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Review Article

Potential of 3'-Fluoro-3' Deoxythymidine as a Cellular Proliferation Marker in PET Oncology Examination

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ABSTRACT

Development of the positron emission tomography (PET) diagnostic radiopharmaceutical (¹⁸F) fluoro-2-deoxy-D-glucose (¹⁸F-FDG) subsequently facilitated the discovery and clinical evaluation of several new tracers as imaging markers for cancer. While ¹⁸F-FDG is a widely employed marker for enhanced intracellular glycolysis and metabolic function, one of the newer tracers, (¹⁸F)-3'-fluoro-3' deoxythymidine (¹⁸F-FLT), has been developed as a biomarker for cell proliferation. In this review, the potential of ¹⁸F-FLT as a biomarker for cancer imaging is discussed.

Keywords: 18F-FLT, 18F-FDG, Positron Emission Tomography (PET), cellular proliferation

INTRODUCTION

Positron emission tomography (PET) is a rapidly developing imaging tool, with a clinical role that exceeds 15 years (Fathinul *et al.*, 2013). It is a quantitative imaging technique that

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produces cross-sectional images that are composites of volume elements (NIH, 2006). The signal intensity for images especially in PET, corresponds to the concentration of radionuclide within the target tissue volume. PET is applied mainly in the clinical areas of cardiology, neurology and oncology, with the latter accounting for about 90% of all PET.

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The glucose derivative, (¹⁸F) fluorodeoxy glucose (¹⁸F-FDG), is the ubiquitous PET marker. However, there are numerous other tracers under development and with proven capability in highlighting a broad range of tissue metabolic functions. In a large meta-analysis, PET technique was found to change patients' management to almost 30% (Gambhir *et al.*, 2001). Though ¹⁸F-FDG is now widely used as a frontier in management of cancer patients, numerous studies have suggested that this marker is not universally selective for tumour imaging. This is because ¹⁸F-FDG is a glucose analogue and it is utilised by many cell types, which limits its specificity (Shields *et al.*, 1998; Yun *et al.*, 2003).

To overcome this limitation, radioisotope-labelled thymidine derivatives have been developed to image cellular proliferation by PET. Radioisotope-labelled thymidine has a long history. Pyrimidine nucleoside was first labelled with radioisotope in 1969 by Langen and his collaborators. In their study, they described the radio-labelled form of pyrimidine nucleoside as a selective inhibitor of DNA synthesis. However, only in 1991, was fluorothymidine (FLT) labelled with ¹⁸F successfully introduced as carrier-added ¹⁸F-FLT (Wilson *et al.*, 1991). Wilson and his collaborators monitored the efficacy of 3'-fluoro-3'-deoxy-thymidine (FDT) in HIV treatment. FDT is a fluorinated analogue of 3'-azido-thymidine (AZT), which was also found to be active against the HIV. However, FDT is more toxic than AZT (Wilson *et al.*, 1991). Wilson and his collaborators successfully labelled the 3'-fluoro-3'-deoxy-thymidine (FDT) with the ¹⁸F to monitor the drug's distribution and targeting in the body (Grierson *et al.*, 1997).

Development of the FLT marker was subsequently continued by Grierson *et al.* in 1997 and it successfully introduced no-carrier-added ¹⁸F-FLT. The next year, ¹⁸F-FLT was first applied in imaging. The study was carried out to investigate animals and non-small cell lung cancer (NSCLC) patients (Shields *et al.*, 1998). From that study, it was found that ¹⁸F-FLT was specifically taken up by tissues that actively proliferate, including bone marrow (Barthel *et al.*, 2003). Although ¹⁸F-FLT appears to be a most promising marker, the major hurdle for its routine use is its low radiochemical yield during production. Nevertheless, it provides greater advantages to the clinicians in management of cancer patients.

THE BASIS OF ¹⁸F-FLT AS A PROLIFERATION MARKER

The ¹⁸F-FLT marker is administered to the patient by intravenous injection. It is taken up in the cell via both passive diffusion and also by Na+-dependent carriers. The ¹⁸F-FLT marker, which is trapped in the cell, will undergo the phosphorylation process by thymidine kinase (TK1) and be converted into ¹⁸F-FLT-monophosphate (Been *et al.*, 2004). Intracellular trapping and accumulation of ¹⁸F makes it possible to be detected by PET camera, which in turn gives a measure of the TK1 activity.

In the physiological pathway, both thymidine and ¹⁸F-FLT encounter the same initial fate (Fig.1). Both of them will be phosphorylated by TK1 for DNA synthesis. However, for the ¹⁸F-FLT marker, the DNA replication is inhibited due to the lack of the hydroxyl (-OH) group attached at the carbon number 3'-position on the sugar ring. Hence, the ¹⁸F-FLT marker will be trapped inside the proliferating cells, and its radioactive signature will continue to accumulate there.

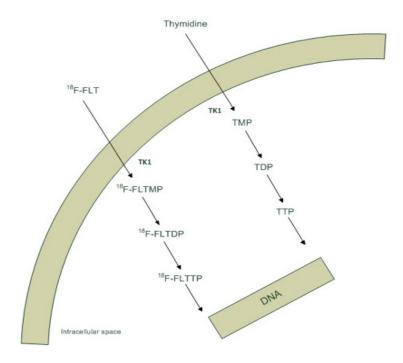


Fig.1: Uptake mechanism of thymidine and ¹⁸F-FLT.

The uptake of ¹⁸F-FLT by cells is correlated with TK1 activity (Barthel *et al.*, 2003; Chen *et al.*, 2005). TK1 activity in proliferating cells is noted to be 3 to 4 times higher in malignant cells compared to benign cells (Been *et al.*, 2004). The enzymatic activity of TK1 reaches maximum level in the late G1 phase and S phase of the cell proliferative cycle. Therefore, monitoring of TK1 activity should give an early indication as to whether a cell population is in proliferative malignant state, or in benign state.

POTENTIALS OF 18F-FLT IN ONCOLOGY

¹⁸F-FDG marker is known for its relative non-specificity. Hence, there are many active inflammatory diseases and some aggressive benign tumours that inevitably give high ¹⁸F-FDG uptake in cells. Furthermore, some disease processes healed by fibrosis leave a significant residual mass, thereby limiting categorisation of a complete response to ¹⁸F-FDG.

In comparison, the FLT marker has the ability to demonstrate an increased rate of cellular proliferation and is potentially helpful in the setting of therapeutic monitoring as it has less affinity to inflammatory conditions. ¹⁸F-FLT is potentially a more specific marker than ¹⁸F-FDG with a high positive predictive value for malignancy. ¹⁸F-FLT marker is also potentially useful in the evaluation of cerebral malignancy due to the lack of background cerebral uptake, unlike the high cerebral activity normally seen in ¹⁸F-FDG.

In addition, there is good evidence that ¹⁸F-FLT uptake is closely correlated with cellular proliferation with correlation between the intensity of uptake in lung cancer as measured by SUV with proliferation indices such as Ki-67 staining in a resected specimen (Hofman *et al.*,

2012). The ability of ¹⁸F-FLT to identify tissue with a high proliferative rate has potential applications in the assessment of haemopoietic tissue and high grade disease transformation in haematological malignancy. The assessment of bone marrow reserve is important in considering patients for chemotherapy or radionuclide therapy, which is potentially myelotoxic. ¹⁸F-FLT has the ability to document the extent and distribution of haemopoietic tissue, including the presence of extramedullary haemopoiesis, which can guide subsequent treatment choice. There are occasions when bone marrow sampling does not provide a representative picture of the true haemopoietic status due to sampling error and heterogeneous distribution of haemopoietic tissue (Fig.2).

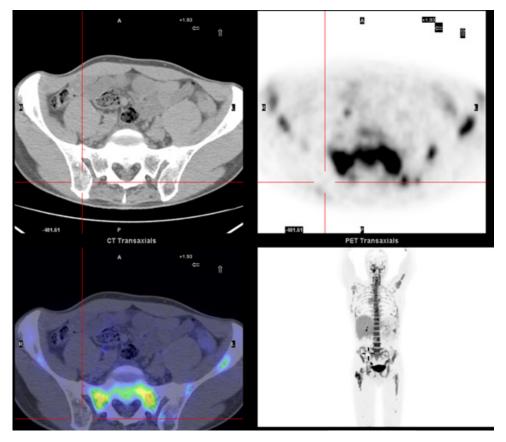


Fig.2: A 55-year-old man with stage IV diffuse large B cell lymphoma with nodal and multifocal bony disease and the pre-treatment bone marrow biopsy from the right posterior ilium showing hypocellular marrow and aplasia, in the presence of normal peripheral blood counts. 18F-FLT PET/CT was performed, which demonstrated absence of proliferative tissue in the right posterior ilium but fairly normal distribution of hyperproliferative bone marrow elsewhere with no evidence of extramedullary haemopoiesis in the spleen or elsewhere. The 18F-FLT PET/CT findings suggested that the initial bone marrow biopsy result was non-representative of his true bone marrow status and the patient went on to undergo systemic chemotherapy treatment without any myelotoxicity problem (Image courtesy of The Peter Mac Callum Centre).

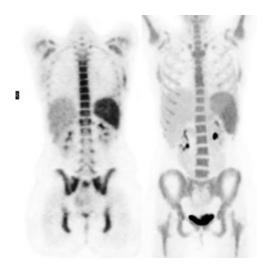


Fig. 3: Coronal MIP-PET images using ¹⁸F-FLT in the assessment of skeletal sarcoma before (left) and after treatment (right). The images show an increased ¹⁸F-FLT intensity in the left lateral chest wall (marked), which appears less proliferative after treatment (right). (Image courtesy of The Peter Mac Callum Centre).

DIAGNOSIS EVALUATION: 18F-FLT VERSUS 18F-FDG

Pancreatic Cancer

¹⁸F-FLT has been used in imaging pancreatic-cancer-specific cell lines, SW-979 and BxPc-3. The study was performed by Seitz *et al.* (2002) to prove that ¹⁸F-FLT has greater specificity than ¹⁸F-FDG. In that study, the ¹⁸F-FLT uptake was 18.4% and 5.2%, respectively. In comparison, ¹⁸F-FDG was also administered to the same cell lines. It was observed that the ¹⁸F-FDG uptake was only 0.6% and 0.3% for the corresponding cells. Evidence such as no increased ¹⁸F-FLT uptake was observed in normal pancreatic lobules in comparison with large ¹⁸F-FDG uptake detected in normal pancreatic lobules confirmed that ¹⁸F-FLT has higher specificity for pancreatic cancer.

The previous work by Seitz was supported by the Herrmann group's findings in 2008. In the study consisting of 21 patients diagnosed with malignant pancreatic tumours, 15 patients had an increased ¹⁸F-FLT uptake. Herrmann and his colleagues were able to demonstrate that ¹⁸F-FLT was a specific marker for pancreatic cancer (Herrmann *et al.*, 2008). They also suggested that ¹⁸F-FLT may be used to differentiate pancreatic cancer from pancreatic pseudotumors that were subjected to arise from chronic pancreatitis (Herrmann *et al.*, 2008). As any other studies, Herrmann acknowledged that although the ¹⁸F-FLT showed high specificity for pancreatic cancer, it turned out that the sensitivity was reduced for malignant lesions (Herrmann *et al.*, 2008).

Contrary to the Seitz *et al.* (2002) and Herrmann *et al.* (2008) studies, initial evaluation of ¹⁸F-FLT for primary pancreatic study led by Quon *et al.* (2007) demonstrated the opposite. In a pilot study consisting of five patients who were newly diagnosed with unresectable pancreatic

cancer, the visual interpretation of the primary site was assessed using ¹⁸F-FLT PET/CT and ¹⁸F-FDG PET/CT. In ¹⁸F-FLT PET/CT, the primary lesion was detectable in only two of the five patients, while all five showed lesions in the ¹⁸F-FDG PET/CT imaging (Quon *et al.*, 2007). Throughout the study, ¹⁸F-FLT showed poor lesion detectability and low levels of uptake in the primary tumour compared to ¹⁸F-FDG. Hence, it was suggested that the use of ¹⁸F-FLT was not promising for characterisation of pancreatic cancer and it offerred no benefit in monitoring therapy due to poor baseline scan (Quon *et al.*, 2007).

Pulmonary Nodes and Lung Cancer

In pulmonary nodes and lung cancer, one expected difficulty in differentiating malignant from benign solitary pulmonary nodes (Been *et al.*, 2004). Although ¹⁸F-FDG has proved to be a helpful and accurate diagnostic tool, with excellent sensitivity of 96.8% and good specificity of 77.8%, reports of false-positives still originate, mainly from granulomatous and inflammatory disease. Thus, a more specific tracer that does not show uptake in inflammatory tissues would be useful. In the non-small cell lung cancer (NSCLC) patient, image with low background activity was acquired with the administration of ¹⁸F-FLT on patients (Been *et al.*, 2004). Buck *et al.* (2003) further investigated 30 patients diagnosed with solitary pulmonary nodes (SPN) and deployed ¹⁸F-FLT as a marker. They reported 86% ¹⁸F-FLT uptake by the malignant lesions, whereas no ¹⁸F-FLT uptake was observed in benign lesions. This demonstrated that in distinguishing the malignant SPN, ¹⁸F-FLT had higher specificity than ¹⁸F-FDG.

Breast Cancer

On the contrary, in breast cancer cases, although multiple studies have been carried out by many groups using ¹⁸F-FLT, the results have not been consistent. In an early investigation in patients diagnosed with breast cancer, it was observed that ¹⁸F-FLT was taken up in breast cancer cells (Been et al., 2004). In another study by Silverman and his colleagues in 2002, there was a 1.3 to 2.3 times higher ¹⁸F-FLT uptake reported in primary breast cancers as compared to ¹⁸F-FDG uptake. However, in 2004, Smyczek-Gargya and his colleagues, investigated 12 patients with breast cancer with ¹⁸F-FLT and compared it to ¹⁸F-FDG, concluding that ¹⁸F-FDG uptake was higher than ¹⁸F-FLT. Scientists agree that it is still unclear what will be the role for ¹⁸F-FLT in patients with breast cancer as inconsistent findings from multiple studies have led to uncertainty in the role of ¹⁸F-FLT in breast cancer management. Interestingly, the study of Been and his colleagues showed that ¹⁸F-FLT uptake predicted tumour marker response to chemotherapy better than ¹⁸F-FDG (Been et al., 2004). The latter work was supported by Pio and his colleagues in 2006 and also Kenny et al. (2007). Pio and his colleagues had evaluated the treatment response with ¹⁸F-FLT PET in patients diagnosed with breast cancer over ¹⁸F-FDG PET. Scans were done prior to chemotherapy treatment or anti-hormonal therapy two weeks after completion of the first treatment cycle and after the end of treatment or over a year if the treatment had not yet been completed. In the study, they found that changes in levels of the serum marker, CA27.29, were more strongly correlated with tumour ¹⁸F-FLT uptake than with ¹⁸F-FDG (Pio et al., 2006). Meanwhile, Kenny and his colleagues reported a significant association between tumour ¹⁸F-FLT uptakes with the Ki-67 labelling index (Kenny et al.,

2007). Hence, it was proposed that measuring the early response to chemotherapy for locally advanced breast cancer is probably the most interesting research question for ¹⁸F-FLT studies in patients with breast cancer (Been *et al.*, 2004; Pio *et al.*, 2006; Kumar, 2007; Kenny *et al.*, 2007).

Brain Cancer

In the management of brain tumours, PET provides information on the tumour grade and also assists in assessing the optimal site for biopsy. Several PET radiopharmaceutical markers have been used for brain cancer imaging. These include ¹⁸F-FDG, ¹⁸F-FLT, and 11C-Met (L-methyl-[11C] methionine). Chen and his colleagues systematically compared ¹⁸F-FLT with ¹⁸F-FDG in human gliomas, in relation to sensitivity, in the evaluation of recurrent high-grade glioma (Chen *et al.*, 2005). They discovered that uptake of ¹⁸F-FLT in glioma was relatively rapid. ¹⁸F-FLT typically showed a similar uptake as for ¹⁸F-FDG. However, an interesting finding was that the ¹⁸F-FLT background uptake in normal brain tissue was low, and this could be due to a slow proliferation rate. This feature significantly showed that ¹⁸F-FLT has potential to derive a better mean standardised uptake value (SUV) in PET imaging of a tumour, as compared with ¹⁸F-FDG.

Although ¹⁸F-FDG has been used extensively in brain tumour imaging, one of the several major drawbacks of ¹⁸F-FDG in this context is its difficulty in characterising tumours in the brain. This is due to the high basal glucose metabolic rate of normal brain tissue. ¹⁸F-FDG uptake of low-grade tumours is generally similar to that of normal white matter, whereas high-grade tumour uptake can be similar to that of normal grey matter, resulting in limited sensitivity of lesion detection. In addition, in recurrent tumours the ¹⁸F-FDG uptake could be lower than the normal white matter, whereas in necrotic cells the ¹⁸F-FDG uptake could be higher than the normal white matter. It can be assumed that ¹⁸F-FLT has a theoretical advantage in detecting tumour recurrence as there is little uptake in normal brain. It has been agreed that ¹⁸F-FLT may help to define tumour activity by imaging tumours with greater sensitivity than ¹⁸F-FDG (Nitzsche *et al.*, 2003). Another significant finding arose from a study by Nitzsche and his colleagues in 2003, who determined that ¹⁸F-FLT was greater to ¹⁸F-FDG for the detection of recurrent brain tumours after brachytherapy.

A study by Dohmen and colleagues in 2000 compared the use of ¹⁸F-FLT with L-methyl-11C-methionine (11C-MET) for the detection of brain tumours. They discovered that ¹⁸F-FLT showed higher tumour contrast compared to 11C-MET. However, low-grade brain tumours limit the application of ¹⁸F-FLT in brain tumour imaging as it showed poor visual distinction in that case (Been *et al.*, 2004). Garlip (2003) discovered that the ¹⁸F-FLT standardised uptake value was higher than that of 11C-MET. Even though ¹⁸F-FLT has shown some advantages compared with ¹⁸F-FDG and 11C-MET, relatively small and therefore inconclusive studies have been published. There is a need to provide anatomical information and to further determine whether ¹⁸F-FLT is able to differentiate between benign and malignant tissues and between residual tumour and radionecrosis. If ¹⁸F-FLT proves to be a sensitive and specific marker for the brain, it will be very useful for the next stages in management; namely establishing the best site for tumour biopsy and for planning of radiotherapy in heterogeneous tumour (Been *et al.*, 2004).

Colorectal Cancer (CRC)

Francis and his colleagues in 2003 successfully imaged colorectal cancer using both ¹⁸F-FDG and ¹⁸F-FLT. Both markers displayed 100% sensitivity when imaging primary colorectal cancer. For visualisation of extra hepatic lesions, ¹⁸F-FDG and ¹⁸F-FLT demonstrated sensitivities of 100% and 92% respectively (Francis *et al.*, 2003).

However, his study also demonstrated an increased uptake of ¹⁸F-FDG from non-malignant inflammatory peritoneal lesions, which were thus presumed to be malignant. This would lead to false-positive scans when using ¹⁸F-FDG. In contrast, such lesions showed no avidity for ¹⁸F-FLT, demonstrating a specificity that may be useful for further characterisation of equivocal lesions (Francis *et al.*, 2003). His study concluded that in colorectal cancer, ¹⁸F-FLT demonstrated lower cellular trapping compared to ¹⁸F-FDG. The poor sensitivity displayed by ¹⁸F-FLT makes it a poor candidate as a diagnostic tool for colorectal cancer. Although it lacks in sensitivity (inclusion of free positives), ¹⁸F-FLT has the potential to improve the specificity (rejection of false positives) for the detection of colorectal cancer.

Another study led by Wang *et al.* (2009) investigated whether the use of dual-tracers, ¹⁸F-FDG and ¹⁸F-FLT, could predict the biologic character of metastases in colorectal cancer. Wang *et al.* (2009) used animal modelling to prove that higher uptake of ¹⁸F-FLT could be correlated to a higher incidence of metastasis. The human colorectal cancer cell lines SW480 and SW620 were generated in 20 mice, whereby the former was generated in the left front leg and latter was generated in the right front leg. Wang observed high uptake of ¹⁸F-FLT in mice from small animal PET/CT which correlated well with the overexpression of HSP27 and integrin β3 in the left front leg of the mice (SW480) (Wang *et al.*, 2009). On the other hand, high uptake of ¹⁸F-FDG was observed in the right front leg, which had been generated with SW620 cell lines but not by ¹⁸F-FLT. The overexpression of HSP27 and integrin β3 in SW480, which observed higher uptake of ¹⁸F-FLT, was believed to reflect a higher rate of metastasis to lung and liver (Wang *et al.*, 2009). Meanwhile, high uptake of ¹⁸F-FDG in SW620 cell lines can possibly correlate with lymphatic metastases (Wang *et al.*, 2009). From the study, Wang and his colleagues (2009) suggested that a combination of the dual-tracers ¹⁸F-FLT and ¹⁸F-FDG could be used to predict the biologic behaviour of colorectal cancer.

Lymphoma

Lymphoma is a type of malignancy that originates in lymphocytes of the immune system; particularly in lymph nodes and presenting as an enlargement of these nodes. For high-grade lymphoma visualisation, there is no dispute that ¹⁸F-FDG has been proven to be a sensitive method. However, for the low-grade (indolent) lymphoma, the value of ¹⁸F-FDG is still unclear (Been *et al.*, 2004). Hence, ¹⁸F-FLT could in theory have an additional value as a tracer of proliferative tissues. Been (2004) also compared ¹⁸F-FLT and ¹⁸F-FDG in lymphoma patients. It was found that the mean standardised uptake value (SUV) for ¹⁸F-FLT was 4.5 whereas the mean SUV for ¹⁸F-FDG was 5. This showed that ¹⁸F-FDG had higher uptake in lymphoma. In terms of sensitivity, both markers were found to be comparable. As ¹⁸F-FLT uptake in lymphoma is closely correlated with the rate of proliferation, problems may arise during the prognosis in lymphoma. In the case of prognosis in lymphoma, the rate of proliferation is not always

correlated with lymphoma's prediction (Been *et al.*, 2004). In haematopoietic dysfunction cases, the ¹⁸F-FLT marker is able to determine the activity, extent and distribution of bone marrow reserve and hence, assist in decision making for a variety of clinical indications. ¹⁸F-FLT findings complement results of bone marrow aspiration and trephine biopsy (BMAT) and could be a useful tool for assessing response to novel treatments in patients with myeloproliferative diseases (Hofman *et al.*, 2012).

Melanoma

Melanoma is a malignant tumour of pigment cells (melanocytes), which are found predominantly in skin but also in the bowel and eye. Cobben and colleagues in 2003 used ¹⁸F-FLT in imaging of melanoma to compare with ¹⁸F-FDG. They discovered that the specificity and sensitivity of ¹⁸F-FLT in imaging of melanoma was 60% and 88%, respectively. In contrast, the specificity and sensitivity using ¹⁸F-FDG was 83% and 92%, respectively. This indicates that the specificity and sensitivity of ¹⁸F-FLT for melanoma are lower than those of ¹⁸F-FDG. It appears that ¹⁸F-FLT is not a preferential marker when it comes to detection of melanoma.

LIMIT OF 18F-FLT AS A CELLULAR PROLIFERATION MARKER

The extent of the agreement on whether ¹⁸F-FLT shows a net benefit in cellular proliferation has been continuously debated. The dispute arises due to the nature of DNA synthesis mechanisms: the thymidine salvage pathway and de novo synthesis pathway. In thymidine salvage pathway, thymidine is transported across the cell membrane and phosphorylated by TK1 into thymidine monosphosphate (TMP) before it is further phosphorylated into thymidine diphosphate (TDP) and thymidine triphosphate (TTP) (McKinley *et al.*, 2013). TTP then is incorporated into the DNA.

In contrast to thymidine salvage, the de novo synthesis pathway uses deoxyuridine monophosphate as an alternative for conversion into TMP through the action of the thymidylate synthase (TS) enzyme. TPM is then further phosphorylated and incorporated into the DNA. Due to this nature of the DNA synthesis mechanism, it is assumed that previous studies using ¹⁸F-FLT may underestimate cell proliferation in de novo pathway-dependent tumours. In 2013, McKinley and his colleagues conducted a study to demonstrate that ¹⁸F-FLT is poorly reflected as a proliferative index in some tumours that utilise the de novo pathway. They generated the human colorectal cancer cell lines, HCT-116 (parental line) and HCT-116p21 in the cell lines and also in the xenografts to explore the effect of p21 deletion on ¹⁸F-FLT. Interestingly, in HCT-116p21 cells, elevated levels of the TS enzyme was observed. Meanwhile, the level of TK1 diminished. When ¹⁸F-FLT PET was performed on the xenografts to demonstrate the sensitivity of ¹⁸F-FLT to de novo pathway utilisation, the HCT-116 xenografts manifested greater uptake than the analogous HCT-116p21 xenografts (McKinley et al., 2013). The finding were supported by the findings of a previous study led by Moroz et al. (2011) who suggested that ¹⁸F-FLT uptake was unrepresentative of xenografts growth in tumours utilising the de novo pathway. From the findings, McKinley et al. (2013) concluded that ¹⁸F-FLT PET cannot discriminate moderately proliferative, thymidine salvage-driven tumours from highproliferative index tumours that rely primarily upon the de novo pathway.

CONCLUSION

It is well accepted that ¹⁸F-FDG is the ubiquitous marker in PET oncological practice. Nevertheless, ¹⁸F-FLT is an exciting marker with improved specificity that could be the number one candidate for therapeutic monitoring. Thereby, future research should continue to probe the potential of ¹⁸F-FLT as a powerful marker for cellular proliferation.

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