
Is 3'-deoxy-3'-¹⁸F-fluorothymidine a better marker for tumour response than ¹⁸F-fluorodeoxyglucose?

Sven N. Reske¹, Sandra Deisenhofer¹

¹ Universitätsklinik Ulm, Robert-Koch-Strasse 8, 89081 Ulm, Germany

Published online: 24 May 2006

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Abstract. 3'-Deoxy-3'-¹⁸F-fluorothymidine (FLT) was developed in 1998 by Shields and co-workers because monitoring of treatment response would be facilitated by imaging agents able to provide measures of tissue and tumour proliferation. Since then, FLT metabolism has been clarified in more detail in cell culture and experimental animal tumour models and also in clinical studies. Recently, FLT has increasingly been used for the assessment of response to anticancer treatment, mainly in tumour xenograft SCID mouse models; in contrast, clinical data are scarce. In this article we briefly summarise the intermediary metabolism of FLT and its application as an anticancer treatment response probe. The potential value and limitations of FLT as a highly promising proliferation imaging probe and its use for monitoring of treatment response are discussed.

Keywords: Fluorothymidine – Treatment response – Metabolism – Monitoring – Clinical studies

Eur J Nucl Med Mol Imaging (2006) 33:S38–S43
DOI 10.1007/s00259-006-0134-2

Introduction

Assessment of tumour response to cytoreductive treatment is an essential component of the management of patients with malignant disease in clinical practice and serves as an objective end point in clinical trials. The response of solid tumours to anticancer agents and/or radiotherapy is commonly assessed by radiological imaging techniques. The WHO first established response assessment criteria in 1979 to standardise the recording and reporting of response

assessment of solid tumours [1]. In 1994, several international health organisations formulated the revised RECIST criteria for solid tumour response assessment [2] (Table 1).

As shown in Table 1, the employed criteria are mostly anatomically defined. Recently, functional and molecular imaging MR and PET techniques have been developed and introduced into clinical practice [3–5]. PET has demonstrated the ability to detect changes in tumour metabolism prior to changes in the actual size of the tumour, and to predict outcome earlier and more precisely [6–8]. The performance of ¹⁸F-fluorodeoxyglucose (FDG) PET in evaluating tumour response is reviewed in detail in this issue by Lammertsma, Mikhael and Stroobants. Here, we shall briefly summarise the intermediary metabolism of 3'-deoxy-3'-¹⁸F-fluorothymidine (FLT) and review its application as an anticancer treatment response probe.

Metabolism of FLT

The concept of using tumour uptake of FLT as a surrogate marker for tumour proliferation was developed because early experiments using ¹¹C-thymidine PET to measure proliferation were not successful. Rapid degradation of ¹¹C-thymidine by thymidine phosphorylase (EC 2.4.2.4), present in blood, liver, spleen and tumour tissue, resulted in a high blood radioactivity background owing to the many ¹¹C-labelled metabolites [9]. For the same reason, iodine-labelled desoxyuridine was inappropriate for measuring proliferation in vivo [10, 11]. FLT is more stable against thymidine phosphorylase-mediated degradation and was thus developed and tested as a potential proliferation marker for PET [12].

After intravenous injection, FLT distributes rapidly in the extracellular fluid and is transported from there into the cytosol via nucleoside transporters, probably mostly via the equilibrative nucleoside transporter ENT1 and in some tissues, such as the intestines, also via the Na⁺-dependent concentrative nucleoside transporter CNT1. The transmembranous flux of FLT, however, has not yet been well characterised. It has been shown that ENT1 expression is upregulated severalfold in various tumour cell lines [13, 14], fostering the concept of using radiolabelled

Sven N. Reske (✉)
Universitätsklinik Ulm,
Robert-Koch-Strasse 8,
89081 Ulm, Germany
e-mail: sven.reske@uniklinik-ulm.de
Tel.: +49-731-50024500; Fax: +49-731-50024503

Table 1. Definition of best response according to WHO or RECIST criteria

Best response	WHO change in sum of the products	RECIST change in sum of the longest diameters
CR	Disappearance; confirmed at 4 wks	Disappearance; confirmed at 4 wks
PR	50% decrease; confirmed at 4 wks	30% decrease; confirmed at 4 wks
SD	Neither PR nor PD criteria met	Neither PR nor PD criteria met
PD	25% increase; no CR, PR or SD documented before increased disease	20% increase; no CR, PR or SD documented before increased disease

WHO World Health Organisation, RECIST Response Evaluation Criteria in Solid Tumours, CR complete response, PR partial response, SD stable disease, PD progressive disease

nucleosides for imaging and functional characterisation of malignant tumours.

Within the cytosol, FLT is phosphorylated by thymidine kinase 1 (TK1) (EC 2.7.1.21). TK1 is a cell cycle regulated enzyme with a severalfold increased expression during the S phase [15–17]. In many malignant tumours, TK1 is constitutively upregulated. FLT is a selective substrate for TK1 [18]. In contrast, thymidine and other 2-arabinofuranosyl-nucleosides such as FIAU [5-iodo-(2-deoxy-2-fluoro- β -D-arabinofuranosyl)uracil] are also phosphorylated by the mitochondrial enzyme thymidine kinase 2 (TK2) (EC 2.7.1.21), which is expressed independently of the cell cycle, leading to significant cardiac and liver toxicity when they are used as antiviral agents with therapeutic intent [19].

In proliferating cells, FLT is metabolised through the anabolic arm of the thymidine salvage pathway. This pathway has an important role in balancing intracellular nucleotide pools. It operates by using futile phosphorylation/dephosphorylation cycles to adjust intracellular nucleotide pools, derived from de novo synthesis and nucleoside influx from extracellular fluid and phosphorylated by TK1 [20]. The catabolic arm of these futile cycles involves a set of nucleotidases (e-NT, dNT-1, dNT-2, cN-I, cN-II), dephosphorylating excess deoxynucleotides. These nucleotidases have different substrate specificities and tissue distributions [21–24]. Nucleosides may efflux from the cytosol through facilitated, nucleoside transporter-mediated transport. Additional outward transport may be accomplished through a nucleoside transporter efflux pump [25].

FLT monophosphate (FLTMP) is phosphorylated by thymidylate kinase (TMPK) to FLT diphosphate (FLTDP) and subsequently to FLT triphosphate (FLTTP) by nucleotide diphosphate kinase (NDPK) [26–29]. Phosphorylation of thymidine monophosphate by TMPK is considered irreversible, whereas non-specific phosphorylation of nucleotide diphosphates by NDPK is probably reversible

[20, 30–32]. NDPK is a ubiquitous, S phase-regulated kinase [32], overexpressed in some tumours [33].

It has been shown that in A549 lung adenocarcinoma cells, TMPK is the rate-limiting enzyme for FLT metabolism within the anabolic arm of the thymidine salvage pathway [25]. FLTMP NT-mediated dephosphorylation and subsequent FLT efflux was about 50% of total FLT initially accumulated within this tumour cell line [25].

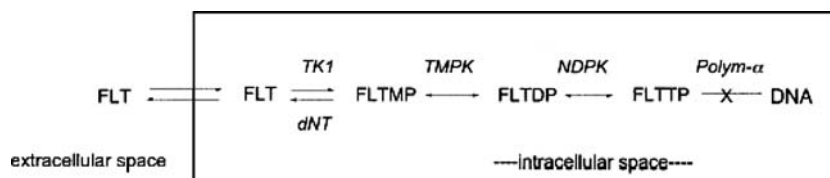
Due to the 3' substitution with ^{18}F , FLT cannot be incorporated into the growing DNA chain but leads to DNA chain termination [26, 27]. Whether FLT, bound to the end of the growing DNA chain, is a substrate for DNA repair enzymes, for instance nucleoside excision repair enzymes, has, to our knowledge, not yet been examined.

In summary, FLT enters the cell mainly through ENT1-mediated facilitated transport. Intracellular radioactivity accumulation results exclusively from formation of phosphorylated FLT mono-, di- and triphosphate nucleotides in competition with dNT-1-mediated dephosphorylation and FLT efflux [25]. The kinetics of this process is dominated by the expression and activities of TK1, dNT-1 and TMPK, the rate-limiting step in the net conversion of FLT to FLTTP being the activity of TMPK [25] (Fig. 1).

Treatment evaluation in preclinical studies

The suitability of FLT for assessing anticancer treatment response has been assessed in several cell culture and tumour xenograft mouse models (Table 2, modified from ref. [34]). In most cell culture and virtually all tumour xenograft mouse models, a decrease in cellular FLT uptake was reported (Table 2, Fig. 2). Interestingly, increased FLT uptake was observed in several cell culture models when the thymidine synthesis pathway was blocked with 5-fluorouracil (5-FU) and/or methotrexate [35], suggesting that the salvage pathway might have been upregulated to

Fig. 1. Metabolic pathway of FLT according to Grierson et al. [25]



adjust thymidine demands to actual supply. It might be hypothesised that this rather fragile metabolic situation can only be maintained within a post-therapeutic time window, when thymidine flux through the salvage pathway can really meet the demands for DNA synthesis and cell viability is not compromised. In those studies comparing treatment-associated changes in FLT and FDG uptake, an earlier and more pronounced post-treatment decrease in cellular or tumour uptake of FLT was observed. In addition, the temporary increase in FDG uptake after radiation therapy was not observed with FLT.

Clinical studies

Only very limited clinical data are available on treatment response assessment with FLT alone or in comparison to FDG. As reviewed by Beets et al. [36] and re-assessed in several recent studies, tumour uptake of FLT has been compared with that of FDG in several clinical tumour entities [37–41]. In general, FLT uptake is considerably lower than that of FDG, and in the case of non-small cell lung cancer (NSCLC), FLT uptake has been found in only 50% of FDG-positive nodal metastases (Buck, submitted). Therefore it is currently unclear whether the sensitivity of FLT imaging of primary assessable tumour lesions according to RECIST criteria is sufficient for reliable assessment of tumour response. In addition, physiological high uptake in haematopoietic bone marrow and the liver will certainly render assessment of small bone/bone marrow and liver metastases difficult.

Buck et al. and Vesselle et al. were the first to demonstrate a clear-cut correlation of tumour FLT uptake, measured with PET, and tumour proliferation rate, measured with Ki67 immunostaining of proliferating cells in NSCLC resection or biopsy specimens [38, 42].

These findings were recently confirmed by Muzi et al. [43] and Yap et al. [44].

In follicular lymphoma, a close correlation of FLT uptake with proliferation rate was recently observed by our group (Buck et al., submitted, Fig. 3). The results of this study indicated that progression of indolent to aggressive lymphoma might be assessed with FLT PET (Buck et al., submitted). Also in brain tumours, tumour FLT uptake correlated with Ki67-measured tumour proliferation rate [45]. In contrast, Smyczek-Gargya et al. did not observe a significant correlation of tumour FLT uptake and Ki67-assessed tumour proliferation rate in patients with breast cancer [46]. In summary, also in a clinical setting, most researchers have observed a correlation of tumour proliferation rate and tumour FLT uptake, measured with PET. These observations clearly support the concept of using FLT for the evaluation of cancer treatment.

A literature search has identified only one published study on the assessment of treatment response with FLT in breast cancer [47]. In a very small sample of 14 patients with newly diagnosed primary or metastatic breast cancer, patients had baseline FDG and FLT scans, scans with each tracer at 2 weeks after termination of the first treatment cycle (chemotherapy or antihormonal therapy), and final scans with each tracer after the end of treatment or after 1 year if treatment had not yet been terminated. Treatment-related changes in FLT and FDG uptake were generally concordant [47]. The authors found that alteration in the blood concentration of the tumour marker Ca 27.29 was more strongly associated with treatment-induced changes in tumour FLT uptake than with changes in FDG uptake. The authors felt that FLT PET performed 2 weeks after the end of the first treatment course was useful in predicting long-term efficacy of the applied treatment [47]. Although the results of this study are encouraging, it is clear that a much more comprehensive understanding of the major

Table 2. Summary of treatment-related changes in post-treatment tumour uptake of FLT

Tumour	Treatment	[¹⁸ F]FDG: difference in tumour uptake after therapy ^a	[¹⁸ F]FLT: difference in tumour uptake after therapy
Follicular lymphoma (Buck, submitted)	Chemotherapy (cyclophosphamide)	n.d. ^b	–76% (48 h)
Follicular lymphoma (Buck, submitted)	Immunotherapy (CD20 mAb, ibritumomab-tiuxetan)	n.d.	–28% (48 h)
Follicular lymphoma (Buck, submitted)	Radioimmunotherapy ([⁹⁰ Y]CD20 mAb, Zevalin)	n.d.	+7% (48 h)
Epidermoid carcinoma A431 [51]	PKI-166	–50% (1 wk)	–79% (1 wk)
SCCVII tumour [52]	Single-dose X-ray radiation, 20 Gy	–43% (3 days)	–34% (3 days)
Fibrosarcoma 1 (RIF-1) [53]	Cis-platin	–63% (48 h)	–50% (48 h)
Fibrosarcoma 1 (RIF-1) [54]	5-FU	–46% (48 h)	–74% (48 h)
Prostate tumour CWR22 [55]	Diethylstilboestrol	n.d.	–63% (1 wk)

5-FU 5-fluorouracil, n.d. not determined

^aDue to inconsistent designation of measurement units in cited references, the differences in tumour uptake before and after therapy are given in % of control

Fig. 2. Treatment response to 5-FU evaluated with FLT and a small animal PET scanner in a fibrosarcoma SCID mouse xenograft model [54]

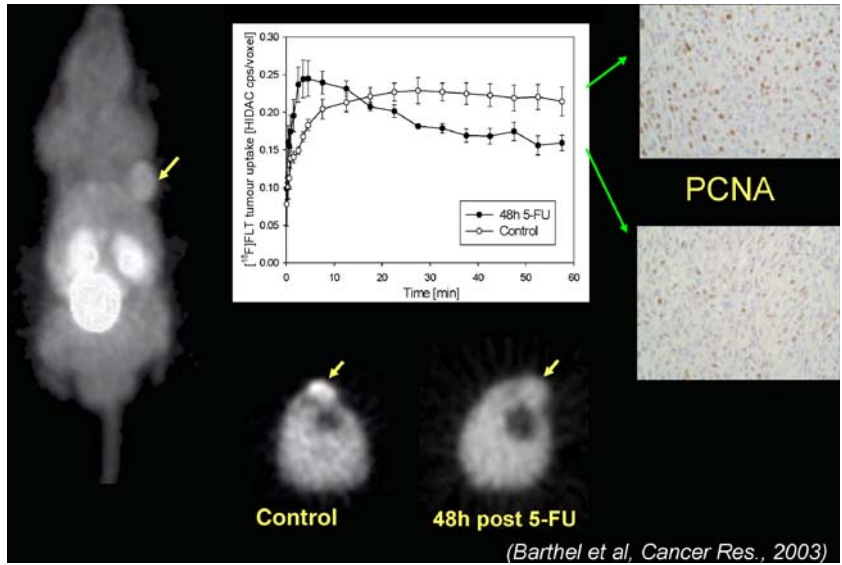
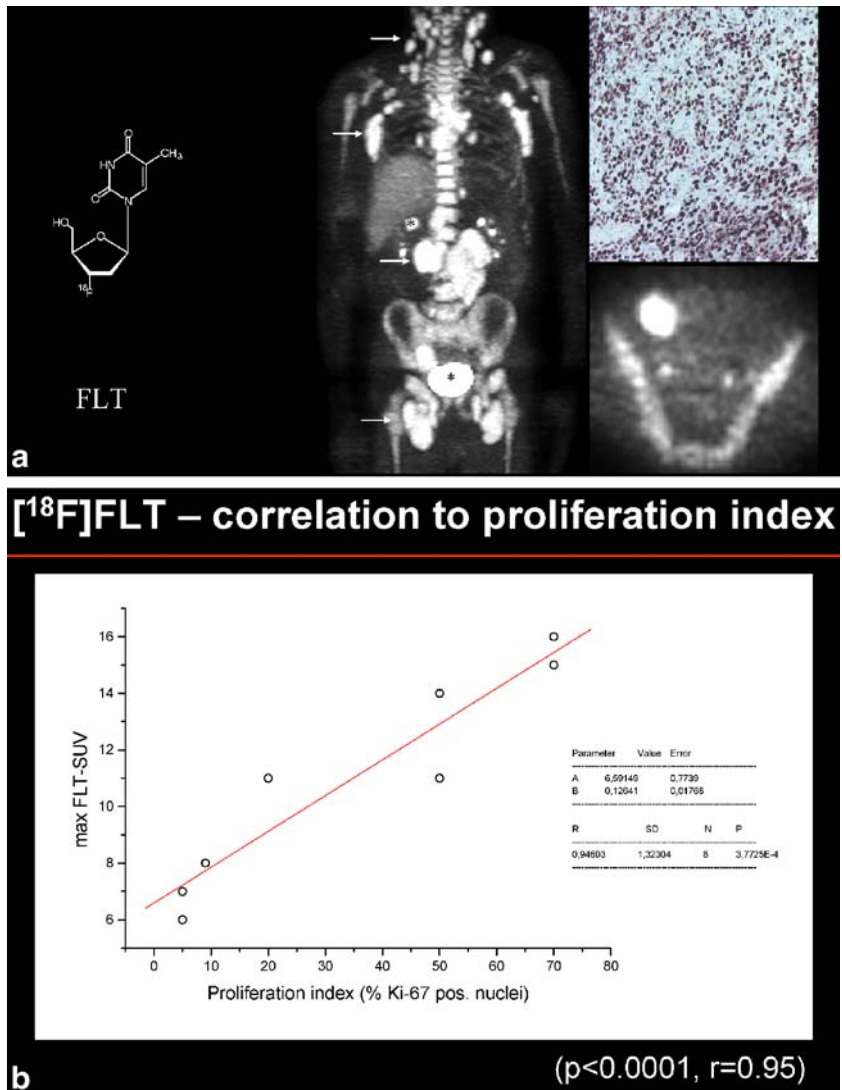


Fig. 3. a FLT PET whole-body scan of a patient with an aggressive NHL. Supra- and infradiaphragmatic nodal disease with intense FLT accumulation and a high proliferation rate shown by immunohistology (Ki67 staining) of an excised node. **b** Close correlation of FLT uptake with Ki67-positive cells in patients with NHL (Buck et al., submitted)



determinants of tumour FLT metabolism in a clinical setting is necessary before a solid judgement can be made on the ability of FLT to measure anticancer treatment response.

For example, key enzymes of thymidine synthesis such as thymidylate synthase can be upregulated severalfold during 5-FU or methotrexate treatment [48, 49]. Treatment-induced changes in tumour protein expression profiles are considered a major mechanism of resistance to radio-chemotherapy [50]. It is conceivable that nucleoside transporters and enzymes of thymidine metabolism display similar treatment-induced changes in their expression pattern. Therefore, anticancer treatment-related changes in the function and expression of key proteins and enzymes of thymidine metabolism need to be known before quantitative measures of the flux of FLT through the thymidine salvage pathway can be used as a reliable treatment response parameter.

In addition, to the best of our knowledge, the uptake properties of FLT in individual tumour cells *in vivo* have not been examined as yet. Therefore, the intrinsic assumption underlying the use of FLT as a treatment response probe, namely that all tumour cells are equally and homogeneously labelled by FLT *in vivo*, remains to be proven. Also, the minimum tumour cell mass that can be imaged with FLT *in vivo* in a clinical setting, which will define the sensitivity of this novel imaging test, needs to be determined.

In summary, it is clear that we are just beginning the process of defining the role of FLT as an anticancer treatment response probe. Nevertheless, its ability to specifically label an important pathway of tumour cell metabolism renders FLT a highly promising and potentially important novel radiopharmaceutical. Rapid progress in our knowledge of basic treatment-related changes in tumour FLT metabolism and in the definition of its clinical utility is foreseeable in the near future.

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