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Non-invasive in vivo imaging with radiolabeled FIAU for monitoring cancer gene therapy using herpes simplex virus type 1 thymidine kinase and ganciclovir **(NSC 91-NU-7-038-001)**

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Monitoring gene expression in vivo to evaluate the gene therapy efficacy is a critical issue for scientists and physicians. Non-invasive nuclear imaging can offer information regarding the level of gene expression and its location when an appropriate reporter gene is constructed in the therapeutic cassette[1]. Two approaches in non-invasive imaging development include: (1) enzyme-mediated trapping, i.e. by transducing the cells with reporter genes that encode enzymes able to phosphorylate or metabolize the radiolabeled substrates, so that the metabolites are subsequently trapped in transduced cells[2-5]; (2) receptor-ligand-mediated trapping on the cell surface[6, 7]. Radiolabeled substrates or ligands (reporter probe or marker substrate) are then used for imaging to determine the expression of reporter gene with a gamma camera, single photon emission computed tomography (SPECT) or positron emission tomography (PET) depending on the isotope used[8-10]. If the reporter gene is co-localized with a therapeutic gene, the expression of the therapeutic gene could be monitored indirectly.

HSV1-tk (herpes simplex virus type 1 thymidine kinase) is the most common reporter gene and is used in cancer gene therapy because of cell death by activating relatively non-toxic prodrugs, such as ACV or GCV[11, 12]. Moreover, various radiolabeled nucleoside analogues are used as specific probes for *HSV1-tk* and can be freely diffusible across cell membranes. When phosphorylated by the transduced *HSV1-tk* gene, the metabolite of probes subsequently accumulated within the transduced cells. Mammalian cellular thymidine kinase, however, shows relatively low phosphorylation activity and low accumulation levels of these probes in nontransduced cells. Improved sensitivity and specificity has also been demonstrated with the use of a mutant *HSV1-tk* as a reporter gene transferred by adenovirus vector for the in vivo non-invasive imaging purpose [3].

FIAU, an anticancer drug widely used in clinic, is an analogue of thymidine [13-15]. In a series of studies using adenovirus vector for gene transfer, Tjuvajev et al. [1, 2, 16] described the appropriate combination of exogenously introduced *HSV1-tk* as a “marker/reporter gene” and radiolabeled FIAU as a “marker substrate/reporter probe” for monitoring gene therapy and gene expression. Successful specific imaging of *HSV1-tk* expression in an animal tumor models were accomplished noninvasively with [^{131}I]FIAU and a clinical gamma camera system[16], or with [^{124}I]FIAU and PET[17]. Recently, Tjuvajev et al.[18] compared the efficiency of 3 radiolabeled probes (FIAU, FHBG, and FHPG) for in vivo imaging of *HSV1-tk* expression with PET, and demonstrated that FIAU is a substantially more efficient probe than FHBG or FHPG for imaging *HSV1-tk* expression, with greater sensitivity and contrast as well as low levels of abdominal background radioactivity at 2 and 24 hr. The initial accumulation and the elimination kinetics of radiolabeled FIAU was reported by Haubner et al.[19] in a tumor-bearing mouse model to suggest that sufficient tumor/background ratios for in vivo imaging of *HSV1-tk* expression were reached as early as 1 hr. Therefore, a simplicity and

ready-to-use method for preparing this radiopharmaceutical would be of value and of importance.

In the literature, radiolabeled FIAU was prepared from (i) its unsubstituted precursor FAU (1-(2-deoxy-2-fluoro- β-D-arabinofuranisyl)uracil) by direct radioiodination, and gave a 93% pure [^{131}I]FIAU[16]. N.c.a. [^{124}I]FIAU was prepared by reacting FAU with [^{124}I]NaI and the synthesis yield was 95%[17]. (ii) Alternatively, using tributyltin as precursor, [^{124}I]FIAU was prepared from FTAU and got more than 90% of radioactivity in the radiochromatogram eluted as a single peak[20]. In this study, we have developed a simplified synthetic methodology using tributylstannyl derivative as the precursor[21] to synthesize radiolabeled FIAU in lyophilized form (the "hot kit"). The simplicity, high radiochemical yield and high stability of n.c.a. [^{131}I]FIAU preparation has been demonstrated. In order to investigate the biological characteristics of n.c.a. [^{131}I]FIAU and the possibility to monitor cancer gene therapy using retroviral vector-transduced *HSV1-tk* and prodrug ganciclovir, in vitro cellular uptake and in vivo animal studies, including biodistribution and planar gamma camera imaging, were performed in *HSV1-tk*-transduced NG4TL4-STK and parental non-transduced NG4TL4 murine sarcoma cell lines.

Note: tk refers to the thymidine kinase gene and TK refers to the expressed enzyme
The abbreviations used are: *HSV1-tk*, herpes simplex virus type 1 thymidine kinase; FIAU, 2'-fluoro-2'-deoxy-1-b-D-arabinofuranosyl-5-iodo-uridine; HPLC, high performance liquid chromatography; PET, positron emission tomography; ACV, acyclovir; GCV, ganciclovir

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2-Deoxy-2-fluoro-3,5-di-O-benzoyl-£\-D-arabinofuranose and uracil, along with the enzymes alcohol oxidase, catalase and alkaline phosphatase, were purchased from Sigma-Aldrich Corp.(St. Louis, MO, USA). Hexa-n-butylditin and bis(triphenylphosphine) were obtained from Strem Chemicals, Inc.(Newburyport, MA, USA.). Hydrogen bromide (33% solution in acetic acid) and iodine monochloride and other chemicals were purchased from Merck & Co., Inc. (Whitehouse Station, NJ, USA).

The NMR spectra were recorded with a Bruker AC-300 Spectrometer at a proton frequency of 300 MHz and chemical shifts were expressed in ppm. Thin layer chromatography was conducted using an imaging scanner (System 200, Bioscan). High-performance liquid chromatography was conducted using Waters Model 600, Waters Model 600E pumps, a Waters Model 486 tunable UV detector along with a radioisotope detector (Flow Count Detector FC-003, Capintec, Bioscan). Data were collected and analyzed using a computer program (CSW, version 1.7, DataApex Ltd). The radiochemical yields reported are those obtained at the end of synthesis.

*Preparation of 2-Deoxy-2-fluoro-3,5-di-O-benzoyl-£\|-D-arabinofuranosyl Bromide (**2a**)*

Using a modification of a reported procedure (Huges, 1995)[22], we dissolved two grams (4.3 mmol) of 2-deoxy-2-fluoro-1,3,5-tri-O-benzoyl-£\-D-arabinfuranose (1a) (Fig.1) in 50 mL dichloromethane. Under a nitrogen atmosphere, hydrogen bromide (33%) in acetic acid (1.25mL) was slowly added to **1a**. The mixture was stirred at room temperature overnight. After cooling to ambient temperature, the solvent was removed in vacuo. The crude product was purified by silica gel chromatography (eluent: ethyl acetate/hexane = 1/14) to yield 1.65 g of the bromo derivative **2a** (91%). ^{1}H-NMR ($CDCl_3$) £_8.2 ~ 7.4 (m, 10H, ArH), 6.6 (d, 1H, C'$_1$-H; J_{F-H} =12 Hz), 5.6 (d, 1H, C_2-H; J_{F-H} =50 Hz), 5.5 (dd, 1H, C_3-H; J_{F-H} =22 Hz , J =3 Hz), 4.75 (m, 3H, C_4-H, C_5-H_2).

*Preparation of 2,4-Bis-O-(trimethylsilyl)uracil (**2b**)*[23]

A mixture of uracil (1b) (500 mg, 4.46 mmol), ammonium sulfate (0.59g, 4.46 mmol) and hexamethyldisilazane (10mL) was refluxed for 4 hr. After cooling to room temperature, the clear solution was evaporated under reduced pressure to give a thick oil **2b**, which was used without any further purification.

*Preparation of 1-(3,5-Di-O-benzoyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl uracil (**3**)*

Compound **2a** (1.7 g, 4.3 mmol), dissolved in CH_2Cl_2 (15mL), was tubing transferred into a flask containing compound **2b** in CH_2Cl_2 (15mL). The mixture was refluxed overnight. The solvent was removed in vacuo to give a white solid residue. The crude product was purified by silica gel chromatography (eluent: ethyl acetate/hexane = 1/1) to give 1.36 g of the white uracil derivative **3** (75%). ^{1}H-NMR ($CDCl_3$) £_8.41 (br s, 1H, NH), 8.04 (m, 4H, ArH), 7.66 ~ 7.41 (m, 7H, ArH; C_6-H), 6.31 (dd, 1H, C_1`-H; $J_{F-H}$ = 21.7Hz, J = 2.7Hz), 5.67 (dd, 1H, $C_5$-H; J = 17.2Hz, J = 2.2Hz), 5.62 (dd, 1H, $C_3$`-H; J_{F-H} = 18.5Hz, J = 2.7Hz), 5.31 (dd, 1H, C_2`-H ; $J_{F-H}$ = 50.4Hz, J = 2.0Hz), 4.75 (m, 2H, $C_5$`-H_2), 4.50 (m, 1H, C_4`-H).

*Preparation of 1-(3,5-Di-O-benzoyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl-5-iodouracil (**4**)*

A sample of **3** (260mg, 0.58mmol) was dissolved in CH_2Cl_2 (50mL), and ICl (200mg) was added. The solution was heated to reflux for 6 h and the solvent was removed under reduced pressure. The crude product was washed with H_2O (50mL x 2), dried, and evaporated. A 265.6 mg (78%) white solid of ***4*** was obtained after silica gel chromatography purification (eluent: ethyl acetate/hexane = 1/1). ^{1}H-NMR ($CDCl_3$) £_8.43 (br s, 1H, NH), 8.04 (m, 5H, ArH), 7.63 ~ 7.43 (m, 6H, ArH; C_6-H), 6.29 (dd, 1H, C_1`-H; $J_{F-H}$ = 21.7Hz, J = 2.8Hz), 5.60 (dd, 1H, $C_3$`-H; J_{F-H} = 18.2Hz, J = 2.6Hz), 5.35 (dd, 1H, C_2`-H; $J_{F-H}$ = 52.8Hz, J = 2.0Hz), 4.80(m, 2H, $C_5$`-H_2), 4.50 (m, 1H, C_4`-H).

*Preparation of 1-(3,5-Di-O-benzoyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl-5-tributylstannyluracil (**5**)*

A mixture of 146.7mg (0.25 mmol) of **4**, and 261 mg (0.45 mmol) hexabutylditin, and 10mg of bis(triphenylphosphine)palladium dichloride was dissolved in 7.5 mL of anhydrous dioxane. After refluxed for 6 h under a nitrogen atmosphere, the dioxane was removed by rotary evaporation. The light green precipitate was filtered through celite. The filtrate was adsorbed onto silica gel and purified by silica gel gradient chromatography (eluent: ethyl acetate/hexane = 1/3 to 100 % ethyl acetate). The solvent was removed in vacuo to give a white solid of **5** (98.4 mg , 53% yield). ^{1}H-NMR ($CDCl_3$) £_8.89 (s, 1H, NH), 8.04 ~ 7.34 (m, 11H, ArH; C_6-H), 6.33 (dd , 1H , C_1`-H; $J_{F-H}$ = 21.5Hz, J = 2.6Hz), 5.65 (dd, 1H, $C_3$`-H; J_{F-H} = 18.8Hz , J = 2.8Hz), 5.33 (dd, 1H, C_2`-H; $J_{F-H}$ = 52.8Hz, J = 2.0Hz), 4.70(m, 2H, $C_5$`-H_2), 4.48 (m, 1H, C_4`-H), 1.37 ~ 0.78 (m, 27H, $SnBu_3$).

*Preparation of 5-Tributylstannyl-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)uracil (FTAU) (**6**)*

The above product **5** (52.7mg) was added to 10 mL of conc. NH_4OH/MeOH = 80/20 solution. The mixture was stirred for 24 h and the solvent was removed under reduced pressure. The crude oil product was purified by silica gel chromatography (eluent: chloroform/hexane = 11/1) to afford 33 mg of **6** (87%). ^{1}H-NMR ($CDCl_3$) £_7.48 (d, 1H, C_6-H; J = 2.0Hz), 6.24 (dd , 1H, C_1`-H; $J_{F-H}$ =18Hz, J = 3.9Hz), 5.04 (dm, 1H, $C_2$`-H; J_{F-H} = 50Hz), 4.29 (dm, 1H, C_3`-H; $J_{F-H}$ = 16Hz), 3.92 ~ 3.70(m, 3H, $C_4$`-H; C_5`-H_2), 1.54 ~ 0.85 (m, 27H, $SnBu_3$). The elemental analysis of compound **6** was: FTAU (determined): C, 47.10 %; H, 8.53 %; N, 5.62 %, and FTAU (theoretical):C, 47.13%; H, 6.92 % ;N, 5.24 %.

N.c.a. Synthesis of [^{131}I]FIAU from Tributyltin Precursor FTAU

N.c.a 5-[^{131}I]Iodo-1-(2'-deoxy-2-fluoro-β-D-arabinofuranosyl)uracil ([^{131}I]FIAU) was synthesized from its organotin precursor (Fig. 2). 100 μL of oxidizing agent (H_2O_2:1N

HCl:H_2O = 4:1:95) was added to a 300 μL V-vial coated with 15 μg of 5-tributylstannyl-(2'-deoxy-2-fluoro-β-D-arabinofuranosyl)uracil (FTAU) and containing 20 μL ethanol and 0.1~5.0 mCi sodium [^{131}I]iodide. The reaction mixture was vortexed intermittently. After 8 min, the mixture was frozen in dry ice bath, and then lyophilized with a vacuum system (equipped with a charcoal absorber) to give the final product as a "hot kit". The lyophilized [^{131}I]FIAU "hot kit" was redissolved in ethanol and the radiochemical purity was determined using TLC and HPLC. Thin layer chromatography was performed on TLC aluminium sheet (Silica gel 60F_{254}, MERCK), using ethyl acetate/ethanol (90/10, v/v) as the mobile phase. Chromatograms were recorded using an imaging scanner (system 200, BIOSCAN). HPLC analysis was performed on a reversed-phase column (RPR-1, Hamilton), using methanol/water (50/50, v/v) as the eluant at a flow rate of 1 mL/min. A UV detector (tunable absorbance detector 486, Waters) and a radiodetector (CAPINTEC, BIOSCAN) were used to analyze the eluate. Data were collected and analyzed using computer software (CSW, version 1.7, DataApex Ltd). The lyophilized [^{131}I]FIAU product, dissolved in physiological saline and eluted through a 0.22 μm apyrogenic disk, was ready for biological or clinical application.

The theoretical specific activity of [^{131}I]FIAU prepared from n.c.a. synthesis process can be calculated from the equation:

$$\text{Specific activity} = Av / (R \times t_{1/2}) \quad (1)$$

Where Av is Avogadro's number 6.02 x 10^{23}, R is the conversion factor 3.7 x 10^{10} Bq/Ci, $t_{1/2}$ is the physical half-life of the radionuclide in sec. The theoretical specific activity for [^{131}I]FIAU is ~ 2 x 10^7 Ci/mol.

Stability of n.c.a. [^{131}I] FIAU obtained from the simplified synthesis process

In order to evaluate the stability of n.c.a. [^{131}I]FIAU obtained from the simplified synthesis process, the radiochemical purity of [^{131}I]FIAU in the lyophilized "hot kit" product and in the normal saline solution were determined at 1, 2, 3, 5, 7, 10, 14, 21, 28 days after preparation.

Cellular Uptake of n.c.a. [^{131}I]FIAU

Two murine cell lines (NG4TL4 and NG4TL4-STK) were used to evaluate n.c.a. [^{131}I]-FIAU from precursor FTAU. The NG4TL4-STK cell line, as described previously[24], were derived from parental NG4TL4 sarcoma cell [25, 26] by transfection with packaged virions of a bicistronic retroviral vector constructed to contain *HSV1-tk* gene that carries its own promoter and *neo*^{R} gene that carries simian virus 40 early promoter[27, 28]. NG4TL4 and NG4TL4-stk cells were cultured in MEM supplemented with 10% FBS, 100u/ml penicillin, 10 μg/ml streptomycin and 2mM L-glutamine in a humidified atmosphere with 5% CO_2 at 37°C.

For cellular uptake assay, cells of each cell lines were trypsinized and grown over night in 24-well culture plate (3 x 10^5 cells/ 0.5 ml/ well) and medium was changed before experiment. N.c.a. [^{131}I]FIAU (0.08μCi/ 0.5 ml/ well) were added to each well and incubated at 37¢J for 15 min, 30 min, 1 h, 2 h, 4 h, and 8 h. Triplicates were performed at each time point. For [^{131}I]FIAU uptake assay, the supernatants were removed and the cells rinsed with 200 μl HBSS. Then, each well was added 100 μl of trypsin-EDTA, and washed twice with 150 μl HBSS to harvest the cells. Cellular uptake of [^{131}I]FIAU was determined by gamma counting in a Wallac 1470 Wizard gamma counter.

Biodistribution of n.c.a. [^{131}I]FIAU

The animal experiment protocol was approved by the Institutional Animal Care and Use

Committee of Taipei Medical University. Syngenic female FVB/N inbred strain mice were inoculated with 1 x 10^5 NG4TL4 or NG4TL4-STK sarcoma cells subcutaneously to form tumors in the flank. Ten days later, n.c.a. [^{131}I]FIAU (0.01 mCi/animal) was injected via the tail vein. Groups of three animals were sacrificed at 1, 4, 8, and 24 h postinjection. Dissected tissue of interest was harvested, washed, weighed and counted along with injection dose standards using a Wallac 1470 Wizard gamma counter.

Planar Imaging

Planar imaging was performed on the syngenic FVB/N inbred strain mice bearing subcutaneous NG4TL4-STK tumors in the flank, derived from 1 x 10^5 NG4TL4-STK cells as described above. All developed palpable tumors were around 10 mm in diameter. After the injection of n.c.a. [^{131}I]FIAU (0.01 mCi/animal) via the tail vein, static images were obtained from anesthetized animals at day 1, 4, 6, 7 on a digital gamma camera (Elscint SP-6, Hifa, Israel), equipped with a high energy pinhole collimator. The image acquisition was performed at 100 k counts per frame at day 1. The following imaging at day 4, 6 and 7 were acquired by a preset-time acquisition mode. To evaluate the tumor regression, animals were treated with GCV (10mg/kg daily) or 0.09% NaCl by intraperitoneal injection for 7 consecutive days [24,28,29].

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The Preparation and Stability of n.c.a. [^{131}I]FIAU

The final product, lyophilized [^{131}I]FIAU “hot kit”, was redissolved in ethanol and the radiochemical purity was determined using TLC and HPLC. Thin layer chromatography showed the R_f value of [^{131}I]FIAU was 0.82~0.83 (Fig.3a). The retention time of [^{131}I]FIAU was 12.91 min by HPLC analysis (Fig.3b), the same as that obtained from the authentic FIAU standard. The labeling yield was more than 95% and the radiochemical purity was more than 98% (in average from more than 10 runs). The stability of n.c.a. [^{131}I]FIAU in the lyophilized “hot kit” product showed significantly more stable than in the normal saline solution by TLC assay. The shelter life of the final [^{131}I]FIAU “hot kit” product is as long as 3 weeks (table 1).

Cellular Uptake of n.c.a. [^{131}I]FIAU

To evaluate the stability of n.c.a. [^{131}I]FIAU, cellular uptake study was performed in *HSV1-tk*-transduced NG4TL4-STK and its non-transduced parental NG4TL4 murine sarcoma cell lines which have been employed for investigating suicidal prodrug cancer gene therapy in previous studies [24, 27, 28]. Figure 4 showed *in vitro* cellular uptake of n.c.a. [^{131}I]FIAU in NG4TL4-STK and NG4TL4 cells over a incubation period of 8 h. The [^{131}I]FIAU was formulated from the lyophilized “hot kit” in cold storage for 1 day (a), 7 days (b), 28 days (c), and from the [^{131}I]FIAU stock solution that was kept in the refrigerator for 28 days (d).

Compared with the parental NG4TL4 cells that contain no *HSV1-tk* gene, the *HSV1-tk*-transduced NG4TL4-STK cells accumulated more radioactivity in all experimental conditions, and the [^{131}I]FIAU accumulation increased with time up to 8-hour after exposure. The kinetic profile of the cellular uptake of n.c.a. [^{131}I]FIAU formulated from the lyophilized “hot kit” and from the stock solution are qualitatively similar.

Biodistribution and tumor/blood ratio

The accumulation of n.c.a. [^{131}I]FIAU in tumors and normal tissues was determined by *ex vivo* gamma counting. FVB/N mice were inoculated subcutaneously with *HSV1-tk*(+) NG4TL4-STK and *tk*(-) NG4TL4 cells into the flank and 10 days later injected with [^{131}I]FIAU

through tail vein. Biodistribution studies were performed at 1, 4, 8 and 24 h post-injection (Fig.5). As shown in figure 5b, the NG4TL4-STK tumor tissues retained consistently higher radioactivity than all other organs at every time point. The *HSV1-tk*(+) tumor/blood ratio reached maximum at 24 hr. p.i. At this time point, the retained I-131 radioactivity was 9.67 %ID/g in NG4TL4-STK tumors and 0.48 %ID/g in non-transduced NG4TL4 tumors. Tracer clearance from blood was completed in 24 hr. Also, the highest activity of tracer accumulation in *HSV1-tk*(+) tumors was observed at 24 h p.i.

The tumour/blood ratio at 1, 4, 8, and 24 h was 2.0, 3.5, 8.2, and 386, respectively, for the *HSV1-tk*(+) tumors, and 0.6, 0.5, 0.7, and 5.4, respectively, for the *HSV1-tk*(-) control tumor (Table 2). The kinetics of tissue clearance is shown in figure 6. Compared with the blood, liver and tumor(tk-) tissue, NG4TL4-STK tumor(tk+) showed high and lasting accumulation during the study period (Fig. 6A). The retention of radioactivity in the blood, liver and NG4TL4 tumor(tk-) only showed biphasic elimination characteristics (Fig. 6B).

Tumor Regression and Planar Gamma Imaging Studies

Low non-target tissue uptake of radioactivity and high tumor(tk+)/blood ratios suggested that the NG4TL4-STK tumor can be imaged scintigraphically with n.c.a. [^{131}I]FIAU. Pinhole imaging after intravenous injection of n.c.a. [^{131}I]FIAU clearly reflected the relatively high level of radioactivity retained in transduced tumor(tk+) (Fig. 7). Non-specific uptake in the stomach and thyroid was also demonstrated. After daily GCV treatment, the image of the same *HSV1-tk*(+) tumor-bearing mice showed obvious tumor regression at day 4 and virtual tumor disappearance at day 7. As previously noted [28], these mice showed no tumor recurrence nor positive detection by FIAU imaging at the site up to 60 days of observation (data not shown). The results clearly demonstrated that n.c.a. [^{131}I]FIAU is a promising marker substrate for in vivo monitoring of *HSV1-tk* expression in the tumor and therapeutic effects.

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More and more gene therapy protocols and clinical trials are in progress, especially with cancer diseases. Thus, to develop efficient non-invasive methods for monitoring the progress of gene transfer and gene expression in vivo is more and more important. Although elegant exploitation of mutagenized *HSV1-tk* gene showed improved substrate specificity and sensitivity for imaging purposes [3], the use of the wild type *HSV1-tk* as a reporter gene with different ncleoside analogs as marker substrates has been investigated. Tjuvajev et al. [18] used [^{124}I]FIAU, [^{18}F]FHBG, or [^{18}F]FHPG and PET to compare the efficiency of these three radiolabeled probes for in vivo imaging of *HSV1-tk* expression in transduced RG2 rat gliomas. They reported a 20-fold greater sensitivity of FIAU compared with that of FHBG and a >50 fold sensitivity advantage for FIAU compared with that of FHPG for imaging *HSV1-tk* expression. In the present study, we have employed [^{131}I]FIAU and demonstrated the applicability of this non-invasive imaging for monitoring cancer gene therapy in an experimental animal model of *HSV1-tk*-expressing tumor cells transduced with retroviral vector. We have followed and modified the procedure of Dougan et. al. [21] to develope a simpler, faster, and easier synthetic method for [^{131}I]FIAU. With the consideration that the tributylstannyl precursor would be a better choice than the trimethylstannyl precursor due to less toxicity of the tributyltin group compared with that of trimethyltin[30, 31], we synthesized the tributylstannyl precursor and prepared n.c.a. [^{131}I]FIAU in the form of a lyophilized “hot kit”. The convenience and radiochemical yield in [^{131}I]FIAU preparation and the stability of [^{131}I]FIAU “hot kit” product are significantly improved.

Although similarly modified procedures have been reported previously [20, 34], the present study has shown several advantages in our procedure for preparing radioiodinated

tracers from its tributylstannyl precursor: (a) Unreacted [^{131}I]iodide (in form of [^{131}I]I_2 in the presence of oxidizing agent), HCl, solvents (ethanol and H_2O) and oxidizing agent (H_2O_2) were all removed during lyophilization, only [^{131}I]FIAU and a tiny amount of FAU (less than 10 μg) were left in the lyophilized "hot kit" final product, hence no further purification such as by using a C-18 Sep-Park column chromatograph with subsequent methanol elution and removal [34, Ref. Cancer Research 2001, 61, 2983-2995) was needed. (b) The stability of the lyophilized [^{131}I]FIAU "hot kit" is obvious and the shelf life is as long as 3 weeks. (c) N.c.a. [^{131}I]FIAU solution with high radioactivity concentration which is ready for biological application can be readily prepared by dissolving the [^{131}I]FIAU in the "hot kit" with a small amount of physiological saline. (d) The process has wide application in preparing radioiodinated tracers from its organotin precursors to give clean, no-carrier-added and high radiochemical purity product in high yield. Our [^{131}I]FIAU preparations generally had the radiochemical yield of above 95% and the radiochemical purity of more than 98%, which were higher than those reported by Vaidyanathan et. al. (90%)[20] and Tjuvajev et al. (93%)[16]. The higher radiochemical purity may have significantly contribute to successful imaging at earlier time points after tracer injection in this study and that of Haubner et al. [19].

Biodistribution studies were performed at different time period p.i. to examine the kinetic of [^{131}I]FIAU accumulation in vivo. As shown in figure 5b, the radio-tracer retention and accumulation in the NG4TL4-STK tumor tissues increased with time and reached the maximal at 24 h p.i., while the levels in all other organs remained relative low. High radioactivity accumulation was observed in the kidneys and the blood in the early phase (1, 4 hr) due to renal excretion. Tracer clearance from blood was complete in 24hr, and extremely low radioactivity accumulation was observed in the brain during the period, presumably due to the blood-brain barrier.

The tissue/blood ratios at 1, 4, 8, and 24 h illustrated the kinetic accumulation level in *HSV1-tk*(+) tumors. Compared with the *HSV1-tk*-positive tumors (see Table 2), all other organs and the *HSV1-tk*-negative tumors showed low tracer accumulation at different time points postinjection. The increased accumulation is time-dependent and reaches the maximal at 24 h after injection. Haubner et al.[19] used [^{125}I]FIAU for in vivo imaging of *HSV1-tk* gene expression to study the early kinetics of radiolabeled FIAU and reported a bi-exponential tracer clearance from blood. We also observed a similar biokinetics of biphasic [^{131}I]FIAU elimination in the blood and organs of sarcoma-bearing mice except in the *HSV1-tk*(+) NG4TL4-STK tumors. The results showed that the use of the [^{131}I]FIAU have a little influence on further imaging experiment.

Low non-target tissue uptake of radioactivity and high tumor/ blood ratios suggest that the *HSV1-tk* transduced tumors could be imaged scintigraphically with [^{131}I]FIAU. Planar gamma camera imaging after intravenous injection of [^{131}I]FIAU clearly revealed these *HSV1-tk* gene transduced tumors and the expression level of *HSV1-tk* is adequate for GCV therapy.. Our mouse imaging analysis also showed non-specific uptake in the stomach and thyroid. The observation of [^{131}I]FIAU accumulation in the thyroid and stomach in this study indicates the tracer is stable and also resistant to metabolic degradation, in which the concerns related to imaging metabolites in both target and non-target tissue could be diminished.

We also observed the contrast between *HSV1-tk*-expressing tumour tissue and control tumor after GCV treatment. The gamma camera image results clearly demonstrate that successful imaging for cancer gene therapy could be reached after early tracer injection. The therapeutic dose of GCV is adequate to match the levels of *HSV1-tk* expression in tumor tissue. The imaging for predicting the efficacy of *HSV1-tk* with GCV early during cancer therapy is dependable, thus confirming the previous studies [24, 27, 28]. However, it remains to be determined whether or not the present in vivo imaging procedure could be applied to assess the

partial tumor regression as well as the recurrence of *HSV1-tk* transduced tumors.

Recently, PET imaging has been shown to be a preferable system for monitoring gene expression due to its high sensitivity and quantitative character[2, 32, 33]. Several studies have been ongoing to compare the different radiolabelled compounds (pyrimidine nucleoside and acycloguanosine derivatives) in different original cancer cells by SPECT and PET imaging system to demonstrate the sensitivity, dynamic range and background levels of radioactivity in choosing a reporter probe and in monitoring gene expression. Furthermore, nuclear imaging would be useful for assessing the efficacy of various cell-based as well as gene-based therapeutic approaches in immunodeficient mice that bear tumors of *HSV1-tk* transduced human cancer cells.

In conclusion, a tributyltin precursor can be prepared by using a simple synthetic strategy. [^{131}I]FIAU can be synthesized from this precursor in excellent radiochemical yield. As expected, FIAU not only was stable but also showed high specific uptake in *HSV1-tk* gene expressing tumors and fast clearance from normal tissues of tumor-bearing mice. The optimal time points range between 2 and 4 h. The results support that the FIAU may serve as an efficient and selective agent for transduced *HSV1-tk* gene expression in vivo in clinical trials.

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1b **2b**

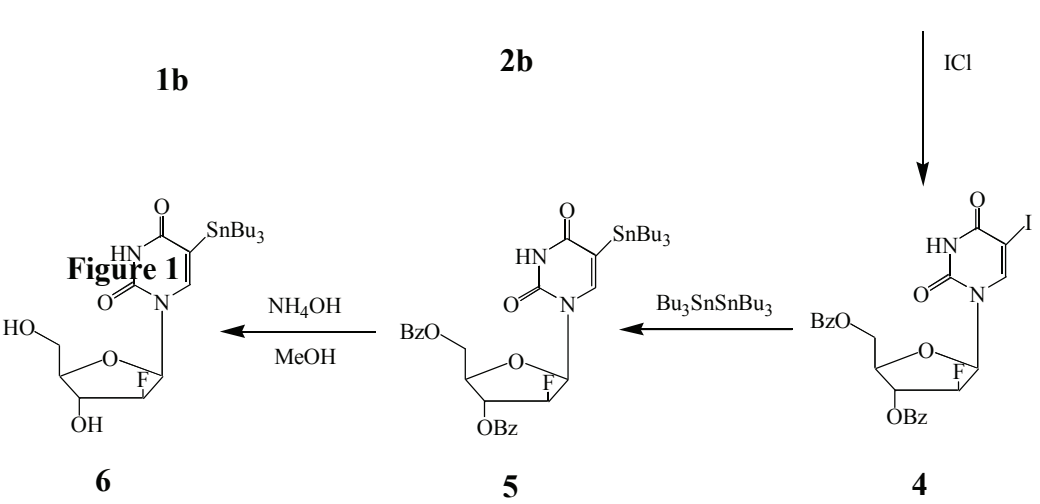
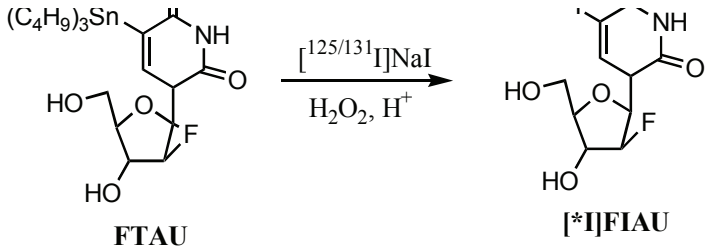


Figure 1

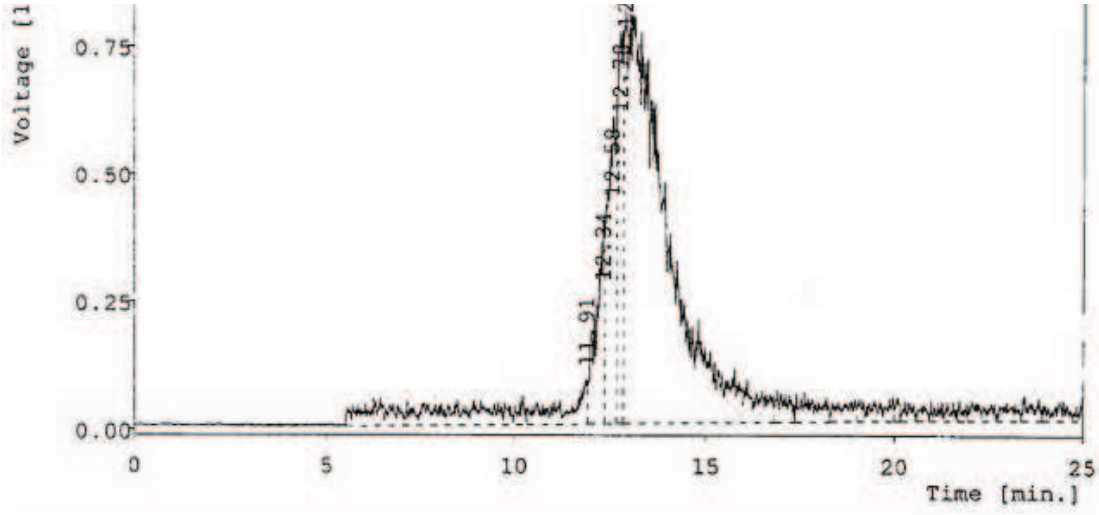
6 **5** **4**

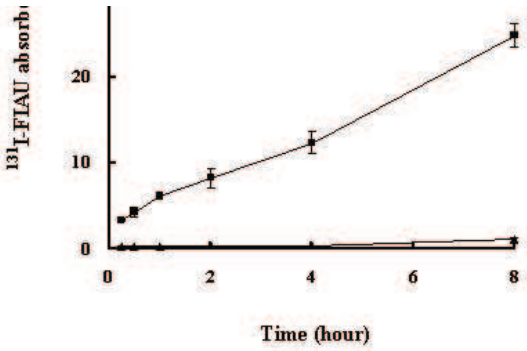
Figure 2



FTAU **[*I]FIAU**

Figure 2. The preparation of no-carrier-added [^{131}I]FIAU from organotin precursor


Voltage [
0.75
0.50
0.25
0.00
11.91
12.34
12.58
0
5
10
15
20
25
Time [min.]


131I-FIAU absorb
20
10
0
0
2
4
6
8
Time (hour)

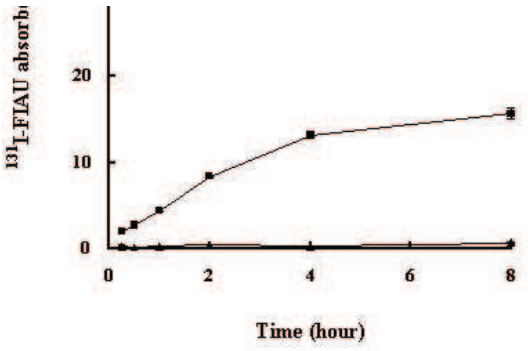
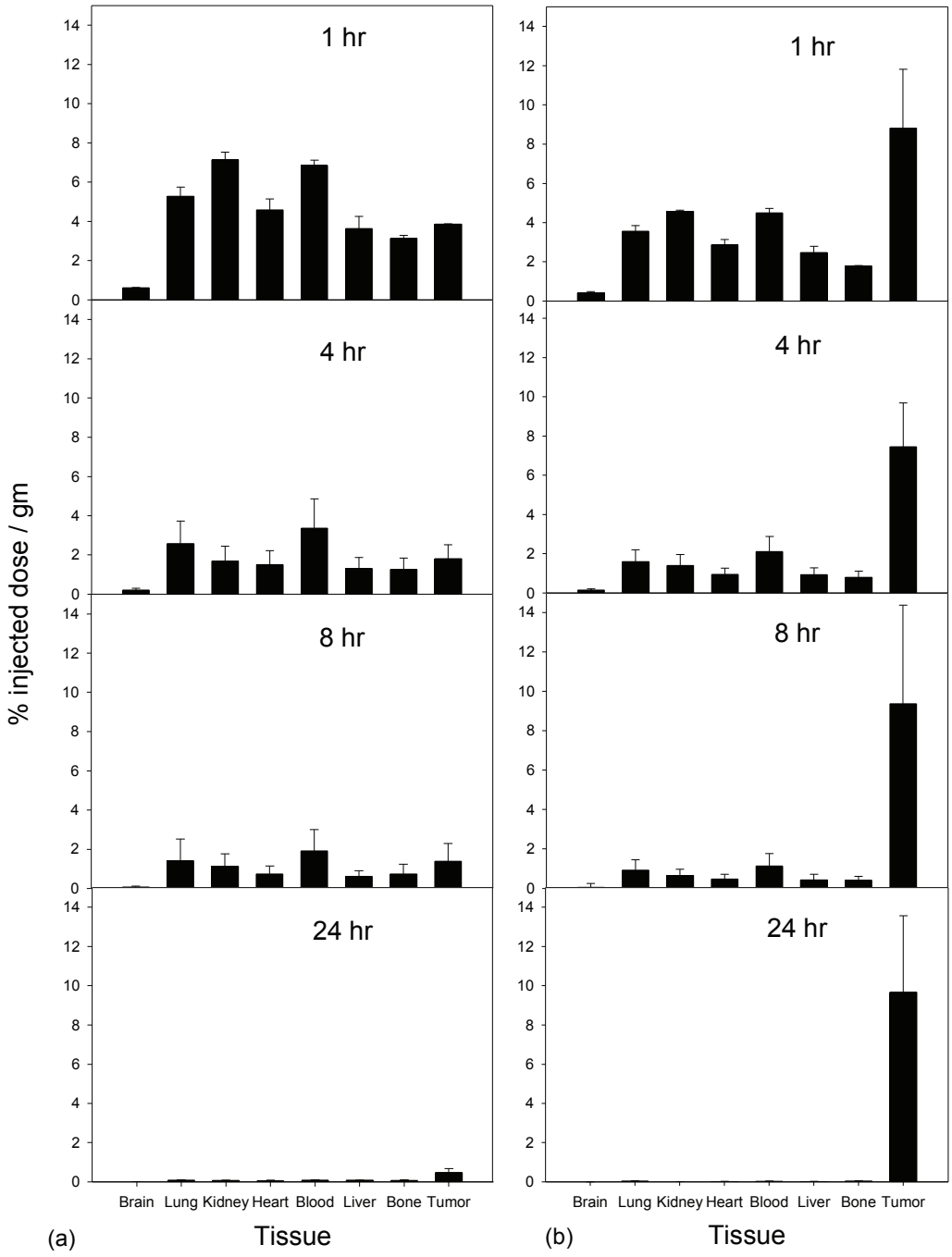
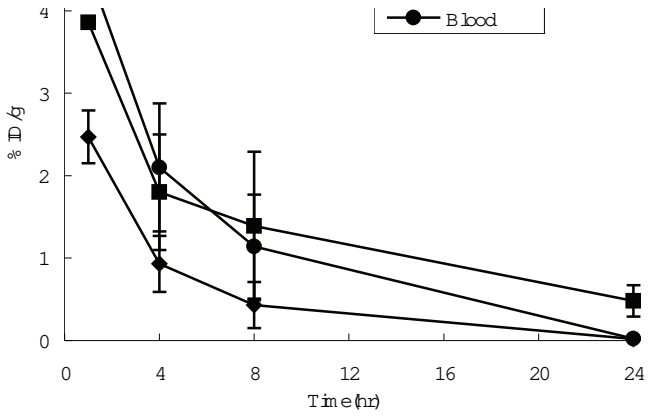

131I-FIAU absorb
20
10
0
0
2
4
6
8
Time (hour)

Figure 5


% injected dose / gm
1 hr
4 hr
8 hr
24 hr
Brain Lung Kidney Heart Blood Liver Bone Tumor
(a)
Tissue
(b)
Tissue



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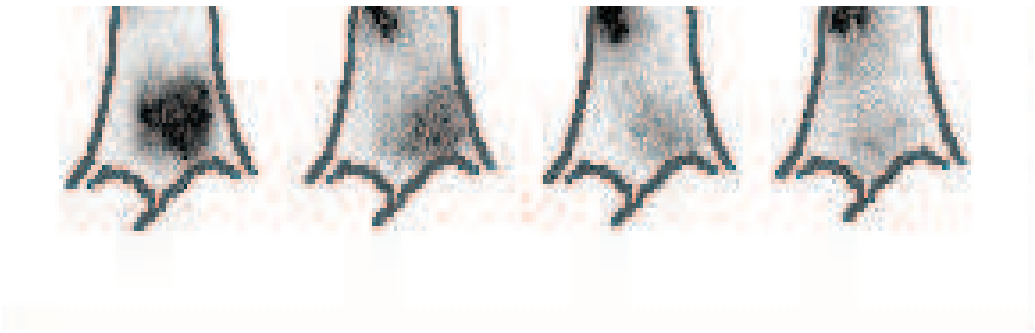

Thyroid

¡ œ

Tumor

¡ œ

Tumor

¡ œ

Tumor

¡ œ

Day 1 Day 4 Day 6 Day 7

Figure Legends

Figure 1. Synthetic scheme for preparation of organotin precursor FTAU.

Figure 2. The preparation of no-carrier-added [^{131}I]-FIAU from organotin precursor.

Figure 3. TLC and HPLC analysis demonstrated high radiochemical purity and specific activity. (a)Thin layer chromatography was performed on TLC aluminium sheet, using ethyl acetate/ethanol (90/10, v/v) as the mobile phase. The R_f value of [^{131}I]FIAU was 0.82~0.83. (b) Quality control of the FAU labelling with radio-HPLC, using methanol/water (50/50, v/v) as the eluant at a flow rate of 1 mL / min. The retention time of [^{131}I]FIAU was 12.91 min., the same as that obtained from the cold FIAU standard. The labeling yield was more than 95% and the radiochemical purity was more than 98%.

Figure 4. Evaluation of the stability of. no-carrier-added synthesis [^{131}I]FIAU. *In vitro* cellular uptake of (a) 1, (b) 7, (c) 28 day's storage of n.c.a [^{131}I]FIAU in lyophilized "hot kit" and (d) 28 day stability in normal saline solution in murine sarcoma cell lines NG4TL4-STK and NG4TL4. The cellular uptake is shown over a period of 8 h.

Figure 5. Biodistribution of [^{131}I]FIAU in mice bearing NG4TL4-STK and NG4TL4 tumors. Radioactivity, as % dose per gram tissue, in (a) NG4TL4 and (b) NG4TL4-STK tumor-bearing mice 1h, 4h, 8h, 24h following injection of [^{131}I]-FIAU.

Figure 6. The kinetics of tissue clearance. (a) Compared with blood, liver and tumor(tk-) tissue, NG4TL4-STK tumor(tk+) showed high and lasting accumulation during the period. (b) In blood, liver and NG4TL4 tumor(tk-) only showed biphasic elimination characteristics.

Figure 7. Pinhole images of FVB/N bearing NG4TL4-STK (right side) tumors. Syngeneic FVB/N inbred strain mice bearing subcutaneous NG4TL4-STK tumors illustrates the selective uptake of [^{131}I]FIAU in NG4TL4-STK tumor expressing HSV1-TK (day1), and progressive decreased tumor uptake of [^{131}I]FIAU after GCV treatment (day4, 6, and 7).

Table 1. Stability of no-carrier-added synthesis [^{131}I]FIAU at storage

Form of [^{131}I]FIAU	Storage time (day) / Radiochemical purity (%)									
	0	1	3	5	7	10	14	17	21	28
in lyophilized "hot kit"	>99.5	>99.5	>99.5	>99.5	>99.5	>99.5	>99.5	99.1	98.5	97.7
in normal saline solution	>99.5	97.8	95.4	91.3	89.3	84.1	-	-	-	-

1. No stabilizer was added in either lyophilized "hot kit" or normal saline solution of [^{131}I]FIAU.
2. The radioactivity of [^{131}I]FIAU in lyophilized "hot kit" or aqueous solution is among 100~200£gCi/mL.

Table 2. The kinetic tumor/blood ratio of the NG4TL4-STK (+) and NG4TL4 (-) tumors

Time post-injection (hr)	Tumor(+)/blood	Tumor(-)/blood
1	2.0±0.77	0.5
4	3.5±1.7	0.5
8	8.2±5.8	0.7
24	386.8±221.7	5.4