercaptoethylamino)ethyl)amino)-propoxy)phenyl)-3H-imidazo[1,2-f]-purin-9(5H)-one-oxo-[Tc-99m] **14** (Tc-TGCV) (Figure 1) based on the fact of some of tricyclic analogs have been studied as potential fluorescent cellular imaging probes for herpes virus assay and demonstrated the similar activity and selectivity to HSV1-TK as ACV or GCV [5-7]. The synthesis of FTcAU and Tc-TGCV have been published

[8, 9] and, however, both of compounds demonstrated limit uptake in thymidine kinase transfected EL4 cells. Further exploration of Tc-99m conjugated potential HSV1-TK substrates is still undergoing in our laboratory.

The second aim is to monitor prostate-cancer-specific tk gene expression using the newly developed imaging probes with a SPECT imaging modality. While waiting for obtaining a potent Tc-labeled TK substrate, we decide to test the hypothesis that the distribution, expression, and persistence of the prostate specific Ad-PSA-Luc in C4-2 tumor xenograft model can be non-invasively and repeatedly determined *in vivo* by our newly acquired Xenogen small animal optical system.

The 5837 bp prostate-specific antigen promoter, which is highly active in both androgen-dependent and androgen-independent prostate cancer cells, has been demonstrated the utility for tissue-specific toxic gene therapy for prostate cancer[10, 11]. Therefore, an adenovirus vector (AdPSA-Luc), which can express firefly luciferase under the control of was constructed and tested in mice with C4-2 xenograft and normal nude mice. However, our results showed that, instead of targeting prostate, the AdPSA-Luc led to highly specific luciferase expression in lungs of both human prostate cancer mouse models and naïve mice from 7 days after systemic injection, although the luciferase level of expression in human prostate cancer mouse models was significantly higher than in naïve mice. These results indicate the potential limitations of the suicide gene therapy of prostate cancer based on the selectivity of PSA promoter. By contrary, it has encouraging implications for the further development of vectors via PSA to enable gene therapy for pulmonary vascular diseases.

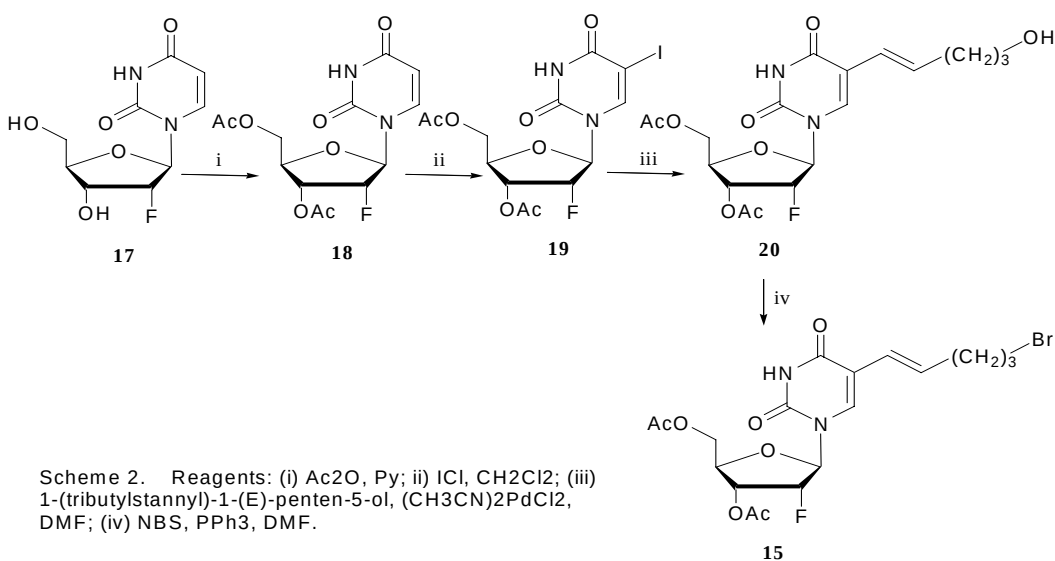


Scheme 1

Body

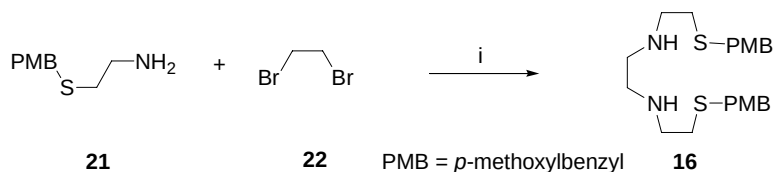
1. Synthesis and Characterization of FTcAU and Tc-TGCV

a. FTcAU



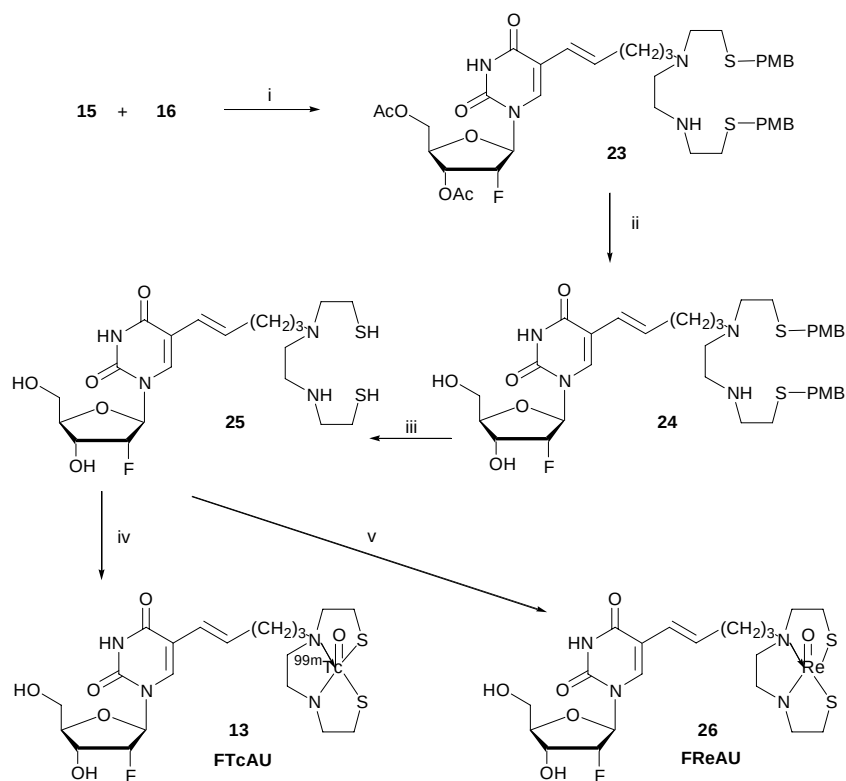
The target molecule, 2'-Deoxy-2'-fluoro-5-{3-oxo[N,N-bis(2-mercaptoethyl)ethylenediaminato] [Tc-99m] technetium(V)-1(E)-propenyl}uridine **13**, was convergently synthesized from synthons **15** and **16**. The detail of the synthesis was reported in *Tetrahedron Letters*, 2004, 45, 8673-8676.

Briefly, the synthesis of 5-(5-bromopent-1(E)-enyl)-1-(3,5-diacetyl-2-fluoro-2-deoxy-1-β-D-ribofuranosyl)uracil **15** and *N,N'*-bis-[2-(4-methoxybenzylsulfanyl)ethyl]ethylenediamine **16** are outlined in scheme 2 and 3.



Scheme 3. Reagents: (i) CeOH, molecular sieve (4Å), DMF.

Coupling of thymidine analog **15** and chelator fragment **16** produces **23**. The removal of acetyl protecting groups of **23** with potassium carbonate in aqueous methanol yields **24**. The thiol protecting groups, 4-methoxybenzyl, of **24** are removed with Hg(OAc)₂ in TFA to give trifluoroacetate salts of **25**. The crude air-sensitive compound **25** is conjugated with technetium immediately, without purification. Addition of [^{99m}Tc]pertechnetate in PBS into the aqueous methanol of the crude **25** in the presence of Sn-glucoheptonate in 80°C water bath for 30 min and thereafter HPLC purification yields the target compound, FTcAU **13** with radiochemical yield of 42%. To characterize the chemical structure of the FTcAU **13**, its analog of rhenium-188 conjugate, FReAU **26**, is synthesized with modifying a similar reaction condition by adding tetrabutylammonium tetrachlorooxorhenate(V) into a solution of compound **25** in methanol and stirring for 12 hours. The rhenium conjugate **26** is purified by flash chromatography and its chemical structure is characterized with ¹HNMR and high resolution ESI-MS. The characterization of FTcAU is carried out using reverse phase HPLC by co-injection with FReAU (scheme 4).



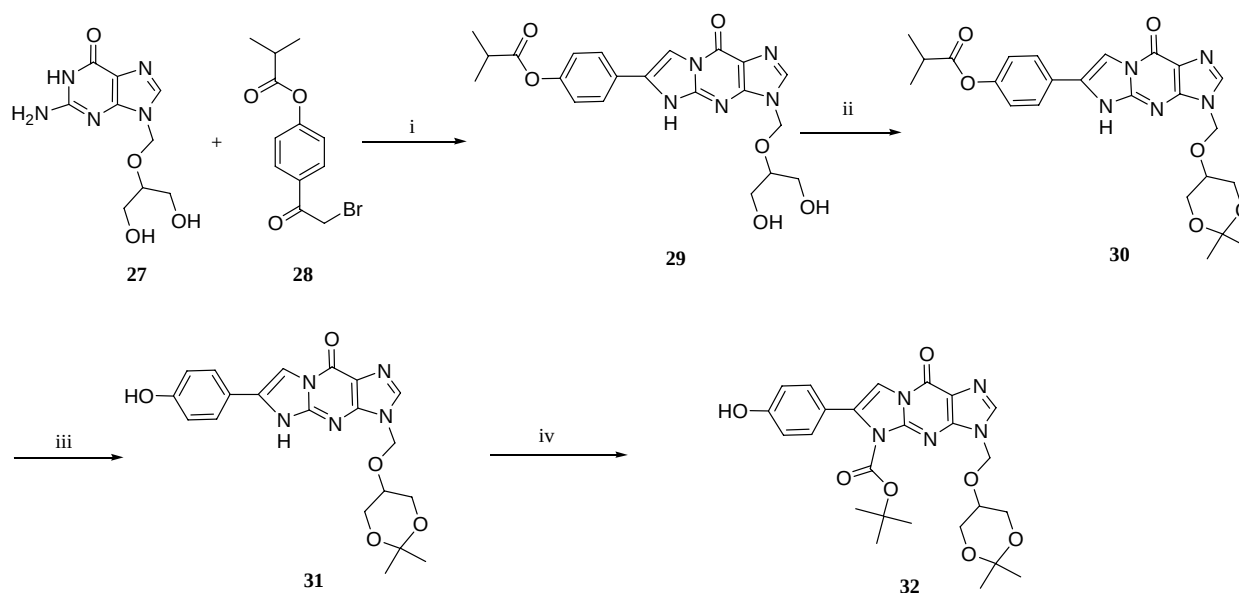
Reagents: (i) DIEA, CH₃CN; (ii) K₂CO₃; (iii) Hg(OAc)₂/TFA, H₂S; (iv) [^{99m}Tc]NaTcO₄, Sn-glucate; (v) (Bu₄N)⁺(ReOCl₄)⁻.

Scheme 4

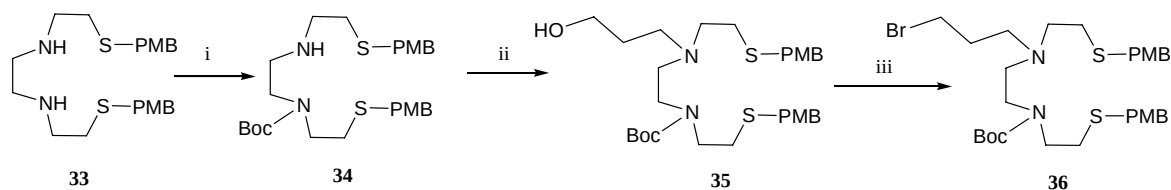
b. Tc-TGCV

Synthesis of 3-((1,3-dihydroxypropan-2-yloxy)methyl)-6-(4-(3-((2-mercaptoethyl)(2-(2-mercaptoethylamino)ethyl)amino)propoxy)phenyl)-3H-imidazo[1,2-f]purin-9(5H)-one [^{99m}Tc-**14** (Tc^{99m}-TGCV)], was started from gancyclovir **27**.

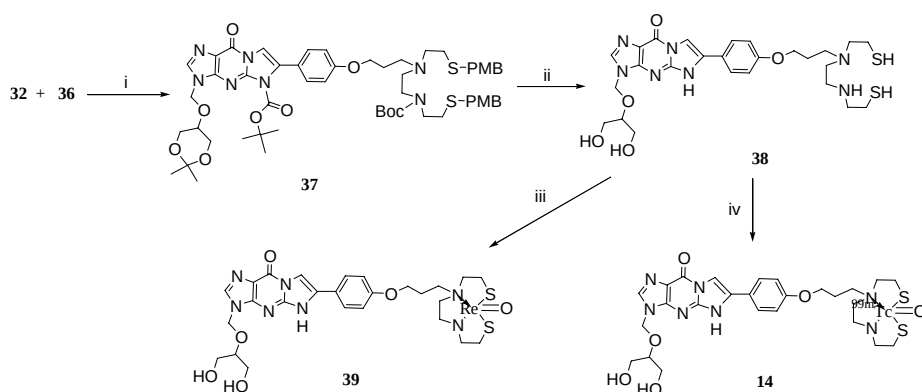
The synthesis is briefly described as schemes 5, 6, and 7. Coupling GCV **27** and phenacyl bromide **28** yielded the tricyclic TGCV **29**, followed by protecting the free hydroxyls with 2-methoxypropene in presence of TsOH to give isopropylidene ketal **30**. Removal of the isobutanoyl group of **30** with saturated methanolic ammonia gave phenol **31**. The secondary amino group at position 5 was protected with t-Boc by mixing compound **31** with di-tert-butyl dicarbonate in 1,4-dioxane and aqueous Na₂CO₃ to yield synthon **32** (Scheme 5). The synthesis of radiometal-chelating moiety **36** started from N,N'-bis-[2-(4-methoxybenzylsulfanyl)-ethyl]-ethylenediamine **33**. After blocking one of the secondary amino groups of **33** with t-Boc to produce **34**, the monoalkylated **35** was obtained by coupling of **34** with 3-bromo-1-propanol. The subsequent bromination of **35** with NBS/triphenylphosphine yielded **36** (Scheme 6). Coupling of TGCV derivative **32** and chelator fragment **36** produced **37**. After deprotection of **37** with Hg(OAc)₂ in TFA, to the crude intermediate **38** (50 ug) in aqueous methanol in the presence of Sn-glucose heptonate was added 2.1 mCi of ^{99m}Tc pertechnetate in PBS buffer, and 1.5 mCi of the target radiochemical **14** was obtained after HPLC purification with radiochemical yield of 73% (Scheme 7). To characterize the chemical structure of the radiochemical **14**, a rhenium conjugated analog **39** was synthesized by adding tetrabutylammonium tetrachlorooxorhenate(V) into compound **38** in methanol and stirring for 12 h. The rhenium conjugated **39** was characterized with ¹H NMR and mass spectrum. The characterization of Tc-^{99m} tracer **14** was carried out by co-injection with rhenium analog **39** using reverse phase HPLC.



Scheme 5. Reagents: (i) NaH/DMF; ii) DMF, 2-methoxypropene, TsOH/H₂O; (iii) NH₃-MeOH; (iv) Boc₂O, 1,4-dioxane, aq Na₂CO₃.



Scheme 6. Reagents: (i) Boc₂O, 1,4-dioxane, aq Na₂CO₃; (ii) acetonitrile, 3-bromo-1-propanol, DIEA; (iii) NBS, PPh₃, DMF.



Scheme 7. Reagents: (i) CHCl₃, aq Na₂CO₃, Bu₄N⁺Br⁻; (ii) Hg(OAc)₂/TFA, H₂S; (iii) (Bu₄N)⁺(ReOCl₄)⁻; (iv) [^{99m}Tc]NaTcO₄, Sn-glucate

2. Monitoring PSA-specific reporter gene expression in mouse models

a. Construction of recombinant adenoviruses.

All plasmid constructs were prepared using standard methods[12]. The original pPSA-Luc containing the 5.8-kb PSA promoter was obtained from Dr. Leland W.K. Chung. It was generated by inserting a HindIII fragment of the PSA promoter in the multiple cloning site (MCS) of pGL3-basic vector (Promega, Madison, WI). pShuttle-PSA-Luc was generated by inserting a KpnI/SalI fragment of pPSA-Luc, which contains PSA promoter, luciferase gene, and SV40 late poly(A) signal, in the MCS of pShuttle vector. AdEasy Vector

System was used for construction of recombinant adenovirus of the firefly luciferase under the control of the 5837 bp prostate-specific antigen promoter. Briefly, pShuttle-PSA-Luc was linearized with PmeI and co-transformed into Escherichia coli strain BJ5183 together with pAdeasy-1, the viral DNA plasmid. The pAdeasy-1 is E1 and E3 deleted, its E1 function can be complemented in 293A cells. The recombinant adenoviral construct, which was named pAd-PSA-Luc was then cleaved with PacI to expose its inverted terminal repeats (ITR) and transfected into 293A cells to produce viral particles. The recombinant virus was identified with restriction analysis, PCR, RT-PCR, and detection of luciferase activity. The recombinant virus Ad-PSA-Luc was purified through two cesium chloride gradients, and then purified virus was desalted by dialysis at 4 °C against 10 mM Tris–hydrochloric acid buffer with 10% glycerol and stored in aliquots in liquid nitrogen. The titer of virus preparations was determined by plaque assay according to the application manual of the pAdEasy Vector System. As a control, AdCMV-Luc, which contains CMV promoter and luciferase gene, was constructed as described above.

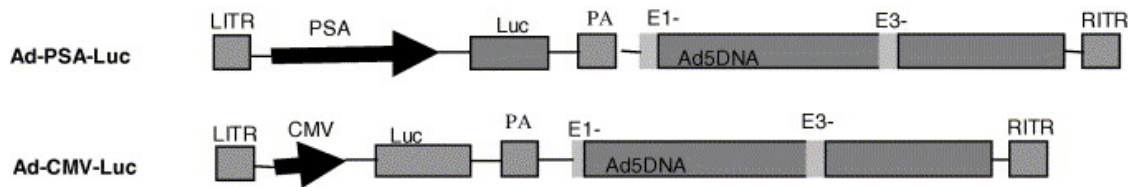


Fig. 2. Schematic representation of Ad vectors used in this study. ITR, Ad inverted terminal repeats; CMV, human cytomegalovirus promoter; PSA, prostate specific antigen promoter; Luc, firefly luciferase gene.

b. Evaluation of expression of AdPSA-Luc in prostate and non-prostate cancer cells[12].

Cells C4-2, derived from human LNCaP tumor, or non-PSA-producing cells, WH, at 80% confluency in 24-well plates were infected with AdPSA-Luc or AdCMV-Luc at the specified PFU per cell. From 24 h after transduction, an aqueous solution of substrate, D-firefly luciferin (Xenogen, Alameda, CA) was added into cells (5ul/well, 15 mg/ml) and the imaging was performed by an IVIS CCD camera (Xenogen). Fig. 3A shows that AdPSA-Luc led to significantly higher level of luciferase expression in C4-2 than in WH on days 2 after transduction when they were transduced at 80, 40, 20, 10, 5, and 2.5 PFU of AdPSA-Luc per cell. We imaged the infected cells from 2 to 5 days after transduction and the similar results were obtained. The infectivities of C4-2 and WH were compared by using the same range of AdCMV-Luc as the infecting viruses (Fig. 3B). The high tissue-specificity of the long PSA promoter has been well determined in its recombinant plasmids, transgenic animals as well as adenoviral vectors. Our results are consistent with these publications. In contrast, Shi et al. [L1, 18] reported that the PSA promoter cassette in helper-dependent adenoviral vectors maintained strict tissue-specific expression, but lost specificity when expressed from first-generation adenoviral vectors. The difference amongst these results may be due to variations in sequence of the promoters or difference in methodology. In addition, we observed that AdPSA-Luc just elicited higher level of Luc expression in C4-2 cells than in WH cells in a short period (24 h) after transduction beyond 200 PFU per cell and decreased rapidly Luc expression in C4-2 cells into an extent even less than in WH cells because of higher adenoviral toxicity in C4-2.

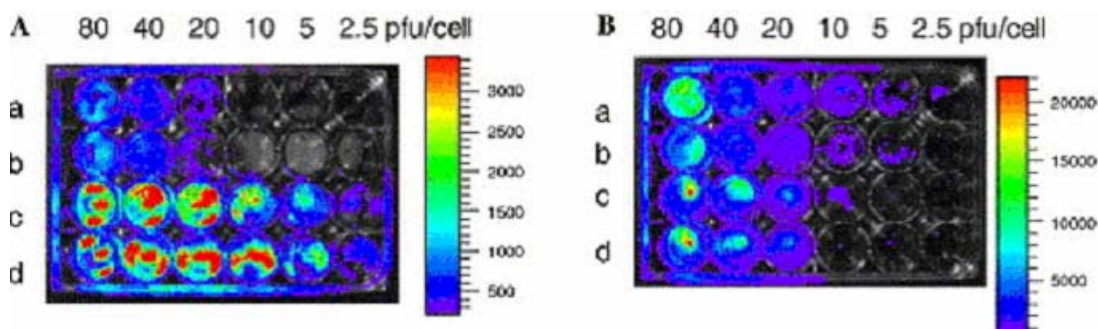


Fig. 3. Comparison of luciferase expression following transduction of prostate and non-prostate cell line with AdPSA-Luc. The PSA-positive cell line C4-2 (c,d) and non-prostate cell line WH (a,b) were transduced with AdPSA-Luc (A) or AdCMV-Luc (B) and imaged by CCD camera 2 days later. The acquisition times: (A) 5 min; (B) 10 s.

c. Specific expression of luciferase gene in lungs of naïve nude mice directed by prostate-specific antigen promoter

Six- to eight-week-old male athymic nude mice (nu/nu strain [Balb/c background], Harlan Sprague-Dawley, Indianapolis, IN) were used for all in vivo experiments. Adenoviruses (2.0×10^9 PFU) AdPSA-Luc and AdCMV-Luc were injected into naïve nude mice ($n = 4$), respectively, via tail vein. On days 3, 7, and 11, the CCD images were obtained using a cooled IVIS CCD camera (Xenogen, Alameda, CA) and images were analyzed. Light was monitored in all of the experiments described at 5 min after injection of luciferin. The CCD signals were quantified as total relative light units per minute of acquisition time (RLU/min) in the region of interest (ROI). On day 11, mice were sacrificed and isolated organs were imaged.

On day 3, only weak liver and chest signals can be detected. On day 7, light signals in the chests and lower abdomens were apparent. The chest signals were emitted by 1.8×10^3 , 1.2×10^4 , and 6.0×10^4 RLU/min respectively from day 3 to 11 (Figs. 4A–D). On day 7, the outlines of lungs were imaged distinctly (Fig. 4B). As a control, AdCMV-Luc containing CMV promoter and luciferase gene was injected into normal nude mice via tail vein. The strong liver signals (5.3×10^6 , 3.6×10^7 , and 4.1×10^7 RLU/min, respectively) could be seen from 3 to 11 days after injection (Figs. 4E–G). On day 11, mice were sacrificed and isolated organs were imaged. The signals in the chest injected with AdPSA-Luc were found to originate from lungs (Fig. 5). The signal in whole lung from AdPSA-Luc injected mice displayed about 190-fold higher than from AdCMV-Luc injected mice (Fig. 5). However, the signals in the isolated livers of the mice injected with AdCMV-Luc appeared to be 1.0×10^5 -fold higher than in the lungs while the signals could not be detected in the isolated livers of the mice injected with AdPSA-Luc (Fig. 5). According to these results, AdPSA-Luc displayed high level of specific expression in lungs compared to AdCMV-Luc.

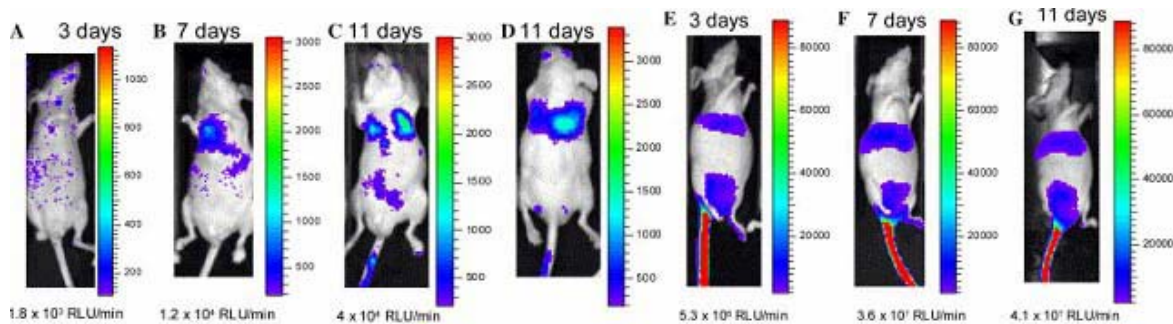


Fig. 4. Adenoviral vector-mediated luciferase gene delivery and expression in the lung and liver after systemic administration. CCD images of mice injected with either the prostate-specific AdPSA-Luc (A–D) or AdCMV-Luc (E–G) via tail vein. The images represent the results from one animal of each cohort at 3, 7, and 11 days post-injection. The relative light intensity emitted from the animal was quantified by image analysis software and represented by the color scale, shown next to the images. The acquisition times: (A–D) 5 min; (E) 30 s; and (F,G) 5 s. (D) The mouse was imaged from the back. The acquisition times were reduced to offset saturated liver signal intensities in AdCMV-Luc cohort.

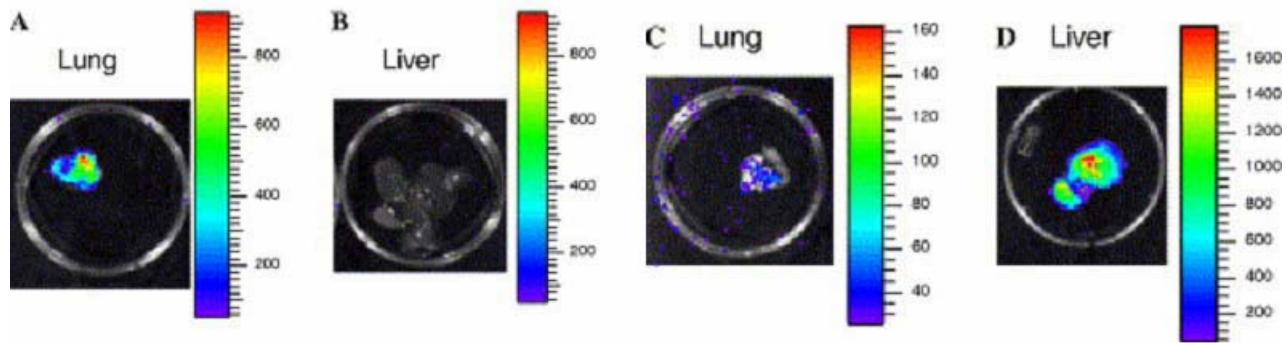


Fig. 5. Imaging of the isolated organs from mice in Fig. 4. (A,B) The lung and liver from the AdPSA-Luc injected mouse; (C,D) the lung and liver from the AdCMV-Luc injected mouse. The acquisition times: (A–C) 5 min; (D) 5 s.

d. PSA-specific gene expression of mouse model of human prostate cancer[13]

Male athymic nude mice, 6–8 weeks old [nu/nu strain (Balb/c background); Harlan Sprague–Dawley, Indianapolis, IN, U.S.A.], were injected of C4-2 cell suspension (1×10^6 cells) in $50 \mu\text{l}$ of medium, mixed with $50 \mu\text{l}$ of Matrigel (Collaborative Biomedical Products, Bedford, MA, U.S.A.) in the left flank ($n=20$). After tumor grown to 5 to 10 mm AdPSA-Luc [$100 \mu\text{l}$, 1×10^9 pfu (plaque-forming units)] was either injected directly into the tumour site ($n=4$) (Fig. 6) or vian tail vein. The virus was allowed to distribute throughout the tumour for 2 days, following which the mice were anaesthetized (ketamine/xylazine, 4:1) and imaged using the CCD camera with D-luciferin as the substrate. The mice were imaged repeatedly every 2–5 days for 30 days.

For mice with intra-tumoral injection of AdPSA-Luc, we detected luciferase gene expression in the tumour from day 2 and observed it to persist strongly until day 30 (bioluminescence signal ranged from 6.1×10^4 to 3.8×10^5 RLU/min, $n=4$). But light signals in the chests were also apparent from day 7 and the outlines of lungs were imaged distinctly. On day 30, mice were killed and isolated organs were imaged. The signals in the chest were found to originate from lungs

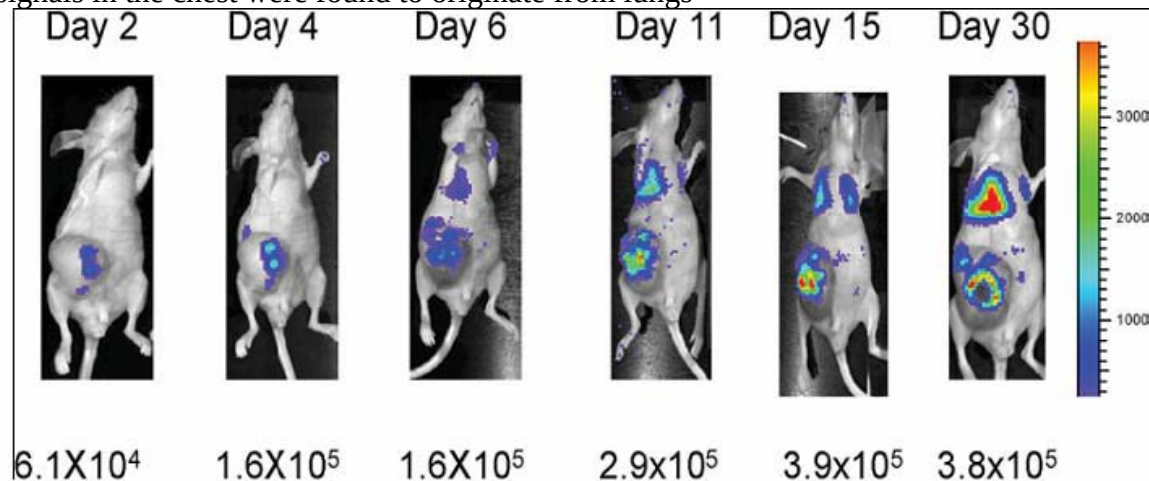


Fig. 6. AdPSA-Luc-mediated expression of luciferase reporter gene in human prostate cancer mouse models post intratumoural injection. AdPSA-Luc ($100 \mu\text{l}$, 1×10^9 pfu) was injected directly into the tumour site. The mice were imaged using the CCD camera on days 2, 4, 6, 11, 15 and 30 by using D-luciferin. Luciferase gene expression in the tumour was detected from day 2 and we observed it to persist strongly until day 30 bioluminescence signal ranged from 6.1×10^4 to 3.8×10^5 RLU/min, $n=4$). But light signals in the chests were also apparent from day 7 and the outlines of lungs were imaged distinctly. The images represent the results for one animal.

Systemic tail-vein injection of AdPSA-Luc led to significant luciferase expression in the lungs of

both human prostate cancer mouse models and naïve mice, but significantly higher in the former (Fig. 7). AdPSA-Luc led to higher light emission in the chests of three mice, in total six human prostate cancer models, than in naïve mice from all of time points (Figures 7A–2F). On day 3, the weak liver signals could only be detected. On day 7, light signals in the chests were apparent. The chest signals emitted by 2.5×10^3 , 2.8×10^4 and 1.3×10^5 RLU/min ($n=4$) respectively from prostate cancer mice and 1.1×10^3 , 9.0×10^3 and 3.0×10^4 RLU/min ($n=4$) respectively from naïve mice on days 3, 7 and 11. On day 7, the outlines of lungs were imaged distinctly. As a control, AdCMV-Luc containing CMV promoter and luciferase gene was injected into nude mice with human prostate tumour xenograft via tail vein. The strong liver signals [8.4×10^6 , 4.3×10^7 and 6.4×10^7 RLU/min ($n=4$) respectively] could be seen on days 3, 7 and 11 after injection (Figures 7G–I). On day 11, mice were killed and isolated organs were imaged. The signals in the chest were found to originate from the lung. The signal in the whole lung from AdPSA-Luc-injected prostate cancer mouse models was approx. 4.5-fold higher than from naïve mice and 855.0-fold higher than from AdCMV-Luc-injected mice (Figure 8). However, the signals in the isolated livers from the mice injected with AdCMV-Luc appeared to be 1.2×10^5 -fold higher than in the lungs, whereas the signals could not be detected in the isolated livers from the mice injected with AdPSA-Luc (Figure 3). Furthermore, in order to identify lung metastases in prostate cancer mouse models, PSA expression in lung was detected with RT–PCR (Figure 9). The expected human PSA cDNA fragment, whose size was expected as 234 bp, was amplified from prostate cancer mouse models but not from normal mice. In addition, we detected luciferase mRNA expression in lungs from both prostate cancer mouse models and naïve mice with RT–PCR (Figure 9).

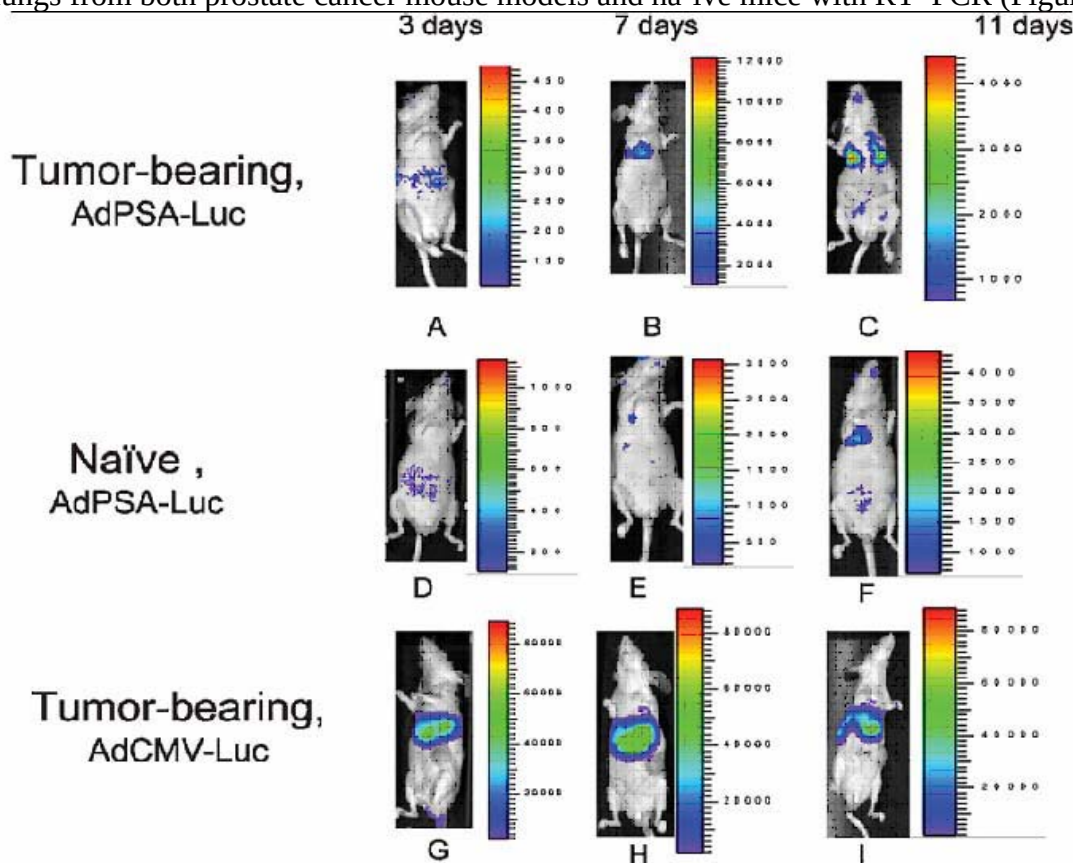


Fig. 7. (A–C, G–I) Human prostate cancer mouse models; (D–F) a naïve mouse. The images represent the results for one animal of each cohort ($n=4$) at 3, 7 and 11 days post injection. The relative light intensity emitted from the animal was quantified by image analysis software (Living Image® 2.50) and is represented by the colour scale, shown next to the images. Acquisition times: (A–F), 5 min; (G–I), 15 s. The acquisition times were reduced to offset saturated liver signal intensities in AdCMV-Luc cohort.

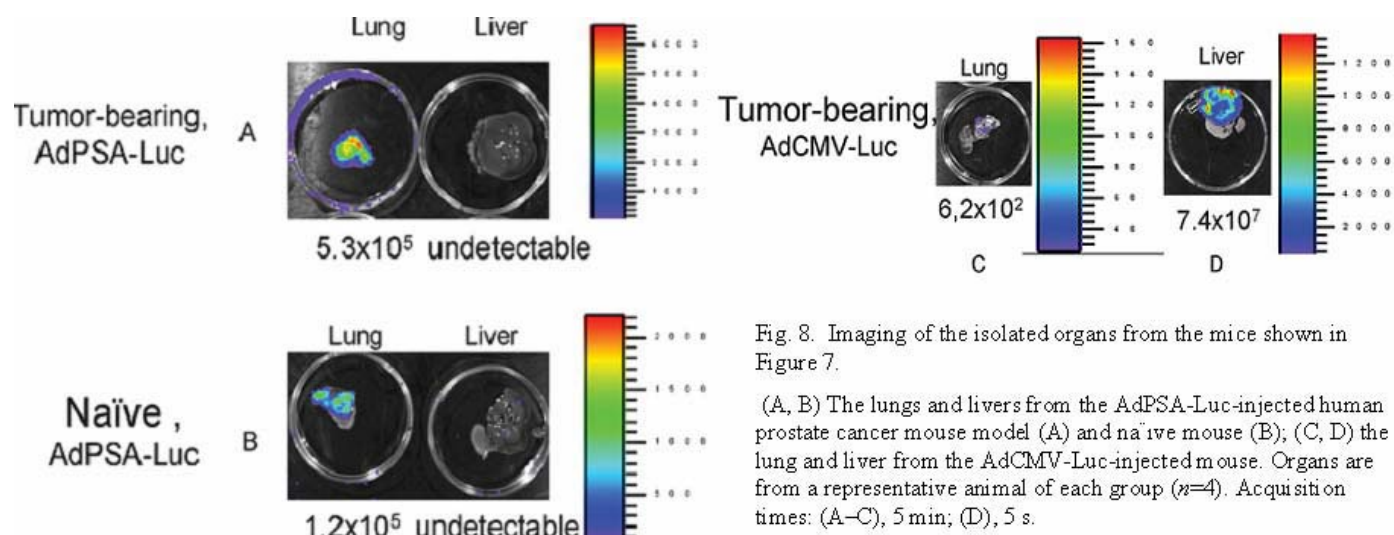


Fig. 8. Imaging of the isolated organs from the mice shown in Figure 7.

(A, B) The lungs and livers from the AdPSA-Luc-injected human prostate cancer mouse model (A) and naïve mouse (B); (C, D) the lung and liver from the AdCMV-Luc-injected mouse. Organs are from a representative animal of each group ($n=4$). Acquisition times: (A–C), 5 min; (D), 5 s.

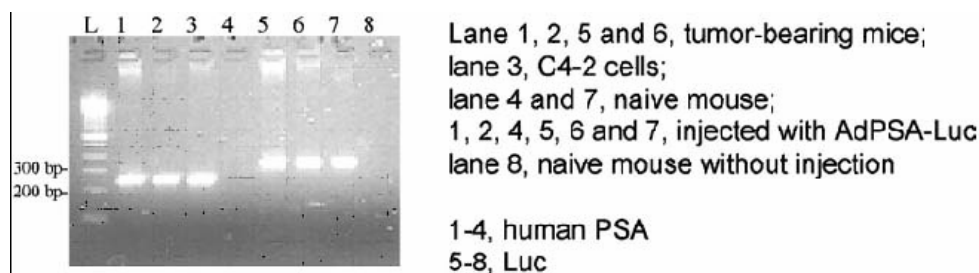


Fig. 9. RT-PCR demonstrating the expression of human PSA and luciferase in lungs from mice injected with AdPSA-Luc via tail vein. Total RNA was isolated from lungs with an RNeasy kit and subjected to RT-PCR with human PSA (lanes 1–4) or luciferase-specific primers (lanes 5–8). Lanes 1, 2, 5 and 6: lungs from human prostate cancer mouse models; lane 3: C4-2 cells; lanes 4 and 7: lungs from normal mice; lane 8: lung from a normal mouse without injection. Lanes 1, 2 and 4–7: mice were injected with AdPSA-Luc. Lane L, 100 bp ladder (New England Biolabs, Ipswich, MA, U.S.A.).

Key Research Accomplishments:

In summary, we demonstrated that the AdPSA-Luc can generate high level expression of luciferase gene under the control of the 5837 bp long PSA promoter in lungs of both normal and prostate cancer mice via tail vein injection. To our knowledge, this is the first report that unequivocally demonstrates specific gene expression in lung tissue elicited by a PSA promoter. This may predict PSA expression in lungs of normal mice. These results indicate the potential limitations of the suicide gene therapy of prostate cancer based on the selectivity of PSA promoter. By contrary, it has encouraging implications for the further development of vectors via PSA to enable gene therapy for pulmonary vascular diseases.

Reportable Outcomes:

The synthesis of the Tc-99m labeled FTcAU and Tc-TGCV were reported in *Tetrahedron Letters* and *Bioorganic and Medicinal Chemical Letters* accordingly. Highly specific and sustained expression of luciferase gene, directed by prostate-specific antigen promoter, in lungs of both naïve nude mice and human prostate cancer mouse model were reported in *Biochemical and Biophysical Research Communications* and *Biotechnology and Applied Biochemistry*.

Conclusions:

1. Tc-99m labeled FTcAU and Tc-TGCV demonstrated limited potency as TK substrates.

2. Highly specific gene expression in lung tissue elicited by a PSA promoter indicates the potential limitations of the suicide gene therapy of prostate cancer based on the selectivity of PSA promoter. By contrary, it has encouraging implications for the further development of vectors via PSA to enable gene therapy for pulmonary vascular diseases

Reference:

1. Morin, K.W., et al., J. Med. Chem., 1997. **40**(14): p. 2184-2190.
2. Spadari, S., et al., Mol. Pharmacology, 1995. **47**(6): p. 1231-1238.
3. Tjuvajev, J.G., et al., Cancer Research, 1995. **55**(24): p. 6126-6132.
4. Watanabi, K.A., et al., J. Med. Chem., 1979. **22**(1): p. 21-24.
5. Golankiewicz, B., et al., *Tricyclic Analogs of Acyclovir and Ganciclovir. Influence of Substituents in the Heterocyclic Moiety on the Antiviral Activity*. J. Med. Chem., 1994. **37**(19): p. 3187-3190.
6. Goslinski, T., et al., *Synthesis and Biological Activity of Strongly Fluorescent Tricyclic Analogues of Acyclovir and Ganciclovir*. J. Med. Chem., 2002. **45**(23): p. 5052-5057.
7. Goslinski, T., et al., *Synthesis and Fluorescent Properties of 6-(4-Biphenyl)-3,9-dihydro-9-oxo-5H-imidazo[1,2-a]purine Analogues of Acyclovir and Ganciclovir*. Nucleosides, Nucleotides and Nucleic Acids, 2003. **22**(5): p. 911-914.
8. Zhang, Y., et al., *Synthesis of a technetium-99m-labeled thymidine analog: a potential HSV1-TK substrate for non-invasive reporter gene expression imaging*. Tetrahedron Letters, 2004. **45**(47): p. 8673-8676.
9. Zhang, Y., J. Lin, and D. Pan, *Synthesis of a technetium-99m labeled tricyclic ganciclovir analog for non-invasive reporter gene expression imaging*. Bioorganic & Medicinal Chemistry Letters, 2007. **17**(3): p. 741-744.
10. Gotoh, A., et al., J. Urol., 1998. **160**: p. 220-229.
11. Latham, J.P., et al., Cancer Res., 2000. **60**: p. 334-341.
12. Li, H.-w., et al., *Highly specific expression of luciferase gene in lungs of naive nude mice directed by prostate-specific antigen promoter*. Biochemical and Biophysical Research Communications, 2005. **334**(4): p. 1287-1291.
13. Li, H., et al., *Non-invasive imaging of firefly luciferase reporter gene expression using bioluminescence imaging in human prostate cancer models*. Biotechnology & Applied Biochemistry, 2007. **46**(Pt 4): p. 179-84.