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[^{18}F]FLT: An imaging biomarker of tumour proliferation for assessment of tumour response to treatment

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Abstract The paradigm of drug development is shifting towards early use of imaging biomarkers as surrogate end-points in clinical trials. *Quantitative Imaging in Cancer: Connecting Cellular Processes* (*QuIC-ConCePT*) is an initiative to qualify complementary imaging biomarkers (IB) of proliferation, cell death and tumour heterogeneity as possible tools in early phase clinical trials to help pharmaceutical developers in 'go, no-go' decisions early in the process of drug development. One of the IBs is [^{18}F]3′-deoxy-3′-fluorothymidine with Positron Emission Tomography (FLT–PET). We review results of recent clinical trials using FLT–PET for monitoring tumour response to drug treatment and discuss the potential and the possible pitfalls of using this IB as a surrogate end-point in early phase clinical trials for assessing tumour response to drug treatment. From first human trial results it seems that the degree of FLT accumulation in tumours is governed not only by the tumour proliferation rate but also by other factors. Nevertheless FLT–PET could potentially be used as a negative predictor of tumour response to chemotherapy, and hence evaluation of this IB is granted in multi-centre clinical trials.

1. Introduction

Novel cancer therapy development is an endeavour that consumes large resources. To counterbalance this in recent years a shift in the drug development paradigm[1] was initiated towards use of biomarkers in early phase clinical trials[2] to provide pharmacodynamic end-points[3] for proof of concept or mechanism of action, as well as Pharmacokinetic-Pharmacodynamic (PK-PD) correlations. The use of Imaging Biomarkers (IB) at the initial stages of drug development can help to select promising drug candidates for further clinical development and thus eliminate ineffective drugs before expensive phase II–III clinical trials commencement.[4]

The QuIC-ConCePT project aims to evaluate a combination of IBs of tumour proliferation, cell death and

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apoptosis (assessed by FLT–PET, Diffusion-Weighted Magnetic Resonance Imaging and PET with [^{18}F] labelled caspase 3/7 inhibitor ICMT-11,[5] respectively) as markers of tumour response to therapeutics. The three tests will measure the effects of therapy by comparing quantitative imaging results in patients treated with various drug classes, as a function of time during neoadjuvant chemotherapy, and will use histopathology as the reference test. QuIC-ConCePT will focus on clinical settings typically encountered in the early phase clinical trials with novel drugs, i.e. primary and metastatic lesions in lung and liver. The primary goal of this project is to provide an imaging-based tool for 'go, no-go' decisions early in the drug development process.

This review will focus on the proliferation IB, [^{18}F]3′-deoxy-3′-fluorothymidine, used in conjunction with Positron Emission Tomography (FLT–PET). Several comprehensive reviews were published on the properties of FLT as a PET tracer of tumour proliferation and its possible use for evaluation of tumour response to therapy.[6–10] In this review we will summarise results of the recent human clinical trials assessing FLT for prediction of tumour response to treatment.

2. Radiotracer characteristics

FLT is a fluorine-modified thymidine analogue.[11] The accumulation of FLT in tissues has been shown to be linked to the cellular proliferation rate,[12] and more specifically to the expression and activity of cytosolic thymidine kinase-1 (TK-1) which is the first enzyme in the exogenous (salvage) pathway of DNA synthesis activated during the S-phase of the cell cycle. A comprehensive review by Buck et al.[8] closely examined the cellular mechanisms of FLT accumulation. It is widely accepted that FLT is a PET marker of cells in the S-phase of the cell cycle.[13] It is not incorporated into DNA to any appreciable extent, but its accumulation in cells shows the sum of the activities of its specific transport and TK-1 phosphorylation. Transport of FLT into the cell is affected by means of specific human nucleoside transporters.[14] Interestingly Buck et al. stress the notion that individual tumours differ considerably regarding the relative fractions of *de novo* synthesis of thymidine monophosphate (TMP) and the salvage pathway and therefore activity of TK1 does not necessarily reflect proliferative activity or S-phase fraction. Having said that significant correlations between tumour FLT uptake and Ki-67 labelling index have been demonstrated by Buck et al. in lung cancer[15] and by Kenny et al. in breast cancer.[16]

Other thymidine analogues have been labelled with radionuclides. [^{11}C] carbon labelled thymidine is the most advanced radiotracer which can provide a measure of DNA synthesis rate *in vivo*,[17] as it becomes incorporated into the DNA and follows all the metabolic routes of natural thymidine. The major drawback of [^{11}C]thymidine is that it is quickly metabolised and requires complicated kinetic image acquisition and analysis.[18] Also the short half-life of [^{11}C] and the need for an on-site cyclotron make it less suitable for multicentre clinical trials. Other radiolabelled tracers targeting DNA synthesis have included iodine-,[19] bromine- and fluorine-analogues of different nucleosides,[20] none of which have yet made their way into clinical practice. One of the most promising candidates, [^{18}F]FMAU,[21] is limited in that it is a better substrate for mitochondrial thymidine kinase-2,[22] and is therefore more relevant for measuring oxidative stress rather than proliferation.

The biochemical and imaging behaviour of FLT is well characterised, and a robust mechanism-based 4-compartment kinetic model has been developed and validated in human studies.[23] This model provides a metabolic flux parameter, K_{FLT}, which has been shown to correlate well with the cell proliferation marker Ki-67. FLT is stable in plasma.[24] The fate of FLT in tissues other than liver is very similar to that of [^{18}F]Fluorodesoxyglucose (FDG): its metabolites accumulate in the cell after phosphorylation to mono-, di-, and triphosphates, which are metabolically trapped in the cytosol. Metabolite correction is an important part of the model, as FLT is metabolised in liver to glucuronide,[25] and approximately 30% of blood circulating radioactivity at 1 h can be attributed to this metabolite. FLT dosimetry has been measured in human whole body studies and compares favourably to other common nuclear medicine and PET radiotracer techniques.[26]

2.1. Complications of measuring tumour response in liver tissue

FLT is actively taken up into the liver and metabolised to [^{18}F]FLT–glucuronide.[23] Images of tumour within the liver are confounded by the presence of activity from at least two distinct mechanisms: active uptake of FLT into tumour due to proliferative activity, and accumulation of [^{18}F] FLT–glucuronide within hepatocytes. The effect of this is that tumour deposits may not be identifiable against background.[27] When FLT–PET is used in liver to assess the tumour response, the existing compartmental models[23,25] cannot be applied directly.

These problems are not insurmountable: there is a significant difference in the pharmacokinetics of the uptake and excretion between tumour cell and normal hepatocyte. Recently novel approaches were proposed to overcome the difficulties of metabolite correction in the liver by applying so called kinetic filtering.[28]

3. Use of FLT–PET for tumour proliferation imaging and response to therapy

An excellent review on the initial validation of FLT–PET was provided by Bading and Shields.[9] FLT accu-

mulation in tumours has been shown to correlate well with cell growth in *in vitro* assays,[29,30] pre-clinical animal studies[31] and different clinical settings in humans including breast cancer,[16] lung cancers,[15] hepatocellular carcinoma,[27] colorectal cancer[32] and many other tumour types.[9] These first results indicated that FLT–PET could be useful for measuring the difference in tumour growth rates in the course of therapy.[33–35]

3.1. Preclinical data

The results of preclinical evaluation of FLT as a predictor of tumour response reviewed by Reske and Deisenhofer[36] indicate its potential but also lead to important questions on the relationship between drug-induced changes in tumour *de novo* and salvage DNA synthesis pathways as well as changes in nucleoside and nucleotide transport mechanisms.[37]

Although FLT–PET has been used extensively in xenograft models to assess response to therapy,[38–43] a number of scientific unknowns still exist. Generally FLT uptake *in vivo* is dependent on the expression and activity of TK1,[44,45] though in specific cases after treatment the role of transport predominates to increase rather than decrease FLT uptake. This temporary increase referred to as the 'flare effect,' is detectable in both xenografts and in clinical tumours.[46,47] Drugs inhibiting the *de novo* pathway of DNA are expected to induce the flare response.[9] Indeed increase of FLT accumulation in human breast cancer was reported as early as 1 h after administration of capecitabine.[46] Significant (7- to 10-fold) flare response of FLT uptake was observed in cultures of oesophageal squamous cell carcinoma 24 h after 5-FU and methotrexate treatment.[48] Redistribution of the human equilibrative nucleoside transporter type 1 (hENT1) to the outer cell membrane was proposed as a mechanism underpinning this flare effect.[37] The exact mechanism that triggers redistribution of the hENT1 after thymidylate synthase inhibition is largely unknown, moreover it is unclear if this phenomenon occurs in all human tumours. Although hENT1 appears to be the most abundant nucleoside transporter and is the most prominent transporter to influence FLT uptake in tumours, it has been shown that FLT is also transported by other types of nucleoside transporter, namely the concentrative nucleoside transporters 1 and 2 (hCNT),[14,37] and a passive diffusion mechanism was also postulated,[49,50] albeit to a lesser extent than hENT1.

Our knowledge is not mature on the use of FLT for monitoring drugs that inhibit cells in the G2/M phase of the cell cycle. Recent work by Contractor et al. suggests that at least in breast cancer patients, FLT might be a useful probe for monitoring the efficacy of docetaxel, an anticancer agent that induces G2/M block.[51] It is unclear how tightly TK1 activity and expression are linked to the cell cycle following changes induced by different chemotherapy regimens in human tumours, though preclinical studies have shed some light on this.[40,41,47] Of note, however, Schwartz et al. have suggested from studies in lung adenocarcinoma cells that functional p53 signalling is needed to maintain a normal relationship between TK1 activity and S-phase percentage following radiation treatment.[52] How the relationship is affected by cytostatic therapies remains to be fully elucidated in humans. Some insight is provided by preclinical studies with therapeutics targeting aurora kinase, histone deacetylase, epidermal growth factor receptor, fibroblast growth factor receptor, and mitogen activated protein kinase.[42,43,53–57] What is clear is that many cancers harbour p53 mutations, and it is not yet known whether they are functional or not.[58,59]

3.2. Clinical study results

The first publications on human trials have been comprehensively reviewed by Salskov et al.[10] Though most of initial reports are optimistic and confirm preclinical findings in noting that early changes in FLT uptake might be useful to monitor tumour response to treatment, there were signs that in some clinical situations, FLT uptake might not be reflecting proliferation alone. For example no correlation with Ki-67 in core biopsies was observed in a pilot breast cancer study,[60] but whether this was due just to the small size of the cohort and biopsy sampling errors remains unclear. A negative correlation found in an oesophageal cancer trial in 10 patients[61] can be attributed not only to the small sample size but also to the redistribution of the hENT1 to the outer membrane of oesophageal epithelial cells, as was observed in mouse intestine.[37,47]

Whilst the first attempts to use FLT–PET as an early marker of tumour response to treatment have shown promise,[10] it is clear that more robust clinical validation is needed.[9] In recent years publications have started to come out on limited (on average 20 patients) clinical trial results directed at assessing FLT–PET as predictor of response to treatment (Table 1). A rather cautious message seems to emerge: in spite of the fact that most studies in a wide range of cancers register significant early changes in FLT uptake between baseline and post-treatment data, these changes do not always translate into prediction of clinical response, and moreover poor correlation with proliferation index (Ki-67) is in some cases observed.[45,62–68] Among the possible reasons could be complex relation between Ki-67 index and pKi-67 mRNA expression levels, which was observed in colorectal cancer.[69]

This notion was spotted and summarised by Weber[70]: '…18F-FLT has so far been more successful in monitoring palliative therapy with rather limited efficacy than in monitoring highly effective, potentially curative treatments. These data may suggest that more effective

Table 1
Recent clinical trials with the use of FLT–PET as predictor of tumour response.

Citation	No. of patients	Cancer	Correlation Ki-67–FLT	Treatment	Response: timing/ criteria	Study results
Contractor[51]	20	Stage II–IV breast	NA	Docetaxel	2 weeks/mid-therapy lesion size	ΔSUV_{FLT} can predict lesion response midtherapy. Sensitive negative predictor of response
Herrmann[62]	66	Aggressive NHL	No statistical significance ($r = 0.21$)	R-CHOP	2 weeks/overall survival	High FLT uptake is a negative predictor of response
Ott[65]	45	Locally advanced gastric	No correlation prior to therapy, nor after treatment ($p = 0.29$)	Cisplatin-leucovorin-5FU	2 weeks/histopathology or clinical response	FLT uptake 2 weeks after initiation of therapy was the only imaging parameter with significant prognostic impact. ΔSUV_{mean} 42% did not correlate with survival
Brockenbrough[45]	25	Lung	$r = 0.57$ Ki-67 and $r = 0.65$ TK1	NA	NA	Absence of correlations with TK1 activity. SUV_{FLT}, K_{FLT} correlated only with overall TK1 expression
Contractor[82]	21	ER-positive breast cancer	Statistically significant correlation	NA	NA	Correlation between choline kinase-alpha and proliferation was observed
Pfannenberg[66]	11	Metastatic germ cell tumours	No correlation SUV_{max} $r = 0.12$ ΔSUV_{mean} $r = 0.13$	Cisplatin-based chemo-therapy	1st cycle, end of therapy/RECIST, EORTC, clinical response	18F-FLT did not correlate with Ki-67 or response
Kwak[83]	82 (few had FLT)	Advanced ALK-positive NSCLC	NA	ALK-inhibitor crisotinib	1 cycle/RECIST, clinico-pathological, CT tumour burden	Exploratory 18FLT–PET scans were performed after one cycle of treatment in several unselected patients, results supporting the clinical impression that responses to ALK inhibition can be rapid
Menda[78]	8	Head and neck	NA, excellent correlation between SUV and K_{FLT}, $r = 0.90$; and K_{Patlak}, $r = 0.99$	Chemo-radiation (RT + cisplatin-based therapy)	5 d (10 Gy RT, chemotherapy at day 3)/Kinetic analysis versus SUV	Relatively intense 18F-FLT uptake, significant ΔSUV_{FLT} after 10 Gy of radiotherapy. SUV is nearly equivalent to *K*-FLT and *K*-Patlak
Kenny[46]	6	Breast	NA	Capecitabine (TS inhibitor)	1 h/pharmaco-kinetic	Flare increased FLT uptake
Eckel[27]	18	HCC	Good correlation at diagnosis $r = 0.66$	Resection	NA/survival	Association between high initial FLT uptake and reduced overall survival needs to be confirmed in a larger prospective trial
Sohn[67]	31	Advanced adenocarcinoma of the lung	NA	Gefitinib	1 week/tumour size (CT 6 weeks) and clinical response WHO criteria[84]	ΔSUV_{FLT} more than -10% is a sensitive (93% sensitivity) and specific (93% specificity) predictor of response
Shields[77]	9	NSCLC	NA	NA	NA Test–retest study	FLT imaging of patients with NSCLC was quite reproducible, worst case SUV mean error 21%
Linecker[64]	20	Head and neck	No correlation found	NA	NA/survival	FLT uptake is inversely correlated with patient survival

(*continued on next page*)

Table 1 (*continued*)

Citation	No. of patients	Cancer	Correlation Ki-67–FLT	Treatment	Response: timing/ criteria	Study results
Herrmann[63]	22	High-grade NHL	NA	R-CHOP	2 d, 7 d, 40 d/survival	Administration of R-CHOP is associated with an early decrease in lymphoma FLT uptake. ΔSUV_{FLT} 70–80%. There was no statistically significant difference
Chen[85]	21	Glioma	NA	Bevacizumab, irinotecan	1–2 weeks, 6 weeks/survival	ΔSUV_{FLT} seems to be predictive of overall survival
Kenny[79]	13	Breast	NA	FEC	1 week/Clinical CT RECIST at 60 d	ΔSUV_{FLT} and K_{FLT} can detect changes prior to tumour size changes
Troost[86]	10	Head and neck	$r^2 = 0.47$	NA	NA	Specificity and positive predictive value were low due to tracer uptake in germinal centres of lymph nodes. Significant correlation between SUV and Ki-67
Wieder[68]	10	Rectal cancer	NA	Neoadjuvant chemoradiotherapy (5-FU)	2 weeks/histopathological tumour regression, clinical response (T-stage)	ΔSUV_{FLT} (−29% at 2 weeks, −55% at the end of therapy) did not correlate with histopathological tumour regression
Pio[87]	14	Breast cancer	NA	Aromasin, 4 cytotoxic, 5 hormonal	2 weeks after first cycle/CA27.29 tumour marker levels at the end of therapy	ΔSUV_{FLT} at 2 weeks is useful for predicting longer-term efficacy

therapeutic agents inhibit 18F-FLT uptake in most tumours but that a complete response is achieved in only a subset of patients.' This observation may reflect the dynamics of proliferation (early and late events).

3.3. Possible pitfalls

It has been argued that most of the studies did not use kinetic modelling, and that SUV analysis introduces a bias in analysis by not fully accounting for contribution of labelled metabolites and perfusion. However, static image acquisition and analysis at later time points is a standard, simple and widely used PET clinical protocol which, when expressed as a standardised uptake value (SUV), provides a sum total measure of tracer delivery to the tissues and its accumulation by trapping in metabolic compartments. Care must be taken when using SUV for evaluating the effects of novel drugs, especially those affected by blood flow, as SUV in this situation might underestimate FLT metabolic flux due to reduced tracer delivery to the tumour. If SUV is measured at later time points (beyond 1 h), the leak of radioactive metabolites from the tumour cells back into the blood will tend to underestimate the proliferation.[71] Other issues such as region of intest definition – manual or automatic, the location and number of core biopsies used for correlation can also influence the relationship between FLT parameters and proliferation.[72]

To overcome these pitfalls the dynamic image acquisition and analysis with validated compartmental kinetic models is recommended.[23,25] One of the best correlations of cell proliferation with FLT uptake has so far been reported for a 120 min 4-compartmental model analysis, where metabolic flux constant K_{FLT} evaluated at 0–120 min was found to have high correlation coefficient ($r = 0.92$) with Ki-67 staining.[23] To simplify the protocols, novel image acquisition and analysis approaches are proposed for the metabolically trapped tracers.[25,73]

Nonetheless, in several clinical studies FLT–PET SUV has been shown to have statistically significant correlation with cell proliferation (Ki-67).[27,74,75] Excellent correlation was shown between different forms of SUV and kinetic parameters, such as flux of the tracer into the tumour (K_{FLT}), derived both from Patlak graphical analysis and non-linear regression kinetic model fitting.[16,76–79]

In fact it seems that the poor correlation of FLT–PET with proliferation markers and survival in recent clinical tumour response studies is not due to technical issues. A surprising result was obtained in a lung cancer study in 25 patients with the analysis of both static and kinetic parameters.[45] Whilst SUV_{FLT} measured at 60–90 min had a good correlation with the maximal Ki-67 score ($\rho = 0.69$) and maximal overall TK1 expression ($\rho = 0.68$); there was no correlation between TK1 enzymatic activity and either SUV or dynamic flux constant

K_{FLT}. Also correlation of K_{FLT} with TK1 expression was poor ($\rho = 0.50$), and with Ki-67 was moderate ($\rho = 0.59$). It was concluded from this study that FLT uptake and retention within tumour cells is governed by a variety of still undetermined factors.

Some of these probable factors were discussed earlier in this review: metabolism of FLT,[25] the relative contributions of the *de novo* and salvage pathways to DNA synthesis,[8] p53 regulation of TK1 activity,[52] cell cycle dependent rearrangement of hENT1,[37] a multitude of transport mechanisms[14,37,49] and flare FLT uptake enhancement.[13,46] There may yet be more undiscovered factors, as in spite of its apparently simple metabolism, FLT is not incorporated into DNA,[11] thus its link with proliferation rate is not direct.

There is only limited knowledge on the interplay between the endogenous and exogenous DNA synthesis pathways and little is known of how activity of important enzymes and transporters will change in tumours after treatment. This notion implicates the importance of the time point when FLT–PET is used to assess tumour response after drug treatment. So far there has been little consistency in the clinical studies, where the FLT–PET was used from 1 d to 2 weeks from the initiation of treatment (Table 1). It will be essential to determine the appropriate time point for FLT–PET studies if the technique is to have any clinical utility in comparative drug development programmes, though this might differ for different drugs.

3.4. Implications for future clinical trials: definition of response criteria

From recent publications it is clear that even if a significant difference between FLT uptake before and after treatment is observed this does not necessarily translate into clinical response and improvement in survival rates. The cancers in which FLT–PET could be useful as a prognostic IB remain to be determined.

The portrait of a robust early negative predictor emerges, i.e. with a good degree of probability that no change in FLT uptake after the first cycle of treatment would mean that the drug either did not reach its target or was ineffective and the tumour would continue to grow.[51] Thus FLT–PET is very likely to become a 'drug terminator,' helping drug developers to eliminate non-efficient candidates in the early phase clinical trials. In this case, a distinction should be made between FLT as an early predictor of drug response and FLT as an objective measure of response.

Most of the studies reported used traditional response criteria – survival, clinical and histological responses. Lack of correlation of ΔSUV_{FLT} with common outcome measures noted in these trials (Table 1) point to the importance of choosing appropriate metrics of response.[3] For example in the breast cancer study with docetaxel therapy[51] FLT–PET was a sensitive negative predictor of lesion response at the middle of therapy (after 3 cycles).

Recently a novel approach was elaborated by the Society of Nuclear Medicine for the use of FDG-PET as a metabolic response assessment – PET Response Criteria in Solid Tumours (PERCIST).[80] The same approach with some modifications for reference tissue and response threshold could be used to assess FLT–PET as a proliferation IB. The main feature of PERCIST is that as much imaging information as possible is recorded along with histological and clinical information in order to provide a database for future choice of the most appropriate matrix of response.

Important components of the proposed PERCIST[80] criteria include assessing normal reference tissue values in a 3-cm-diameter region of interest (liver for FDG), using a consistent PET protocol, using a fixed small region of interest about 1 cm^3 in volume in the most active region of metabolically active tumours to minimise statistical variability, assessing tumour size, treating SUV-lean measurements in the 1 (up to 5 optional) most metabolically active tumour focus as a continuous variable, requiring a 30% decline in SUV for 'response,' and deferring to RECIST 1.1[81] in cases that do not have [^{18}F]tracer avidity or are technically unsuitable.

4. Conclusion

The range of degrees of correlation documented between FLT–PET and not only proliferation measurements but also a variety of end-points for clinical response illustrates the need for a focused programme to determine the most reliable imaging parameters and timepoints in specific tumour types. Such further information will provide evidence to support or otherwise the utility of FLT–PET as an effective IB in future drug development programmes.

Conflict of interest statement

None declared.

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Appendix A

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