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13. ABSTRACT ***(Maximum 200 Words)***

We are developing methods to image her-2-neu oncogene over-expression in breast cancer using positron emission tomography (PET). Small oligodeoxynucleotides (ODNs) that are complementary to the Her-2-neu messenger RNA (mRNA) are being investigated as potential imaging probes. Fluorine-18 (2 hour half-life positron emitter) has been used to label 15-18 mer ODN probes. Furthermore, several breast and ovarian cancer cell lines which over-express ODNs have been used to study trapping of 18F-ODNs in cell culture. We have also preliminary studied the biodistribution of ODNs in 2 living nude mice using microPET in order to understand the limitations of imaging *in vivo* with the antisense approach. Currently the yield of radiolabeled ODNs is very low (<20 μCi), and therefore more work will have to be performed to optimize yields of the ODNs prior to proceeding further. Once ODN yields can be brought into the 150-300 μCi range, then more cell culture and in vivo experiments will be able to be performed to optimize an antisense based approach for imaging breast cancer.

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PI - Signature

12/1/1999
Date

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Introduction

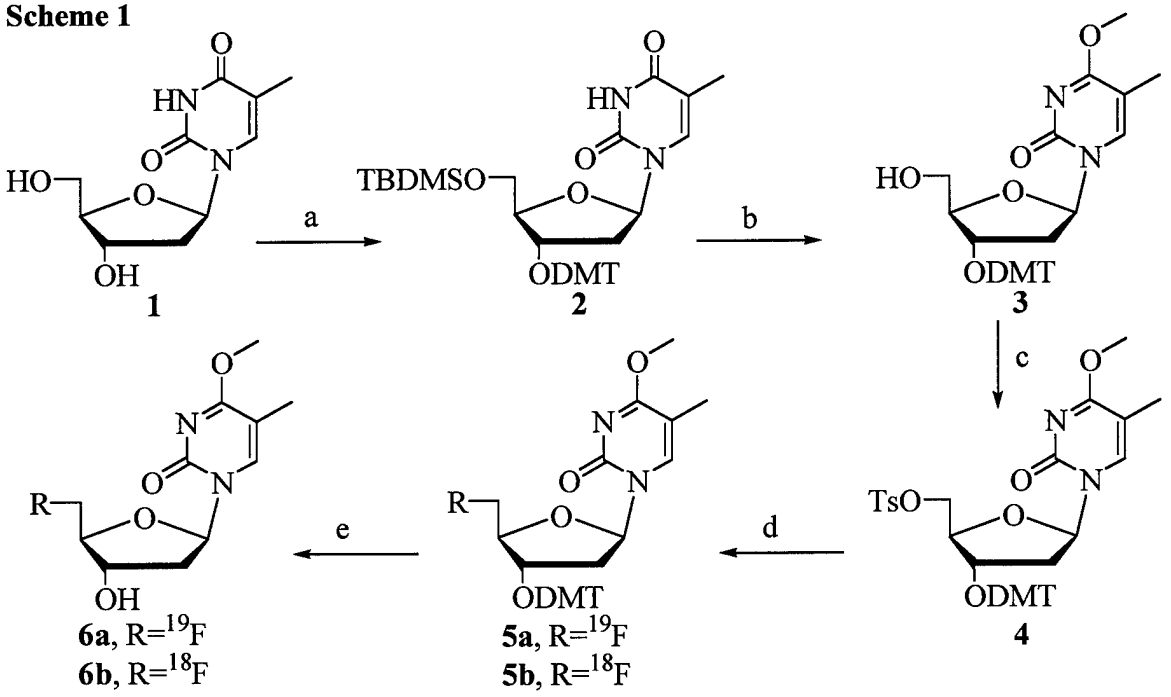
Improved methods to image breast cancer are critically needed in order to lead to earlier initial detection, earlier detection for recurrence, and better management of patients undergoing treatment. Most approaches to date have focused on anatomical changes due to tumor growth (e.g., mammography, computerized tomography, magnetic resonance imaging) or metabolic changes in the tumor (e.g., FDG Positron emission tomography). As molecular oncology continues to shed insight into the molecular basis for breast cancer, methods are needed to directly image molecular abberations in breast cancer cells. We are developing methods using radiolabeled antisense oligodoeoxynucleotides (RASONs) which can be injected via the blood-stream and then accumulate in cells that have sufficient levels of a particular target messenger RNA (mRNA). Normal cells (breast and other tissues) which don't have high level of target mRNA would not lead to intracellular trapping of the RASONs. One known molecular abnormality in about 25% of breast cancer patients is the over-expression of the Her-2-neu (c-erb-B2) oncogene. We have selected this gene as our first target using RASONs labeled with fluorine-18 (a positron emitter). We seek to develop RASONs that can be validated using nude mice carrying human breast cancer tumor xenografts imaged using microPET technology. With pre-clinical proof of their ability to home to breast cancer tumors over-expressing Her-2-neu we hope to have sufficient proof to eventually transition to human applications. It is hoped that this approach will lead to more specific and sensitive detection of breast cancer with over-expression of Her-2-neu and set the foundation for a new antisense based imaging approach which could potentially be applied to many different oncogenes.

Body

<u>Aim 1:</u> The development of ^{18}F (a positron emitter, half-life of 110 minutes) labeled oligodeoxynucleotides. The bulk of the effort to date has been towards this goal. Because all other goals are dependent on the successful completion of this goal, most of our efforts have been towards radiolabeling with Fluorine-18 our oligodeoxynucleotide probes.

The details of the chemistry developed to date is described next.

Scheme 1



Reagents: a. (1) TBDMCl, imidazole. (2) DMTCl; b. (1) $POCl_3$, Et_3N, 1,2,4-triazol. (2) CH_3OH,DBU. (3) Bu_4NF; c. TsCl; d. KF (or [F-18]KF)/Kryptofix[2,2,2]; e. TCA

Figure 1
The top trace lines are recorded by γ-detectors and the bottoms are by UV260 detectors. Column: Alltima 5μ silica (4.6 x 250 mm); flow rate: 1 mL/ min.; buffer: acetonitrile.

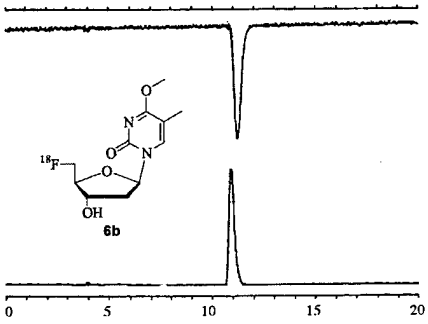
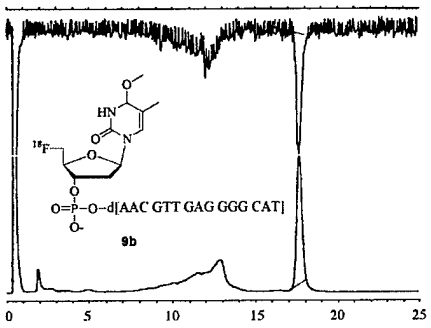


Figure 2.
The top trace lines are recorded by γ-detectors and the bottoms are by UV260 detectors. Column: PerSeptive 20 HQ (4.6 x 100 mm); flow rate: 4 mL/ min.; gradient: 20% to 70%.

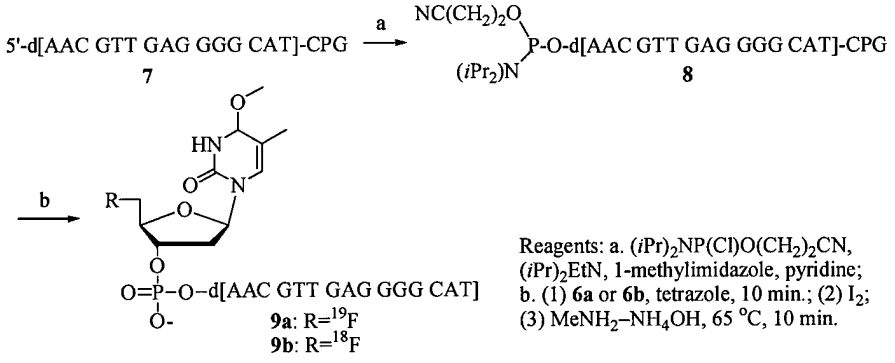


Our synthetic strategy is comprised of two key steps: synthesis of a 5'-deoxy-5'-fluoro-nucleoside followed by its incorporation into a CPG-bound ODN by reverse-activated phosphoramidite chemistry.[1] In our earlier communication,[2] we reported nucleophilic fluorination of 3'-acetyloxy-5'-*p*-methylphenylsulfonyl-4-*O*-methyl thymidine followed by deacetylation in hot NH_4OH to produce 5'-deoxy-5'-fluoro-4-*O*-methyl thymidine **6a**. But it is a tedious practice to heat a sealed reaction vessel with conc. NH_4OH and F-18 labeled chemicals at 100 °C. We further simplified the deprotection by using the 4,4'-dimethoxytrityl group to protect 3'-OH of the nucleoside analog, which can be removed by acid treatment. The new precursor, 5'-*O*-tosyl-3'-(4,4'-dimethoxytrityl)-O^4-methylthymidine **4**, was synthesized from thymidine **1** in a total chemical yield of 22% (Scheme 1). The **4** was then subjected to nucleophilic [F-18]fluorination at 98~100 °C for 5 min., followed by deprotection with trichloroacetic acid (TCA). Purification of the reaction mixture by normal-phase HPLC afforded 5'-deoxy-5'-[F-18]fluoro-O^4-methylthymidine **6b** in 15 ~ 20% (decay corrected) of radiochemical yield. The chemical identity and radiochemical purity of F-18 labeled nucleoside **6b** was confirmed by HPLC co-injection with **6a** (Figure 1).

Coupling of **3b** to the CPG-bound ODN **7** was carried out by the reverse-activation protocol (Scheme 2). Phosphitylation of **7** was conducted by treatment with 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite. The resulting phosphoramidite **8** was then reacted with **6b** (50 ~ 100 mCi) in MeCN containing 1*H* -tetrazole at RTfor 10 min. After oxidation with I_2, the F-18 labeled ODN was cleaved from CPG beads by NH_4OH/CH_3NH_2 and deprotected at 65 °C for 10 min. The crude mixture was purified by anion-exchange HPLC (POROS 20 HQ) to yield the desired 5'-[F-18]fluorinated ODN **9b** (10-70 μCi) (Figure 2).

Both duplexes of fully deprotected ODN of **7** and fluorine modified **9a** against complementary RNA 25-mer, 5'-CGA CGA UGC CCC UCA ACG UUA GCU U, showed identical T_m values, ~ 48 °C. It indicates that a nucleoside tag group attached to 5'-end of the 15-mer ODN doesn't significantly change its hybridization affinity.

Scheme 2



The key issues that remain in order to complete Aim 1 are to improve yields of the final product. Although we can get on average 20 μCi of the radiolabeled ODN, we will need ten times more in order to do routine cell culture testing as well as *in vivo* imaging in our mice models. Current work is attempting to optimize the yields further. In another 3-5 months we hope to have sufficient yields to proceed further.

Aim 2: The development of (15-20)-mer oligodeoxynucleotides for targeting the Her-2-neu (c-erbB-2) proto-oncogene mRNA. We have studied several candidate sequences for targeting the Her-2-neu mRNA. Through structural analysis we have now defined several optimal sequence that we feel should be accessible by our RASON probes. We have also started synthesis of modified-backbone ODNs in order to improve their plasma stability. We find that 2' O-methyl modified ODNs may be optimal for eventual use *in vivo*.

Aim 3: Tissue culture testing of the developed probes to determine the specificity and kinetics of the probe for the c-erbB-2 mRNA. We have studied 4 cell lines for their levels of Her-2-neu expression. These include a MCF-7 control cell line, a MCF-7 over expressing Her-2-neu, SK-BR-3, and SK-OV-3. Utilizing both Westerns and PCR we have shown that there is Her-2-neu over-expression in 3 of 4 of these cell lines. Furthermore, we have performed ODN uptake studies (using pharmacological levels of ODN) in order to show that a particular antisense sequence is capable of reducing levels of Her-2-neu protein in a cell culture assay. The best antisense sequence to date is a 18-mer with the sequence 5' gca caa ggc cgc cag ctc 3'. This sequence has been compared to several control and mis-match sequences in order to show that it is effective in reducing levels of Her-2-neu in cell culture. Furthermore, the control sequence does not reduce levels of Her-2-neu as expected. We now await the labeling of this ODN sequence with Fluorine-18 after Aim 1 leads to improved yields for our RASON probes. Then cell culture uptake and efflux studies will be performed with the RASON probes (antisense and control probes).

<u>Aim 4:</u> To study the targeting properties of ^{18}F-labeled antisense-oligodeoxynucleotides *in vivo* in a mouse animal model using PET.

Towards Aim 4 we have performed some very preliminary studies in two control nude mice in order to understand the limitations of injecting our 18F-ODN probes into mice and imaging with a microPET. Because the yields of 18F-ODN are still very low (see also Aim 1), we have not been able to get satisfactory images of biodistribution of the tracer. We will be able to better characterize the biodistribution when more tracer is routinely available. We have also been able to grow xenografted tumors in mice (e.g., MCF-7) in order to eventually use these tumor models to image with microPET and our 18F-ODN probes.

Key Research Accomplishments

- Synthesis of 18F-oligodeoxynucleotide (ODN) probes in low yields
- Purification of 18F-ODN probes for cell culture testing and *in vivo* testing
- Assessment of hybridization potential of 18F-ODN with target mRNA through T_m measurements
- Synthesis of 2'-o-methyl modified ODNs for improved plasma stability
- Specific Activity measurements of 18F-ODN probes
- Isolation of an 18-mer antisense sequence that should have optimal targeting properties for Her-2-neu
- Study of cell lines for levels of Her-2-neu over-expression
- Demonstration that pharmacological levels of antisense probe targeted towards Her-2-neu leads to decrease in levels of Her-2-neu protein in cell culture
- Preliminary biodistribution studies of 18F-ODN probes in control mice using microPET

Reportable Outcomes

Presentation of 18F radiolabeling approach utilizing newly developed chemistry. Presented by Dr. Dongfen Pan at Annual meeting of the Society of Nuclear Medicine in June, 1999.

D. Pan, T. Toyokuni, J.R. Barrio, N. Satyamurthy, M.E. Phelps, S.S. Gambhir. Synthesis of a Fluorine-18 Labeled Antisense Oligodeoxynucleotide as a Probe for Imaging Gene Expression. J. Nucl. Med 40(5)82P, 1999.

Conclusions

The results to date demonstrate that it is possible to label oligodexoynucleotide molecules with Fluorine-18 (a positron emitter). This is a key step which must take place prior to the use of these probes as imaging agents for breast cancer. With further improvements in yield and specific activity of these probes many additional experiments can be undertaken. These include study of cell culture models and *in vivo* animal tumor models using microPET imaging technology. The groundwork has also been set for further study in cell culture models, and *in vivo* animal models. With continued funding of this work it should be possible to understand the feasibility of using RASON probes to image Her-2-neu over-expression in breast cancer with PET imaging.

References

1. Tan, W.; Iyer, R. P.; Ziang, Z.; Yu, D. and Agrawal, S. *Tetrahedron Lett.,* 1995, *36,* 5323.

2. Pan D., Gambhir S., Toyokuni T., Iyer M. Acharya N., Phelps M. and Barrio J. *Bioorganic & Medicinal Chemistry Letters* 1998, *8*, 1317.

Appendices

A SUPPLEMENT TO

The Journal of Nuclear Medicine

JNM

ABSTRACT BOOK

Scientific Abstracts of the 46th Annual Meeting of the Society of Nuclear Medicine
Los Angeles, California
June 6-10, 1999

LOS ANGELES

uptake was similar for the two radiolabels, and in most normal tissues the ^{86}Y and ^{111}In concentrations differed by less than 10%. In bone, however, the ^{86}Y uptake exceeded that of ^{111}In by 19±6%. **Conclusions:** The quantitative information offered by PET, combined with the presumably identical biodistribution of an ^{86}Y and an ^{90}Y radiolabel, should enable more accurate absorbed dose estimates in ^{90}Y radioimmunotherapy.

No. 329

PREPARATION AND *IN VIVO* STABILITY EVALUATION OF LINKERS FOR ^{211}AT LABELING OF HUMANIZED ANTI-TAC(ANTI IL-2Rα). K. Garmestani*, A. T. Yordanov, K. E. Phillips. M. P. Beitzel, U. P. Schwarz, M. S. Rickford, O. A. Gansow, P. S. Plascjak, W. C. Eckelman, M. W. Brechbiel, T. A. Waldman, National Institutes of Health, Bethesda, MD. (100480)

Objectives: Radionuclide-labeled Mabs directed against tumor-associated antigens have been investigated as immunotherapeutic agents in human cancer. The α-emitter ^{211}At ($t_{1/2}$ = 7.21 h) is a particularly promising candidate for radioimmunotherapeutic applications. A pivotal issue to consider in designing an optimal radioimmunotherapeutic agent is the choice of linker to couple the radionuclide to the MAb. **Methods:** The linkers *N*-hydroxysuccinimidyl 4-[^{211}At]astatobenzoate(**1**), *N*-hydroxysuccinimidyl 3-[^{211}At]astato-4-methylbenzoate(**2**), *N*-hydroxysuccinimidyl 4-[^{211}At]astato-3-methylbenzoate(**3**) and *N*-hydroxysuccinimidyl *N*-(4-[^{211}At]astatophenetyl)succinamate(**4**) were prepared and employed for ^{211}At-labeling of the antibody. The anti-Tac (anti IL-2Rα) antibody that reacts with select leukemia cells but not resting normal cells was utilized for this study. The plasma survival of these compounds in normal mice was studied vs ^{125}I labeled humanized anti-Tac. **Results:** The comparison of the blood clearance curves of the ^{211}At and ^{125}I-labeled anti-Tac and free ^{211}At indicating the stability of compounds **1-4** was in the following order $^{125}I \cong 4 > 3 > 2 > 1$. **Conclusion:** This study showed that linker **4** is the superior compound prepared to date for ^{211}At-labeling of humanized anti-Tac and its plasma survival appeared to be essentially equivalent to that of directly labeled ^{125}I-antibody. These results also suggest that humanized anti-Tac can be successfully labeled with ^{211}At using linker **4** and should be further evaluated for therapeutic applications.

Radiopharmaceutical Chemistry Track
New Chemistry: Oncology - Hypoxia, Nucleosides

4:00 PM-5:30 PM **Session 48** **Room: 403 B**

Moderator: Chyng-Yann Shiue, PhD
Co-Moderator: Janet F. Eary, MD

No. 330

NON ENZYMATIC REDUCTION AS A POSSIBLE RETENTION MECHANISM OF TC-99M-HL91 IN HYPOXIC TISSUES. Y. Fujibayashi*, M. Ohno, A. Waki, K. S. Horiuchi, Y. Yonekura, Fukui Medical University, Fukui, Japan; Kyoto University, Kyoto, Japan. (210)

A novel hypoxia imaging agent, Tc-99m-4,9-diaza-3,3,10,10,-tetramethyldodecan-2,11-dione dioxime (Tc-99m-HL91), shows hypoxia-selective accumulation in myocardium as well as tumors, but its retention mechanism has not been clarified. In our previous work, it was found that Cu-diacetyl-bis(N4-methylthiosemicarbazone) (Cu-ATSM) showed enzymatic and NADH-dependent reduction in hypoxic non-tumor tissues. Thus, in the present work, metabolic analysis was performed to clarify the reductive retention mehcanism of Tc-99m-HL91 in in-vitro system. Methods: Metabolism of Tc-99m-HL91 and Cu-ATSM was comparatively evaluated using reversed-phrase HPLC system. Each sample was incubated with biological reductants, glutathione reduced form or NADH, then analyzed. For controls, oxidized forms of the reductants were used. To evaluate the possible contribution of enzymatic systems, subcellular fractions obtained from Ehrlich ascites tumor cells were added to the incubation medium. The effect of NADH on the enzymatic reduction of each samples was also studied. Results: The reduction of Cu-ATSM required microsomal enzymes and was NADH/NADPH dependent in tumor cells. Without enzymes, no reduction could be found. On the other hand, Tc-99m-HL91showed chemical reduction when only NADH or glutathione reduced form was added to the incubation medium. This reduction was dose-dependent, but there seemed to be threshold levels of reductant concentration. More interestingly, enzyme system inhibited the reductive metabolism of Tc-99m-HL91 but electron transport inhibitors recalled the reduction of Tc-99m-HL91 in the medium containing the microsomal enzymes. Conclusion: Cu-ATSM could be considered as a marker of reversible hypoxia, bacause it required biological reductants as well as intact enzyme system(s). On the other hand, Tc-99m-HL91 only required abnormally high concentration of biological reductants, indicating it as a hypoxia imaging agent with wider spectrum, rather than Cu-ATSM. This finding will bring us a new sight of hypoxia diagnosis using Tc-99m-HL91 as well as Cu-ATSM in clinical level.

No. 331

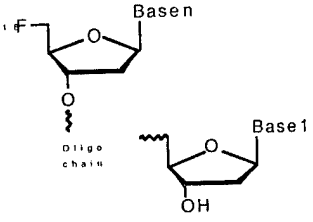
TARGETTING HYPOXIA IN TUMOURS USING 2-NITROIMIDAZOLES WITH PEPTIDIC CHELATORS FOR TECHNETIUM-99M: EFFECT OF LIPOPHILICITY. X. Zhang, Z. F. Su, J. R. Ballinger*, A. M. Rauth, A. Pollak, J. R. Thornback, Ontario Cancer Institute/University of Toronto, Toronto, ON, Canada; Resolution Pharmaceuticals, Mississauga, ON, Canada. (100536)

Objectives: Hypoxia in tumours is an important prognostic factor for response. Radiolabelled 2-nitroimidazoles (2-NI) have been used for imaging hypoxia and partition coefficient (*P*) appears to play a crucial role in suitability for imaging. We developed a series of eleven 2-NI containing a peptidic chelator for ^{99m}Tc with divergent *P* and evaluated them in an *in vitro* system. **Methods:** Two classes of N_3S chelators were used: dialkyl-Gly-Ser-Cys-linker-2-NI and dialkyl-Gly-Lys(2-NI)-Cys. Prepared by automated solid-phase peptide synthesis, the chelators were labelled by transchelation from ^{99m}Tc-gluconate at temperatures between 20 and 100°C. The reaction mixtures were analysed by HPLC. The accumulation of each complex in suspension cultures of Chinese hamster ovary cells incubated under aerobic or extremely hypoxic conditions was determined. **Results:** Radiochemical yields ranged from 5% to 80% for the 11 compounds. HPLC showed that some compounds formed two complexes with ^{99m}Tc, possibly syn- and anti-conformations with respect to the Tc=O bond. In general, the Gly-Ser-Cys chelator labelled more readily than the Gly-Lys-Cys chelator. The *P* values varied from 0.001 to 5, and were generally in accordance with predictions based on structure. There were also differences in *P* as a function of pH; the free acids had a lower *P* at pH 7.4 than at pH 2.0 due to ionisation, whereas the amides did not show this effect. Accumulation levels in cells were related to *P* but varied over a narrower range. Six of the 11 compounds showed selective localisation in hypoxic cells, with 1.8- to 3.6-fold higher accumulation in hypoxic *vs* aerobic cells. **Conclusions:** The peptidic class of 2-NI, with flexible and convenient solid-phase synthesis, deserves further study as agents for imaging hypoxia in tumours.

No. 332

SYNTHESIS OF A FLUORINE-18 LABELED ANTISENSE OLIGODEOXYNUCLEOTIDE AS A PROBE FOR IMAGING GENE EXPRESSION. D. Pan*, T. Toyokuni, J. R. Barrio, N. Satyamurthy, M. E. Phelps, S. S. Gambhir, University of California at Los Angeles School of Medicine, Los Angeles, CA. (500498)

We are developing methods to image gene expression *in vivo* by positron emission tomography (PET). Antisense oligodeoxynucletoides (ODN) and their derivatives complementary towards a small region of mRNA are being studied for targeting the mRNA of various amplified oncogenes. Here, we describe the synthesis of a PET ODN probe in which 5′-OH group of the ODN is replaced by [F-18]fluorine. The synthesis involves radiofluorination of a modified nucleoside followed by its coupling to a fully protected CPG-bound ODN. The key precursor, 5′-*O*-tosyl-3-*O*-di (*p*-methoxyphenyl) phenylmethyl-4-*O*-methyl-thymidine, was prepared in six steps from thymidine in a 22% overall yield. Nucleophilic fluorination of the precursor with [F-18]fluoride ion in the presence of K+/Kryptofix and subsequent deprotection gave 5'-deoxy-5'-[F-18]fluoro-4-*O*-methyl-thymidine. Coupling to the 5′-phosphoramidite-activated CPG-bound ODN, simultaneous cleavage from the CPG and de-protection, and HPLC purification furnished the target 5′-deoxy-5′-[F-18]fluoro-ODN probe. The HPLC spectrum was identical to that of the



F-19 counterpart. The replacement of 5′-OH by a fluorine atom did not cause any significant changes in hybridization affinity to complementary RNA sequence as determined by measurements of Tm. Biodistribution studies with various fluorine-18 labeled ODN derivatives in mice imaged in a microPET are currently underway.

No. 333

AN IMPROVED SYNTHESIS OF 9-[(3-[F-18]FLUORO-1-HYDROXY-2-PROPOXY)METHYL]GUANINE ([F-18]FHPG). C.-Y. Shiue*, G. G. Shiue, R. Hustinx, A. A. Alavi, S. L. Eck, University of Pennsylvania, Philadelphia, PA. (500488)

Gene transfer, especially herpes simplex virus thymidine kinase gene transfer has shown significant potential in treating several common cancers. The principal obstacle to successful gene therapy has been the development of genetic vectors capable of achieving efficient gene transfer and the methods of assessing their transfers *in vivo* non-invasively. [F-18]FHPG(**1**) has been synthesized and suggested as a potential agent for monitoring the efficiency of gene therapy. The purpose of this study was to improve and simplify the synthesis of [F-18]FHPG. **Methods:** [F-18]FHPG was synthesized by nucleophilic substitution of N2-(p-Anisyldiphenylmethyl)-9-{[1-(p-anisyldiphenylmethoxy)-3-toluenesulfonyloxy-2-propoxy]methyl}guanine with [F-18]fluoride at different temperatures. The resulting intermediate was deprotected in 1N HCl at different temperatures and the product was isolated with either HPLC (Alltech, C18, 10x250 mm; CH3CN/H2O; 5/95; 2 mL/min) or silica Sep-Pak (The by-product was washed out first with CH2Cl2/MeOH, 9/1 and the product **1** was isolated with CH3CN/H2O, 8/2). For stability studies, **1** was dissolved in 1N HCl and heated at 90°C and 120°C, respectively, for different time intervals and monitored with TLC. *In vitro* activity of **1** synthesized and purified by HPLC and TLC was evaluated with 9L (gliomas) cells. **Results:** The yield of **1** decreases as the reaction temperature increases. At 120°C and 90°C, and the product was purified with HPLC, the yield of **1** was 2 and 5-10%, respectively. The synthesis time was 90 min. from EOB. The yield of **1** increased to 10-15% when the reaction temperature was 90°C and the product was purified by silica Sep-Pak. The synthesis time was 60 min from EOB. [F-18]FHPG was unstable in 1N HCl at high temperature. At 120°C, 50% of **1** was decomposed in 10 min while 90% of **1** remained intact at 90°C. [F-18]FHPG purified either by HPLC or silica Sep-Pak has the same *in vitro* activity. **Conclusion:** The yield of [F-18]FHPG can be improved by carrying out the reaction at lower temperature (90°C instead of 120°C) and purified with silica Sep-Pak. The same procedure probably can be applied to prepare similar radiotracers (eg. penciclovir).

No. 334

SYNTHESIS OF F-18 2-FLUORO-5-METHYL-1-β-D-ARABINOFURANOSYLURACIL ([F-18]FMAU). P. S. Conti*, M. M. Alauddin, J. D. Fissekis, K. A. Watanabe, University of Southern California, Los Angeles, CA; Memorial Sloan-Kettering Cancer Center, New York, NY. (500282)

Objectives: 2′-Fluoro-5-(C-11)methyl-1-β-D-arabinofuranosyluracil (C-11 FMAU) is a potential marker for cell proliferation by positron emission tomography (PET). The presence of the fluorine atom at the 2′-position prevents catabolism *in vivo*. The short half-life of C-11 and the air-sensitive organometallic synthesis, limits the production and use of the C-11 compound. Fluorine-18 labeled FMAU may potentially be more advantageous in certain applications. The direct, stereospecific (*arabino*) introduction of fluorine at the 2′-position of the furanosyl moiety in a uracil nucleoside has not been possible. Here we are exploring the incorporation of F-18 fluorine at the C-2 (*arabino*) position of the sugar followed by coupling with the pyrimidine. **Methods:** 2-Fluoro-1,3,5-tri-O-benzoyl-α-D-ribofuranose was coupled with thymine silyl ether in MeCN using $SnCl_4$ by heating at 70°C for 40 minutes. The coupled product was characterized by NMR spectroscopy, then hydrolyzed by NaOM in MeOH. FMAU was purified by HPLC and characterized by NMR spectroscopy. Radiosyntheses were performed with F-18 2-fluoro-1,3,5-tri-O-benzoyl-α-D-ribofuranose which was prepared following a method developed in our laboratory. The F-18 fluoro-sugar was purified by HPLC, dried and coupled with thymine silyl ether. The crude coupled product was extracted with CH_2Cl_2, evaporated and heated with NaOMe in MeOH for 7 min to hydrolyze the protecting groups in the sugar moiety. **Results:** The coupling reaction produced a mixture of α- and β-isomers which could be separated by HPLC. Radiolabed FMAU was isolated by HPLC purification using 7.5% MeCN in water. The product was co-eluted with an authentic sample of unlabeled FMAU. In preliminary runs (n=3) the decay corrected radiochemical yield was low (2-5%) although radiochemical purity was >99%. Synthesis time was 3.75h from the end of bombardment. **Conclusion:** F-18 FMAU has been successfully prepared although optimization of reaction conditions is required in order to improve yield.

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OPTIMIZING LABELING SUBSTRATE STRUCTURE FOR 3′-DEOXY-3′-[F-18]FLUOROTHYMIDINE: [F-18]FLT. J. R. Grierson*, A. F. Shields, University of Washington, Seattle, WA; Wayne State University, Detroit, MI. (500381)

Objectives: We recently demonstrated that FLT can be used with PET to provide images of proliferation in vivo. We have established a working synthesis for [F-18]FLT, however, we desired a more efficient process suitable for multi-dosing. **Methods:** Our original labeling substrate for [F-18]FLT synthesis was compound (1): 1-(2-deoxy-3-O-methanesulfonyl-5-O-(4,4′-dimethoxytrityl)-β-D-threo-pentofuranosyl)-3-(2,4-dimethoxybenzyl)thymine. We compared the labeling of a variety of newer substrates against (1) to discover an optimum structure (see Table). Trial labeling experiments were done with the same batch of pre-solubilized fluoride (substrate/KRY/carbonate 1.6-2:2:1) in MeCN, and portions were used (some sets used different batches but always included Cpd (1) for comparison, no yields included unless Cpd 1 gave >20 percent). Production level experiments = yields in parentheses (substrate/KRY/carbonate 1.3:2:1) were done with whole batches of dried, complexed fluoride. **Results** (Table) **and Conclusions**: The N-alkylated substrate performed better than the N-acylated case (1 vs. 2). Of all the leaving groups used the 3-NO2-PhSO2-ester was superior, because the 4-NO2-PhSO2-ester was not displaced from the sugar, rather, the 4-NO2-on the leaving group itself was displaced from the aromatic ring (i.e. nucleophilic aromatic substitution). The triflate compound (5) yield was low, due to its poor stability during synthesis.

Labeling substrate structure and r.c. yields (decay corr.)

Compound	N-group	5-O-group	3-O-ester	Average yield
1	2,4-Di-MeOBn	Di-MeO-trityl	mesylate (Mes)	23.5 (17)
2	Alloxycarbonyl	Di-MeO-trityl	mesylate	17 (10)
3	2,4-Di-MeOBn	Di-MeO-trityl	3-NO2-PhSO2-	42
4	p-MeOPh	p-MeOPh	4-NO2-PhSO2-	(60)-not FLT
5	Alloxycarbonyl	Di-MeO-trityl	triflate	21

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A METABOLIC STUDIES OF 18F- ALPHA-METHYL TYROSINE: FRACTIONATION OF ITS INCORPORATION INTO BRAIN AND TUMOR IN MICE BEARING LCI-180. K. Tomiyoshi*, T. Inoue, K. Endo, Gunma University, Maebashi, Japan. (212)

Objectives: 18F- alpha-methyl tyrosine(18FAmT) has been clinically used and proved to be a very promising agent as determined from our PET studies. However little information on metabolism of 18FAmT is known. We investigated the metabolism in tumor and brain of mice bearing LCI-180 colorectal carcinoma. Methods : Homogenized tissues of brain and tumor in postinjection of 18FAmT at 5, 30 and 60 minutes were analyzed by fractionation method into acid soluble fraction(ASF) and acid precipitable fraction(APS). APS was further investigated to assess the incorporation of 18FAmT into each fraction by HPLC and TLC. Results : 18FAmT was stable up to 6 hours in Saline and plasma in vivo study. Incorporation into four fractions of brain and tumor at 60 minute post injection were 20% and 12%. Among them, 10% of the activity were incorporated to lipid in brain and 5% in tumor. There was 5%, 2%, 2% in RNA, DNA and protein. Conclusion : The uptake of 18FAmT in tissue was rapid and accomplished before 30 minutes and then slowly diffused in blood. These results implied that 18FAmT was little metabolized to protein and trapped as intact 18FAmT in cell up to 60 minutes. 18FAmT is promising tracer for imaging and quantification of transport rate using two compartment models.