PET a tool for assessing the *in vivo* tumour cell and its microenvironment?

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Introduction and background: Positron emission tomography (PET) has started to develop beyond its roots in glucose imaging, expanding to study other parameters of the tumour and its microenvironment.

Sources of data: A review of imaging literature over the past 5 years has shown that functional imaging with PET is starting to exploit our increasing knowledge of genomics and the phenotypic expression of cells and how they interact with their microenvironment.

Areas of agreement: For most of those working in this field, there is agreement that therapeutic outcomes for patients can only be obtained by the assessment and continued reassessment not only of the tumour microenvironment, but also how it is changed by treatment.

Areas of controversy: Although PET offers a tool by which the tumour and its microenvironment can be assessed *in vivo* without the need for multiple interventions, the cost of PET is high and there is a cumulative radiation burden with repeated studies. As the quantity and quality of PET scans increase, we are able to assess tumour cell turn over, metabolism, hypoxia, angiogenesis and a variety of other factors that might affect tumour survival and response to treatment.

Areas timely for developing research: As it is impossible to do everything for every patient, we need to know what are the critical factors in the tumour cell microenvironment in each patient and need to have the tools to assess that factor.

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Introduction

The public perception is that cancer is now the most feared disease as it is often seen as the unbeaten disease in the developed world. The reality is that cancer represents a whole host of different diseases with varying aetiology, growth patterns and outcomes. Even something as simple as a breast cancer represents a range of diseases that need different approaches. As we enter the age of the genome, we will need new tools to assess and reassess the progress of tumours and how they respond to treatment. These treatments will themselves become targeted to the genomic and phenotypic variation between the tumour cell and the patient’s normal cell. It was reported that Steve Jobs, the co-founder of Apple, spent $100 000 having his tumour cells’ genome sequenced even though there is no evidence that this changed the course of his disease.

However, we do not have the tools needed to translate this information into successful therapy. One approach is to use molecular imaging and this primarily means positron emission tomography (PET).

PET techniques are being applied to many facets of the tumour and its microenvironment, including its vasculature. It is unclear whether PET can answer relevant questions such as: Why do tumours grow? How can we treat tumours optimally? Is PET the correct tool to assess the tumour and its microenvironment before, during and after treatment?

Why PET?

Although there is much discussion about how the human genome might change clinical practice, the tools we use to assess new medication are embedded in the past. This is reflected in many chemotherapy regimes, where evidence is collected in randomized multi-centre trials, and the answers tell the oncologist what on average gives the best result. Although this may be useful to the majority, the oncologist ends up treating populations instead of individuals. For example, all patients with a stage III invasive ductal breast cancer might undergo similar treatment. The data to justify such treatment are derived from a series of randomized clinical trials, often multi-centre. However, individuals may respond differently to treatment because of their expressed genome and genetic variation in their tumour. This has already been recognized, and the targeted approach is used in some patients with breast cancer, assessing oestrogen receptors and HER-2 status.

Another common assumption when biopsy material may only be
available from one site is that this is representative of all sites. This may not be true, and in some patients, the primary breast tumour can be oestrogen receptor positive, but metastases can be receptor negative. If we were able to image the patient at the molecular level, we could understand better what is happening and also if there is any heterogeneity of the tumour that would have an effect on treatment.

Neuroendocrine tumours (NET) as a model for phenotypic diversity

Although using SPECT and not PET, molecular imaging has been able to demonstrate variation in tumour phenotype in neuroendocrine tumours (NET). These tumours tend to be slow growing and may take many years to be fatal. During this time, the tumour may change its nature, and this may be reflected in its imaging characteristics. There are two main methods of imaging NET *in vivo*. The first uses the fact that many of these tumours retain an embryological amine uptake mechanism that can be seen with iodine-123 metaiodobenzyl guanidine (mIBG) in 60–70% of patients.\(^7\)–\(^9\) The second method exploits the fact that most of these tumours also have over-expression of somatostatin receptors, which can be imaged by a radiolabelled octapeptide based on human somatostatin (indium-111 pentetreotide) in 85–90% of NET.\(^8\)–\(^12\)

In a review of 149 NET patients imaged with both iodine-123 mIBG and indium-111 pentreotide, iodine-123 mIBG was positive in 63% of patients and indium-111 pentreotide was positive in 79% of patients.\(^13\) However, in 12% of patients, both radiopharmaceuticals were positive, but they identified different tumour sites. This may be important in deciding optimal therapy as a variety of treatments may be needed.

Recently, PET techniques have been developed using gallium-68-labelled peptides. This enables more lesions to be seen, but is much more dependent on the expression of sub-type 2 somatostatin receptors.\(^13\),\(^14\)

The resolution and sensitivity of these PET techniques allow any variation in somatostatin receptor expression to be visualized (Fig. 1)

As patients live longer and as our treatments become more successful, tumour variation may become more pertinent. While this may be dealt with by multiple biopsies, functional imaging may be the optimal way to see these differences.

Cell division

Most cancer cells divide at a faster rate than surrounding tissues. This is important as cytotoxic drugs disrupt cell division either in mitosis
(M phase) or during DNA synthesis (S phase) before mitosis. This can be exploited to identify if cancer cells are actively dividing before giving potentially toxic chemotherapy. The methods most developed in this field are mainly positron emitters and include fluorine-18 fluoro-L-thymidine (FLT) that looks primarily at tumour cell proliferation and amino acids such as fluorine-18 fluoro-ethyl tyrosine (FET) and carbon-11 methionine looking primarily at tumour cell growth. Uptake of these agents tends to be more S phase-dependent, and they are an effective way of demonstrating the action of anti-proliferative drugs.15

An example of this is in patients with non-small cell lung cancer, where fluorine-18 FLT is more specific in determining residual tumour than fluorine-18 fluoro-deoxy glucose (FDG) as it had no uptake in post treatment inflammation.16

Carbon-11 methionine is expensive to make, and carbon-11 only has a 20 min half-life, so the chemistry must be performed very quickly.17 It is of most use in brain tumours because the normal brain does not

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**Fig. 1** Coronal whole body PET images of the same patient suffering from a neuroendocrine tumour of unknown origin. The left hand FDG image shows multiple sites of lymph node uptake in the thorax and abdomen. The right hand gallium-68 DOTATATE image shows uptake in a single abdominal node that was not positive on fluorine-18 FDG PET. Histologically, both the fluorine-18 FDG and gallium-68 DOTATATE avid nodes looked identical, but after staining with Ki67, the fluorine-18 FDG node had a high proliferation index and the gallium-68 DOTATATE positive node had a low proliferation index.
undertake mitosis or cell growth. This means that any abnormal uptake can only occur in dividing cells in the brain signalling neoplasia (Fig. 2, 17–19). Likewise, fluorine-18 FET has been investigated in brain tumours and has similar accuracy as carbon-11 methionine. More interesting has been the use of fluorine-18 FET to predict whether glioblastomas treated with chemoradiotherapy are likely to progress when compared with pseudo-progression that has a better prognosis, but can look similar on functional MRI.21

**Apoptosis**

In most tissues, cell division and cell destruction are in balance; however, in tumours, there can be a failure of cell destruction leading to cell immortality and growth of the cancer. There are two main methods of cell death, immediate and related to ischaemia (necrosis) or delayed (apoptosis). Most effective treatments of cancer involve the induction of apoptosis. Once this process has started, it is irreversible and cell death in 10 days is inevitable.22 During apoptosis, there is blebbing of the affected cell wall surface and reversal of some components of the cell membrane, so that what was extracellular is now intracellular and vice versa. This results in antigens such as phosphatidylserine, once hidden within the cell, becoming exposed and available to complex with imaging agents such as the protein annexin-V.

![Fig. 2 Carbon-11 methionine coronal PET-CT showing focal uptake of tracer in the pituitary fossa demonstrating uptake into a TSHoma, whereas in the surrounding brain, there is no uptake of tracer.](https://academic.oup.com/bmb/article-abstract/105/1/157/271930)
Annexin-V has been labelled with both single photon and positron emitters.\textsuperscript{23} Although the single photon methods are cheaper, they lack the sensitivity and resolution that may be needed to visualize apoptosis within a tumour mass, especially if the response is not homogeneous. Most of the original work, however, has been performed with single photon imaging using technetium-99m annexin-V. In some haematological tumours and small cell lung cancer, uptake of technetium-99m annexin-V can be seen within 24 h of the first effective treatment. There has been an attempt to replicate this work using fluorine-18 annexin V,\textsuperscript{24} but little clinical work has been done with this agent partly as fluorinated peptides are difficult to make and can be unstable.

Angiogenesis

One of the fascinating facts about tumour growth is that as cell growth is disorganized, the cells may end up further from an arterial blood supply and, thus, become hypoxic. One of the tumour cell’s responses to this is to release substances such as vascular epidermal growth factor (VEGF) that results in new blood vessels growing into the tumour mass, a process known as angiogenesis.\textsuperscript{24–26} Many new anti-cancer treatments have been directed towards this process as the cancer cells are dependent on angiogenesis to maintain oxygenation. Simple anti-angiogenic drugs include thalidomide, although more modern drugs such as sunitinib or bevacizumab are more effective.\textsuperscript{27,28}

It would be useful to have an agent to identify angiogenesis \textit{in vivo}. Re-imaging with the same agent could also be used to monitor the effectiveness of anti-angiogenic treatments. At present, the most specific agents identified for this type of imaging are based on RGD (Arginine–Glycine–Aspartate) peptides that have a high affinity for the alpha-V-beta VEGF receptors on the cell wall.\textsuperscript{29} Radiolabelled RGD peptides have shown good localization in a mouse model of human breast cancer and have also shown inhibition of uptake in the presence of taxanes at sites of angiogenesis.\textsuperscript{30,31} Human studies beyond phase II have not been performed.

PET-based products should offer the possibility of looking more closely at the microenvironment within and around the tumour.\textsuperscript{32} The most promising PET radiopharmaceutical is fluorine-18 fluciclatide which is a stable fluorinated peptide containing a RGD group. This agent has completed animal studies and is now commencing clinical trials.\textsuperscript{33} The use of PET imaging of angiogenesis could be best when combined with data from diffusion-weighted MRI possibly using the next generation of combined PET/MRI devices.
Hypoxia

Hypoxia is the response of any cell to a reduction in oxygen tension. There are several consequences of hypoxia. Firstly, the cells are unable to burn fatty acids or pyruvate both of which is oxygen dependent. However, they can still undergo anaerobic metabolism of glucose. This process is highly inefficient, and as such there is an increased demand for glucose. This increased need for glucose explains why tumours can be avid for fluorine-18 FDG as increased uptake is probably driven by increased intracellular hypoxia-inducible factor. However, uptake of fluorine-18 FDG alone does not confirm the presence of hypoxia as other factors can result in increased uptake.

Therefore, more specific hypoxia markers have been sought. These have normally been based on imidazole esters. These are lipophilic esters that enter the cells and, in the presence of high levels of intracellular oxygen, the ester is broken down, and the hydrophilic components are expelled from the cell. Therefore, the product is only retained within an hypoxic cell. The two main agents used for hypoxia imaging are both ester based and are fluorine-18 fluromisonidazole (FMISO) and copper-64 diacetyl-bis [N4-methylthiosemicarbazone (ATSM)].

Imaging hypoxia is important because hypoxic cells do not divide and may show resistance to chemotherapy drugs that act on dividing cells. In addition, hypoxia inhibits wild p53, an inducer of apoptosis. As radiation therapy requires the production of oxygen radicals as an intermediate step to DNA damage, the lack of oxygen reduces the number of radicals that can be produced reducing the effectiveness of radiation-based therapies. Imaging with an agent such as fluorine-18 FMISO can show which parts of a tumour are hypoxic and hence may require different treatment strategies (Fig. 3).

Receptor imaging

PET has started to exploit tumour receptor imaging, although the identification of unique receptors has remained elusive. The best candidate is the somatostatin receptor that is routinely imaged with SPECT, but PET can double the sensitivity. Gallium-68 offers the promise of being able to label other peptide-based receptor agents, and more developments are expected. Examples are still limited, but they include breast cancer because oestrogen is a very tempting target. Manipulation of the oestrogen receptor is used in both benign and malignant disease, and imaging could be of use. In one study, reduction in uptake of fluorine-18-labelled oestrogens had a better correlation with
response to treatment than using fluorine-18 FDG because breast tissue during treatment often initiates an inflammatory reaction that results in non-specific uptake of fluorine-18 FDG.\textsuperscript{38}

Further work has been based on choline that is taken into the cell through a specific receptor and transporter system. In many ways, it is non-specific for a particular tumour type much like FDG. For example, it has good uptake in prostate cancer and primary liver cancer that are both often negative on fluorine-18 FDG imaging.\textsuperscript{39–42} The lack of uptake in inflamed tissues means that it can be more tumour specific than fluorine-18 FDG. However, it is costly and difficult to make and requires, if carbon-11 is used, an on-site cyclotron.

In addition, more specific agents can be prepared, an example of which is carbon-11 metomidate that has specific uptake into adrenal tumours (Fig. 4,\textsuperscript{43}).
Conclusions

PET techniques are helping us to understand tumours and their microenvironment. At present, they are providing more information than other forms of imaging, including single photon methods. PET may lead us to identify those factors such as angiogenesis, hypoxia, receptor status and apoptosis that could affect tumour growth and its response to treatment. Once we understand more about an individual’s cancer, there is the hope of delivering true personalized medicine for the cancer patient.

Conflicts of interest

The authors have no potential conflicts of interest.

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