

# Fluorine-18 labeled thia fatty acids for PET imaging of fatty acid oxidation in heart and cancer

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## Abstract

Myocardial fatty acid oxidation (FAO) imaging is a noninvasive technique that can measure FAO rates in tissues for research applications in animals and humans, as well as clinical applications in managing patients with metabolic disorders. FAO imaging has great potential in diagnosis and monitoring of patients with ischemic heart disease, cardiomyopathies, myocarditis, acute coronary syndrome, and heart failure. Applications of FAO imaging in oncology and endocrinology are also highly anticipated. For over 20 years, our laboratory has investigated fluorine-18 labeled thia-substituted fatty acid analogs as positron emission tomography (PET) probes of myocardial FAO. These FAO probes share a common design motif of metabolic trapping in the myocardium subsequent to their commitment to the mitochondrial FAO pathway, in analogy to the design of 2-[<sup>18</sup>F]fluoro-2-deoxy-D-glucose (FDG) as a metabolically trapped probe of glucose transport and phosphorylation. This mini-review describes the development of these FAO probes, from the seminal 6-thia substituted analog, 14-[<sup>18</sup>F]fluoro-6-thia-heptadecanoate (FTHA), to the most recently developed oleate-based FAO analog, 18-[<sup>18</sup>F]fluoro-4-thia-oleate (FTO). It is shown that small changes in thia fatty acid analog structure can exert profound differences in the biodisposition and specificity of these probes to indicate myocardial FAO, particularly in conditions of oxygen deprivation. The potential of these probes for imaging of FAO in cancer is supported by initial uptake studies in cultured cancer cells. Thus, <sup>18</sup>F-labeled thia fatty acid analogs have significant potential to play an important role as clinical PET probes of FAO in cardiovascular diseases, oncology, and future anticipated applications in endocrinology and neurology.

**Keywords:** PET; fatty acid oxidation; thia fatty acid; FTHA; FTP; FTO

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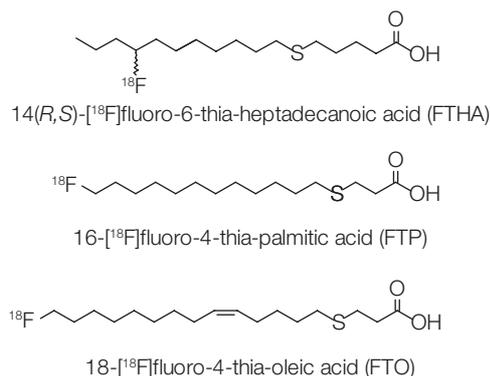
## Introduction

There remains a great demand for advancements of molecular imaging techniques to allow noninvasive assessment of biochemical processes for the metabolic characterization of human diseases. Positron emission tomography (PET), and to a lesser extent, single photon emission computed tomography (SPECT) and magnetic resonance imaging (MRI) can provide important information on ion and metabolic fluxes in human tissues in a noninvasive and quantitative manner. Energy metabolic pathways in heart and tumors are sensitively regulated and immediately reflect any dietary and hormonal changes or abnormalities. Thus, monitoring of

substrate metabolism provides substantial information about the disease state and therapeutic progress. The major energy producing substrates are fatty acids (derived from plasma nonesterified fatty acids or local lipolysis from circulating lipoproteins), glucose, lactate, and ketone bodies. The glucose-based PET probe, FDG, has already set a benchmark in clinical care by indication of glucose utilization in tissues exhibiting high glycolytic rates, including a broad spectrum of cancer types [1] and ischemic but viable myocardium [2]. Fatty acid-based SPECT probes are currently in routine practice in Japan, but not in the United States.  $^{11}\text{C}$ -labeled fatty acid PET radiotracers have been in use for over 35 years, but have been mainly confined to research studies due to their short physical half-life (20.4 m) and the complex nature of the quantitation of the dynamic PET images [3–7]. Quantitation of fatty acid oxidation (FAO) rates is not possible in ischemic myocardium because the diffusion rates of the oxidation product  $^{11}\text{C}$ - $\text{CO}_2$  and unoxidized  $^{11}\text{C}$ -palmitate are both rapid, thereby confounding the model fit [6, 7]. However,  $^{11}\text{C}$ -labeled fatty acid probes have value to indicate accumulation of exogenous fatty acids in the myocardial triglyceride pool, because this component is characterized by a slow turnover. Over the last 20 years, our group has been involved in the development of  $^{18}\text{F}$ -labeled thia fatty acids as metabolically trapped probes of FAO. The half-life of  $^{18}\text{F}$  (110 minutes) allows for regional distribution of probes, while the presence of the sulfur heteroatom blocks the  $\beta$ -oxidation of the fatty acid and also renders the molecule as a poor substrate for incorporation into complex lipids [8, 9]. In this mini-review, we intend to highlight the incremental development and promises offered by thia fatty acids as potential probe molecules for FAO imaging in heart and cancer.

#### 14-(R,S)-[ $^{18}\text{F}$ ]fluoro-6-thia-heptadecanoic acid (FTHA)

FTHA (Fig. 1) was the first-generation thia fatty acid probe synthesized in 1990 and evaluated as a PET probe of fatty acid metabolism in mice [8–9]. After intravenous administration of FTHA, the probe was rapidly taken up by tissues as evident from its rapid blood pool clearance. The heart showed highest uptake of  $39.8 \pm 3.0\% \text{ID/g}$  at 5 minutes and subsequently cleared with a biological half-life of about



**Fig. 1** Structures of  $^{18}\text{F}$  thia fatty acids [ $^{18}\text{F}$ ]FTHA, [ $^{18}\text{F}$ ]FTP and [ $^{18}\text{F}$ ]FTO.

2 hours. The heart:blood ratios of uptake were  $4.3 \pm 0.4$ ,  $20 \pm 6$ ,  $41 \pm 6$ , and  $82 \pm 16$  at 0.25, 1, 5 and 60 minutes respectively. The myocardial trapping of  $^{18}\text{F}$ -radioactivity was drastically reduced by pretreatment of mice with the carnitine palmitoyl-transferase-1 (CPT-1) inhibitor POCA, showing CPT-1 dependent uptake of FTHA. FTHA has shown high myocardial uptake, longer retention and rapid clearance from the bloodstream in humans also, making it a useful tracer of fatty acid metabolism for PET [9–10]. In a mechanistic study, the net retention of FTHA was depressed in ischemic porcine myocardium, but remained unchanged in hypoxic myocardium [11]. The lower retention in ischemia confirmed the applicability of FTHA for imaging ischemic myocardium [11], but the lack of sensitivity to lower FAO rates in hypoxic myocardium motivated further tracer development to improve specificity to monitor FAO rates. Nevertheless, FTHA continues to be the most-investigated thia fatty acid PET probe, and has been used as a fatty acid uptake probe in human studies in heart [12–15], liver [16], skeletal muscle [12], and brain [17].

#### 16-[ $^{18}\text{F}$ ] fluoro-4-thia-palmitate (FTP)

The lack of correspondence of FTHA trapping to FOA rates in hypoxic myocardium was the driving force to develop a second-generation thia fatty acid analog. In 2000, the palmitate-based analog, 16-[ $^{18}\text{F}$ ] fluoro-4-thia-palmitic acid (FTP, Fig. 1), was identified as a FAO probe [18]. FTP was evaluated in a rat model with varying dietary conditions: fed (30 minutes), fasted (30 minutes), fasted (120 minutes), and fasted (30 minutes with CPT-I inhibitor “etomoxir”). The highest uptake was observed in heart, liver and kidneys, regardless of

dietary status [18]. The new tracer FTP was also evaluated in Langendorff perfused rat heart to study the kinetics and relationship of tracer retention to FAO rate in normoxic and hypoxic myocardium. FTP trapping in the rat myocardium correlated well with [9,10-<sup>3</sup>H]palmitate oxidation rates in both normoxic and hypoxic conditions. In the same model, the myocardial accumulation of the 6-thia fatty acid analog, 17-[<sup>18</sup>F]fluoro-6-thia-heptadecanoate, was insensitive to the decrease in palmitate oxidation rate in hypoxic hearts. Thus, the placement of the thia-substituent at the fourth position of the fatty acid analog significantly improved the specificity of the probe for indication of FAO. It was speculated that 4-thia fatty acid analogs have a more rapid turnover in pools of FAO intermediates (i.e., acyl-CoAs and acyl-carnitines) than 6 thia fatty acid analogs that allow these pools to clear the myocardium if they are not further metabolized by mitochondrial  $\beta$ -oxidation. FTP showed high myocardial uptake and retention in porcine heart, consistent with metabolic trapping seen in rats [18]. Detailed mechanistic studies of FTP uptake in isolated rat hearts demonstrated FAO dependent metabolic accumulation that was in proportion to FAO rates as measured by [9,10-<sup>3</sup>H]palmitate oxidation [18]. The ratio of proportionality, which we denoted as the “lumped constant (LC)” in good analogy with FDG modeling methodology, was decreased in hypoxic conditions, suggesting somewhat different affinities of FTP and palmitate at transport and/or metabolic control points of fatty acid disposition within the myocyte [19]. However, changes in the fatty acid composition of the perfusion medium did not influence the LC, which may allow FTP to be a prototypical fatty acid analog for indication of overall FAO [19]. The only major shortcoming observed with FTP during these studies was its lower retention over time in rat myocardium: myocardial clearance was about 70% at 2 hours relative to 30 minutes [18].

### 18-[<sup>18</sup>F] fluoro-4-thia-oleate (FTO)

The suboptimal myocardial retention of FTP in rat myocardium prompted the development of the oleate-based probe 18-[<sup>18</sup>F]fluoro-4-thia-oleic acid (FTO, Fig. 1) [20]. FTO was recently synthesized and evaluated in rats with and without CPT-1 inhibition [20]. FTO was motivated from oleate’s relative abundance in plasma, and a clinical study showing that dietary oleate

had a somewhat higher disposition relative to palmitate toward whole-body FAO [21]. Synthesis of the labeling precursor for FTO required an 11-step synthetic process due to the inclusion of 18-bromo leaving group, 9-*cis*-double bond and the 4-thia substituent [20]. FTO showed excellent myocardial imaging characteristics and superior myocardial retention than the previously developed tracers FTTHA, and FTP. FTO showed three- to four-fold higher heart: background tissue radioactivity ratios than FTP. FTO uptake by heart was approximately reduced to 80% by pretreatment of CPT-1 inhibitor etomoxir indicating high dependence on CPT-1 mediated mitochondrial transport. The microPET images of FTO accumulation in the rat myocardium were clearly superior to those with FTP [20]. The folch-type extraction analysis showed 70–90% of the <sup>18</sup>F-radioactivity to be protein-bound in heart, liver and skeletal muscle. These values were significantly higher than those for FTP and FTTHA, suggesting higher specificity of mitochondrial trapping. The preliminary data with FTO indicate this probe to be the most specific myocardial FAO probe to date based on the thia fatty acid concept.

### Mechanism of metabolic trapping of thia fatty acids

Based on our findings with FTTHA, FTP and FTO, we propose a plausible mechanism for the uptake and metabolic trapping in myocardium of terminally <sup>18</sup>F-labeled 4-thia fatty acid analogs, using FTO as an example (Fig. 2). FTO enters into the cardiomyocyte via fatty acid transporters (CD36 /FATP (fatty acid transporter protein) [22]. In the cytosol, FTO is esterified to FTO-CoA by fatty acyl-CoA synthase (FACS). FTO-CoA is then transferred to carnitine via CPT-1. The acyl carnitine is then shuttled across the inner

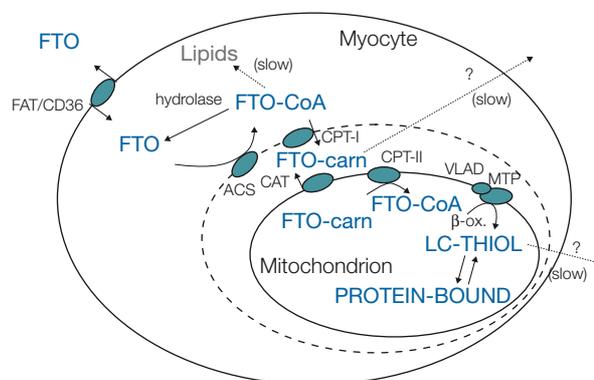
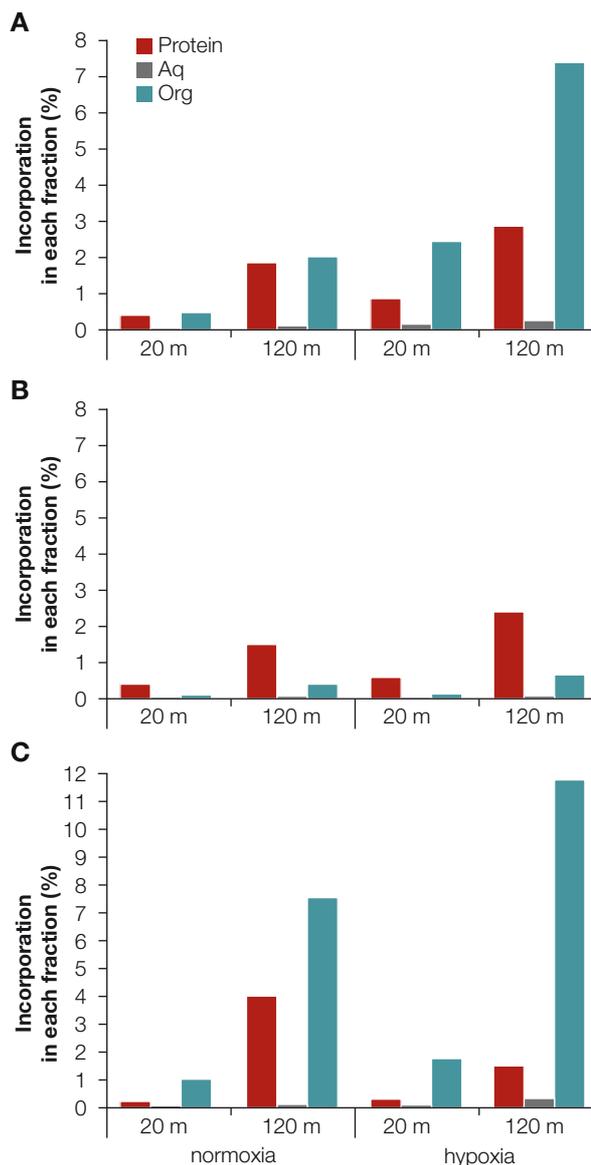


Fig. 2 Proposed metabolism scheme for FTO in the cardiomyocyte.

mitochondrial membrane where it gets converted back to FTO-CoA by CPT-2. In the mitochondrial matrix, FTO-CoA may undergo two subsequent steps of  $\beta$ -oxidation, forming the 3-hydroxy acyl-CoA moiety, and then spontaneously decomposes to a long-chain thiol, 14- $^{18}\text{F}$ fluoro-tetradecane-1-thiol, which in turn covalently or noncovalently binds to various mitochondrial proteins. Differential centrifugation identified the mitochondrial fraction as containing the predominant amount of retained  $^{18}\text{F}$ -radioactivity, while native-gel electrophoresis of heart extracts showed that the  $^{18}\text{F}$ -radioactivity was associated with a broad spectrum of molecular weights, evidencing the non-specific nature of the protein binding (unpublished data). Thus, the accumulation of protein-bound  $^{18}\text{F}$ -radioactivity in tissue is a direct readout for FAO of exogenous fatty acids. Slow clearance processes remain unclarified, particularly for FTHA and FTP. Two likely mechanisms of clearance are slow releases of carnitine esters and/or  $\beta$ -oxidation metabolites (Fig. 2, dotted arrows).

### FAO imaging in cancer

It is well recognized that not all tumor cell types utilize glucose as primary energy substrate. FAO can provide for a predominant fraction of ATP production in tumors that have sufficient oxygen supply, such as prostate cancer [23]. Indeed, the low rate of glycolysis in early-stage prostate cancer severely limits the applicability of the FDG-PET method to staging of patients with newly diagnosed disease. We have performed a preliminary study of the uptake of  $^{18}\text{F}$ FTP in cultured 9L rat glioma, LNCaP human prostate and PC-3 human prostate cancer cell lines [24] (Fig. 3). FTP was taken up avidly by the cancer cells. Hypoxic incubation resulted in an increase in overall uptake, consistent with AMP-kinase activation of fatty acid transport [22]. Folch-type extractions were performed to indicate the FAO-dependent metabolic incorporation of  $^{18}\text{F}$  F-radioactivity into protein. The LNCaP cell line, which is derived from well-differentiated, androgen-dependent prostate cancer, showed the highest fractionation of FTP into the protein-bound phase, corroborating the findings of high levels of FAO expressed in early-stage prostate cancer [23].  $^{18}\text{F}$ -labeled straight-chain and  $\beta$ -methylated fatty acids have been shown to be taken up in rat tumor models [25, 26], however these agents do not metabolically trap in a FAO-



**Fig. 3** Uptake and disposition of  $^{18}\text{F}$ FTP in 9L rat glioma (A), LNCaP human prostate (B) and PC-3 human prostate (C) cancer cells. Uptake is expressed as percentage of total radioactivity added to the culture medium prior to incubation. Cells were incubated for either 20 m or 120 m in normoxic (21%  $\text{O}_2$ ) or hypoxic (1%  $\text{O}_2$ ) conditions, then washed thrice with PBS to remove extracellular radiotracer. Folch-type extractions were performed in chloroform/methanol (2:1) and 50% urea / 5% sulfuric acid, and radioactivity counted in the aqueous, organic and pellet (protein-bound) fractions. In general, hypoxic incubation resulted in increased uptake of FTP and lower fractionation to the protein-bound fractions, indicating a relatively lower partitioning of the probe to FAO. High protein-bound fractions were seen in LNCaP cells, indicating a high relative rate of FAO. Data from Bansal et al [24].

dependent manner. To our knowledge, PET imaging studies in cancer patients with fatty acid analogs have yet to be done, but we believe there exists considerable potential. •

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**References:**

- Poeppel TD, Krause BJ, Heusner TA, Boy C, Bockisch A, Antoch G (2009) PET/CT for the staging and follow-up of patients with malignancies. *Eur J Radiol* 70:382–392
- Knuuti J, Tuunanen H (2010) Metabolic imaging in myocardial ischemia and heart failure. *Q J Nucl Med Mol Imaging* 54: 168–176
- Shoghi KI, Finck BN, Schechtman KB, Sharp T, Herrero P, Gropler RJ, Welch MJ (2009) In vivo metabolic phenotyping of myocardial substrate metabolism in rodents: differential efficacy of metformin and rosiglitazone monotherapy. *Circ Cardiovasc Imaging* 2(5):373–381
- Schelbert HR, Schwaiger M (1986) PET studies of the heart. In: Phelps M, Mazziotta JC, Schelbert HR. eds. *Positron emission tomography and autoradiography. Principles and applications for the brain and heart*. New York: Raven Press, p. 581–661
- Fox KAA, Abendschein DR, Ambos HD, Sobel BE, Bergman SR (1985) Effects of metabolized and nonmetabolized fatty acid from canine myocardium: implications for quantifying myocardial metabolism tomographically. *Circ Res* 57:232–243
- Rosamond TL, Abendschein DR, Sobel BE, Bergmann SR, Fox KAA (1987) Metabolic fate of radiolabeled palmitate in ischemic canine myocardium: implications for positron emission tomography. *J Nucl Med* 28:1322–1329
- Wyns W, Schwaiger M, Huang SC, Buxton DB, Hansen H, Selin C, Keen R, Phelps ME, Schelbert HR (1989) Effects of inhibition of fatty acid oxidation on myocardial kinetics of C-labeled palmitate. *Circ Res* 65:1787–1797
- DeGrado TR (1991) Synthesis of 14 (R, S)-[<sup>18</sup>F] fluoro-6-thia-heptadecanoic acid (FTHA). *Journal of Labeled Compounds and Radiopharmaceuticals* 29:989–995
- DeGrado TR, Coenen HH, Stocklin G (1991) 14(R, S)-[<sup>18</sup>F]fluoro-6-thia-heptadecanoic acid (FTHA): evaluation in mouse of a new probe of myocardial utilization of long chain fatty acids. *J Nucl Med* 32:1888–1896
- Ebert A, Herzog H, Stain GL, Henrich MM, DeGrado TR, Coenen HH, Feinendegen LE (1994) Kinetics of 14(R, S)-fluoro-18-fluoro-6-thia-heptadecanoic acid in normal human hearts at rest, during exercise and after dipyridamole injection. *J Nucl Med* 35:51–56
- Renstrom B, Rommelfanger S, Stone CK, DeGrado TR, Carlson KJ, Scarbrough E, Nickles RJ, Liedtke AJ, Holden JE (1998) Comparison of fatty acid tracers FTHA and BMIPP during myocardial ischemia and hypoxia. *J Nucl Med* 39:1684–1689
- Takala TO, Nuutila P, Pulkki K, Oikonen V, Grönroos T, Savunen T, Vähäsilta T, Luotolahti M, Kallajoki M, Bergman J, Forsback S, Knuuti J (2002) 14(R,S)-[<sup>18</sup>F]Fluoro-6-thia-heptadecanoic acid as a tracer of free fatty acid uptake and oxidation in myocardium and skeletal muscle. *Eur J Nucl Med Mol Imaging* 29: 1617–1622
- Wallhaus TR, Taylor M, DeGrado TR, Russell DC, Stanko P, Nickles RJ, Stone CK (2001) Myocardial free fatty acid and glucose use after carvedilol treatment in patients with congestive heart failure. *Circulation* 103:2441–2446
- Taylor M, Wallhaus TR, DeGrado TR, Russell DC, Stanko P, Nickles RJ, Stone CK (2001) An evaluation of myocardial fatty acid and glucose uptake using PET with [<sup>18</sup>F]fluoro-6-thia-heptadecanoic acid and [<sup>18</sup>F]FDG in patients with congestive heart failure. *J Nucl Med* 42:55–62
- Turpeinen AK, Takala TO, Nuutila P, Axelin T, Luotolahti M, Haaparanta M, Bergman J, Hämäläinen H, Iida H, Mäki M, Uusitupa MI, Knuuti J (1999) Impaired free fatty acid uptake in skeletal muscle but not in myocardium in patients with impaired glucose tolerance: studies with PET and 14(R,S)-[<sup>18</sup>F]fluoro-6-thia-heptadecanoic acid. *Diabetes* 48:1245–1250
- Iozzo P, Turpeinen AK, Takala T, Oikonen V, Solin O, Ferrannini E, Nuutila P, Knuuti J (2003) Liver uptake of free fatty acids in vivo in humans as determined with 14(R, S)-[<sup>18</sup>F] fluoro-6-thia-heptadecanoic acid and PET. *Eur J Nucl Med Mol Imaging* 30:1160–1164
- Karmi A, Iozzo P, Viljanen A, Hirvonen J, Fielding BA, Virtanen K, Oikonen V, Kemppainen J, Viljanen T, Guiducci L, Haaparanta-Solin M, Nägren K, Solin O, Nuutila P (2010) Increased brain fatty acid uptake in metabolic syndrome. *Diabetes* 59:2171–2177
- DeGrado TR, Wang S, Holden JE, Nickles RJ, Taylor M, Stone CK (2000) Synthesis and preliminary evaluation of (18)F-labeled 4-thia palmitate as a PET tracer of myocardial fatty acid oxidation. *Nucl Med Biol* 27:221–231
- DeGrado TR, Kitapci MT, Wang S, Ying J, Lopaschuk GD (2006) Validation of <sup>18</sup>F-fluoro-4-thia-palmitate as a PET probe for myocardial fatty acid oxidation: effects of hypoxia and composition of exogenous fatty acids. *J Nucl Med* 47:173–181
- DeGrado TR, Bhattacharyya F, Pandey MK, Belanger AP, Wang S (2010) Synthesis and preliminary evaluation of 18-<sup>18</sup>F-fluoro-4-thia-oleic Acid (FTO) as a PET probe of fatty acid oxidation. *J Nucl Med* 51:1310–1317
- DeLany JP, Windhauser MM, Champagne CM, Bray GA (2000) Differential oxidation of individual dietary fatty acids in humans. *Am J Clin Nutr* 72:905–911
- Lopaschuk GD, Ussher JR, Folmes CDL, Jaswal JS, Stanley WC (2010) Myocardial fatty acid metabolism in health and disease. *Physiol Rev* 90:207–258
- Liu Y (2006) Fatty acid oxidation is a dominant bioenergetic pathway in prostate cancer. *Prostate Cancer Prostatic Dis* 9:230–234
- Bansal A, DeGrado TR (2010) Comparison of [<sup>14</sup>C]choline and [<sup>18</sup>F]fluoro-4-thia-palmitate (FTP) uptake in hypoxic 9L glioma cells. *J Nucl Med* 51(Suppl 1):198P
- Kubota K, Takahashi T, Fujiwara T, Yamada S, Sato T, Kubota R, Iwata R, Ishiwata K, Ido T, Matsuzawa T (1991) Possibility for tumor detection with fatty acid analogs. *Int J Rad Appl Instrum B* 18:191–195
- Greig NH, Nariai T, Noronha JG, Schmall B, Larson DM, Soncrant TT, Rapoport SI (1991) Brain tumor imaging in rats using the positron emitting fatty acid d-erythro-9,10-[<sup>18</sup>F] difluoropalmitate. *Clin Exp Metastasis* 9:67–73