**Mechanism and non-mechanism based imaging biomarkers for assessing biological response to treatment in non-small cell lung cancer**

**Abstract**

Therapeutic options in locally advanced non-small cell lung cancer (NSCLC) have expanded in the past decade to include a palate of targeted interventions such as high dose targeted thermal ablations, radiotherapy and growing platform of antibody and small molecule therapies and immunotherapies. Although these therapies have varied mechanisms of action, they often induce changes in tumour architecture and microenvironment such that response is not always accompanied by early reduction in tumour mass, and evaluation by criteria other than size is needed to report more effectively on response. Functional imaging techniques, which probe the tumour and its microenvironment through novel PET and MRI techniques, offer more detailed insights into and quantitation of tumour response than is available on anatomical imaging alone. Use of these biomarkers, or other rational combinations as readouts of pathological response in NSCLC have potential to provide more accurate predictors of treatment outcomes. In this article, the robustness of the more commonly available PET and MRI biomarker indices is examined and the evidence for their application in NSCLC is reviewed.

**Introduction**

Lung cancer is the leading cause of cancer related mortality worldwide, with 1.8 million new cases causing 1.6 million deaths in 2012 [1, 2]. Non-small cell lung cancer (NSCLC) accounts for 80-90% of lung cancers, with a greater prevalence of adenocarcinoma (43% of cases) over squamous cell (SCLC; 23%) and other subtypes (34%) [3]. Prior to radical treatment, anatomic evaluation with contrast-enhanced CT and evaluation of metabolic activity with [18F] fluorodeoxyglucose positron emission tomography (18FDG-PET CT) is standard of clinical care, along with pathology for cancer subtype, and molecular profiling in advanced disease. Response evaluation in the majority of clinical trials is provided by CT-based size criteria (Response Evaluation Criteria in Solid Tumours, RECIST). However, size based response evaluation is generally insensitive to early biological changes and often fails to identify responses in patients who experience either cytostasis or pseudoprogression [4-6]. These scenarios are more often encountered with molecularly targeted agents, where inter and intra tumoural heterogeneity lead to more varied treatment responses than is seen for cytotoxic agents [7, 8]. Biological changes that occur for up-to 12 weeks following treatment initiation are dominated by apoptosis, necrosis, cystic degeneration, intra-lesional haemorrhage, oedema and/or immune cell infiltration. They potentially herald survival benefit but may not be identified with anatomical imaging, thus compromising decision clinical making in both within trials and out-with [9].

A range of novel, non-invasive imaging probes have been developed in solid tumours, including NSCLC, with the potential to interrogate a combination of both anatomic (size-based) and functional tumour characteristics of the tumour and its microenvironment [5, 10, 11]. Features evaluable with PET, CT and MRI include metabolism, tissue water diffusion, perfusion, chemical composition and hypoxia. The most widely used of these techniques in the clinical trials setting is 18FDG-PET CT. MRI remains underexploited despite its ability to provide both anatomical and functional (physiological and pathophysiological) information in a single examination [12]. Our centre is currently contributing to coordinated multicentre trials evaluating MRI and PET functional imaging in a range of malignancies, including NSCLC and liver metastases [EORTC Quic-ConCept Innovative Medicines Initiative]. This review considers the requirements of a viable response biomarker for use in clinical trials and provides an overview of the evidence for using mechanism and non-mechanism biomarkers in NSCLC treated with a range of existing and novel treatment options.

**Quantitative Imaging Biomarkers in NSCLC**

For an oncologic biomarker to be viable it must be: 1) Accurate and reproducible over time and across institutions; 2) Closely coupled to presence of disease (sensitivity and specificity of the biomarker) and; 3) Changes in activity should act as a surrogate for the endpoint sought from therapy (survival) [13, 14]. Within clinical trials, imaging biomarker based response criteria aim to inform early ‘go/no-go’ decisions, increasing efficiency, reducing the cost of early phase studies and minimising exposure of patients to futile therapies [10]. From a practical perspective, cost, patient tolerability and availability are important, and rigorous quality control is required for use in multicentre trials.

To date, CT-size based evaluation remains the most commonly used technique in NSCLC drug trials [10], often supplemented by metabolic information from 18FDG-PET CT. Where size and metabolic evaluation yield misleading results, added specificity and sensitivity are potentially afforded by an increasing range of PET and MRI techniques [15] (figure 1). With MRI, standard T1W, T2W or proton density sequences provide anatomic information, while functional techniques allow water diffusion (DW-MRI), tumour perfusion (on DCE MRI), hypoxia (on BOLD MRI), ventilation (using a range of techniques including O2 enhanced MRI), and tissue composition (MR spectroscopy and UTE MRI) to be interrogated [16-19]. Several of these techniques remain investigational at specialist centres only.

*Biomarker Mechanism, Measurement Methodology and Repeatability:*

*Size:* Size-based treatment response was systematised for cross sectional imaging in 1981, and forms the basis of the RECIST criteria (1.0 in 2000, updated to 1.1 in 2009) [14, 20, 21]. RECIST 1.1 are validated against prospectively documented outcome data from >6500 patients with >18000 solid tumour deposits, and provide a balance between ease of application and ability to predict response linked to progression free survival [14]. Following immunotherapy and targeted agents, these criteria can wrongly identify cytostasis or slow growth as stable or progressive disease [22-24] (figure 2). Size based response evaluation is also delayed by up to 12 weeks following therapy. Repeatability of RECIST criteria in NSCLC indicate there is overlap between inter-observer measurement variability and the size change required for progressive disease so that RECIST progression is often misclassified [25], an error that occurs less frequently for partial response, due to the requirement of a larger change in size [25, 26]. Although tumour volumetry produces measurements with smaller variability, its utility in monitoring NSCLC treatment response requires time consuming image analysis and remains to be extensively investigated [27].

*Metabolic markers:* Metabolic response criteria in solid tumours, first proposed by the EORTC in 1999 [28] and followed in 2009 by PET Response Criteria in Solid Tumors (PERCIST) [10], were prompted by the observation that reduced 18FDG uptake in solid tumours (including NSCLC) following treatment conferred a marked improvement in progression-free and overall survival, even in the presence of residual soft tissue on CT [10, 28-31] (figure 3). Limitations remain due to lack of standardisation, with an ongoing debate about whether qualitative, semi-quantified (5 point scoring system), or fully quantitative SUV measures (including SUV mean, max SUL or total lesion glycolysis) are most reliable [10, 32, 33]. Questions also remain surrounding tumour segmentation methodology. Specificity remains low, confounded by tumour associated inflammation, while sensitivity for tumour deposits smaller than the 0.5cm is lacking because of limited spatial resolution (especially problematic in NSCLC mediastinal nodal assessment) [10]. Repeatability coefficients of 18FDG-PET SUVmean and SUVmax, which have been extensively studied for use in treatment response evaluation [32, 34, 35], are in the range 25 to 50% [36-39]. This is improved by measuring the SUV of the 30 most avid voxels rather than SUVmax or SUVpeak [40].

*Proliferation markers:* 18FLT is phosphorylated and trapped within cells by thymidine kinase, a key enzyme in the salvage pathway of DNA synthesis [41], but is insensitive to proliferation where *de novo* thymidine synthesis occurs [42]. From pathological specimens, 18FLT uptake has been shown to correlate positively with both Ki-67 immunohistochemical (IHC) proliferation index (r=0.81, p<0.01) in glioma specimens [43] and post thymidylate synthase inhibition in breast cancer models [44]. Satisfactory within-patient repeatability has been demonstrated for quantitative 18F-FLT measurements in NSCLC, where intra-class correlation (ICC) between SUV values from test-retest studies range from 0.93 to 0.98 [45]. Its use is currently being evaluated at our institution for NSCLC treated with neoadjuvant pemetrexed [EORTC QuIC-ConCePT].

*Tumour Perfusion:* Sequential images through a lesion following contrast administration generate time-intensity plots for either qualitative or quantitative analysis. In DCE-MRI, enhancement relies on the T1-shortening effect of gadolinium based contrast agents [46]. For CT, similar analysis is performed using Hounsfield Unit (HU) density measurements, but with an ionising radiation penalty from serial scans through the lesion [47]. The most commonly used pharmacokinetic models quantify: (a) ve, reflecting contrast leaking into the interstitial space during first pass; (b) Ktrans, the transfer constant describing trans-endothelial contrast diffusion into interstitium (hypothetically reflecting tumour capillary permeability) and; (c) Kep, the rate constant describing contrast diffusion back into the intravascular compartment [48]. These parameters are derived from signal intensity within tumour, normalised to the intensity within the biggest available artery (providing the arterial input function or AIF) [49]. On MRI, further information is available through T2W signal loss from spin dephasing and since this is related to gadolinium contrast agent concentration, it reflects both vessel size and density and allows calculation of relative blood flow (rBF), blood volume (rBV) as well as mean transit time (MTT) [50]. Qualitative and semi-quantitative assessment, include maximum slope of the time-intensity curve, maximum enhancement ratio (MER), washout ratio (WR) and area under the time concentration curve at 60 seconds (AUC60) [refs].

In untreated NSCLC, DCE-CT patterns of blood volume and Ktrans within tumours have been reported to correlate with vessel density and vascular score [49]. In a mouse model of lung cancer, both Ktrans and pathological CD31 staining (reflecting vascularisation) decreased significantly following treatment with anti-angiogenic agent cediranib (VEGFR inhibitor). Similar tumours treated with non-anti-angiogenic Jak1/2 inhibitors show no accompanying change in Ktrans [51].

DCE-CT pulmonary tumour perfusion parameters, derived from images acquired without motion compensation, have reported test-retest coefficients of variation ranging from 10.9-114.4% (BF), 25.3-117.6% (BV), 22.3-51.5% (MTT) and 29.6-134.9% (permeability), with smaller lesions showing largest variability [52-55]. Although variability decreases with motion compensation, CoV of up to 45% nonetheless limits treatment response evaluation [55]. However, better repeatability (18%) has been reported for Ktrans and AUC [56]. In addition to the mixed reports regarding repeatability, different commercially available pharmacokinetic algorithms produce significant variation in DCE parameters; on the same DICOM dataset, Patlak analysis produces permeability and BV values that are 1.34 and 1.65 times greater than those obtained from distributed parameter analysis [57]. This underlines the importance of using the same image acquisition protocol and data processing software for each study, as well as the need to record analysis tools employed when publishing data. By comparison with CT, DCE-MRI test-retest coefficients of variation (CoV) in NSCLC and using non breath-hold technique are in the range of 31% for Ktrans and 27% for tumour initial area under the curve (IAUC) [58]. However, these apply to tumours > 3 cm and increase to 37% and 30% when considering all cases. Very few reports have evaluated the optimal time delay between treatment and dynamic contrast enhanced imaging and whether their use as early (intermediate) response biomarkers is appropriate.

*Tumour microenvironment:* Diffusion weighted MRI is sensitive to thermally driven water motion and influenced by intra and extracellular structures, macromolecules and membranes, all of which impede diffusion at a micrometer (cellular) scale [16]. The most promising DW-MRI parameter, the apparent diffusion coefficient (ADC mm2s-1), is calculated from the slope of the logarithmic plot of signal intensity versus b-value [16]. Low ADC represents restricted diffusion and is found in highly cellular regions such as tumours and normal lymph nodes. Reports correlating ADC with NSCLC pathology specimens have confirmed a decrease in tumour ADC with increasing cellularity (as previously reported for other body parts), and an ADC cut-off can be set such that DW-MRI differentiates grade III cell density from lower grades with a sensitivity of 88.2% and a specificity of 62.5% [59, 60].

Recent data has confirmed satisfactory inter- and intra-observer repeatability for ADC measurements of lung tumours with coefficients of variation in the range 3% to 11%, although variation is greater for lesions smaller than 2 cm and for those in the mid, compared with upper or lower lung zones [61]. As for other modalities, debate remains relating to standardisation of image acquisition sequences and post processing, with different techniques available for the purpose of mitigating respiratory motion [62, 63]. Few reports address the optimal timing of DW-MRI following treatment, but it is informative to note that signal change in cerebral infarction is measurable within 30 minutes of symptom onset, demonstrating a high sensitivity to biological change in this context [64].

*Tissue composition:* Using ultrafast readout of free induction decay, UTE MRI is able to generate contrast between structures with short T2\*. Although satisfactory repeatability of T2\* quantification has been confirmed within lung parenchyma and areas of fibrosis (CoV of around 10-15%), poor repeatability within tumour currently limits its application to treatment response evaluation [65]. Structures that are highlighted by UTE imaging include those rich in collagen, including fibrosis [66], such that T2\* values derived from UTE may identify pulmonary fibrosis following radiotherapy [65]. Other techniques such as MR spectroscopy (MRS) interrogating tumour metabolism have had limited application within the chest to date because of significant problems with magnetic field inhomogeneity in the presence of aerated lung.

*Tumour Hypoxia:* BOLD MRI utilises the increased T2\* relaxation rate, due to field inhomogeneities that accompany increasing concentrations of the paramagnetic deoxyhaemoglobin, compared with oxy-haemoglobin. Increasing hypoxia accelerates T2\* relaxation by dephasing the transverse magnetisation [67]. For BOLD MRI, R2\* variations are evaluated using subtraction images generated from acquisitions for patients when breathing carbogen (95% O2, 5% CO2) compared with air (80% O2, 20% N2). Vasodilation from hypercapnia, combined with higher oxy-haemoglobin concentration when breathing carbogen, create T2\* contrast within hypoxic regions when compared with images acquired breathing room air, due to signal loss on the latter [17]. This technique has not however to our knowledge been used in NSCLC treatment response evaluation. 18F-FAZA, 18F-MISO PET are used for hypoxia imaging in other tumour types, but heterogenous tumour uptake limit their use and there are to date no studies on within-patient repeatability in NSCLC [68].

**Therapeutic Mechanisms in NSCLC and Quantifying Treatment Response**

A mismatch often exists between the mechanisms underlying anti-tumour effects of a therapeutic intervention and the process measured with imaging. However, it is likely that multi-parametric evaluation of NSCLC will provide a more accurate prognostication than is offered by current standard of care imaging [69]. By implementing novel multi-parametric combinations of PET and MRI protocols in Phase I and II trials, using a common region of interest across the different modalities, it should be possible to characterize mechanistic response.

*1. Radiotherapy*: Radiotherapy is used with curative intent in poor surgical candidates with early stage disease [70] and forms the mainstay of treatment in patients with more advanced disease than is amenable to surgery (e.g. inoperable stage IIIa disease). Following radiotherapy, cell death and arrested mitosis arise from ionising radiation related DNA damage and are accompanied by altered interaction between tumour and factors in the microenvironment including hypoxia, tumour microvasculature and host immune cells [71]. The inflammatory changes within both tumour and surrounding parenchyma vary in timing, from transient treatment induced ‘metabolic flare’, to delayed reaction and are observed up to 30-40 months following treatment [72]. Pre-clinical data has confirmed a high metabolic activity on 18FDG-PET following radiotherapy, despite pathological complete remission. Within corresponding surgical specimens from this study, up to 30% of 18FDG uptake was attributable to monocyte/macrophage activity [73] (figures 4 and 5). Frequently, up to 6-12 months following treatment, early pneumonitis and later dense mass-like consolidation and nodularity impair the ability of CT based RECIST and 18FDG-PET CT to differentiate residual or recurrent viable tumour from treatment related inflammation, ‘pseudoprogression’ or evolving fibrosis, especially following stereotactic radiotherapy (figure 5) [15, 70, 72, 74, 75].

Early clinical and recent preclinical reports assessing DW-MRI suggest that ADC may mitigate the confounding effects of inflammation on response evaluation seen for both CT and 18FDG-PET CT following radiotherapy (and immunotherapy; see later) [5, 10, 15]. Preclinical data on a rodent model of gliosarcoma treated with 1,3-bis(2-chloroethyl)-1-nitrosourea in ethanol (BCNU), a regimen that promotes tumoural inflammatory infiltration, confirmed that although response evaluation with 18FDG-PET CT is confounded by inflammatory uptake, ADC increases with pathological response. This is likely due to increased tissue diffusivity from a combination of anti-tumoral activity and inflammatory oedema [76]. In the clinical setting, ADC is able to distinguish between viable lung cancer and both focal inflammatory lesions and pulmonary collapse/consolidation [77-79] and has been reported to be superior to 18FDG-PET CT as a baseline predictor of treatment response to chemoradiotherapy in NSCLC [80], although prospective multicentre data assessing ADC in this setting is pending.

With a view to minimising radiation damage to healthy lung, 18FDG-PET CT uptake in lung parenchyma before radiotherapy is associated with higher risk of developing radiation induced lung toxicity in NSCLC, so that these areas should be spared high doses of ionising radiation during treatment planning [81]. In addition, imaging 3 months after completion of curative radiotherapy, our preliminary data suggests that UTE-MRI derived T2\* differentiates between pneumonitis and evolving fibrosis, at a time when features on CT are non-specific. The potential for this to direct treatment aimed at mitigating fibrosis remains to be evaluated [65].

*2. Chemotherapy*: RECIST measurements at the end of cytotoxic chemotherapy provide not only response assessment but are also a discriminator for survival [82]. Multiple retrospective and prospective studies have however reported reduction in 18FDG uptake earlier following treatment than any size reduction on CT and in many cases, early metabolic response on 18FDG-PET CT also shows better correlation with survival measures than later RECIST assessment [39, 83, 84]. The optimal timing of metabolic response evaluation is subject to debate, since the rate at which 18FDG uptake falls after chemotherapy initiation varies between patients and treatment regimens, depending upon initial avidity of the tumour, frequency of chemotherapy cycles and proportion of cell kill per cycle of therapy [10]. Despite reports of measurable 18FDG-PET treatment effect providing prognostic information as early as 48 hours after treatment initiation [85, 86], some authors advise a 10 day delay before imaging in order to minimise the risk of misdiagnosing transient changes in FDG uptake secondary to tumour flare or treatment-stunning [87-89].

18FDG-PET CT also provides greater accuracy than RECIST for predicting pathological response within surgical specimens following neo-adjuvant chemotherapy [84, 90, 91]; a positive 18FDG-PET scan after several cycles of treatment indicates viable tumour. Microscopic viable tumour residuum below 0.4-1 cm (roughly 0.2-1g or 108-109 cells) is however below the resolution of PET [87]. In addition, the presence of inflammation can be confounding so that a broad range of SUV is seen in complete responders to neo-adjuvant chemotherapy, with a significant correlation between SUVmean prior to surgery and both macrophage infiltration and viable tumour [91]. A recent systematic review confirmed wide ranges of 18FDG-PET CT specificities (0-100%) and positive predictive values (67-100%) for pathological response following neoadjuvant chemotherapy. On DCE-CT, patients demonstrating a decrease in tumour permeability-surface area product have been shown to have 4 times longer PFS and OS compared with patients that experienced an increase [92]. The use of ADC has also indicated the significant potential of this biomarker for demonstrating treatment response, despite disparate image acquisition protocols and post processing and segmentation methodologies between studies (figure 6) [93]. To address limitations of single parameter assessment, multi-parametric evaluation using combined metabolic andsize response has been shown to discriminate responders from non-responders better than SUV or by size criteria alone [84]. Early ADC changes following chemotherapy have been shown to correlate with later changes in lesion size, such that combining size and ADC measurements will potentially improve dynamic monitoring of response, compared with using each biomarker in isolation [94, 95]. The combination of PET tracers (including 18FDG) with anatomic and functional evaluation on MRI warrants further evaluation.

*3) Molecularly targeted therapy and immunotherapy:* The advent of molecularly targeted agents has seen the use in NSCLC of signalling pathway inhibitors, antiangiogenic agents and immunotherapies. In patients with sensitising EGFR mutations, response rates of up to 70% are seen following erlotinib, gefitinib and afatbinib EGFR tyrosine kinase inhibitors (TKIs) [96-100]. ALK rearranged NSCLC is strongly associated with responses to ALK inhibitors, including crizotinib, ceritinib and alectinib [101]. In some patients, a significant survival benefit is achieved despite RECIST stable disease [102-104] and for patients with slow growing lesions, survival benefit can be achieved by delaying change of treatment away from EGFR TKI, despite RECIST progression. As for cytotoxic agents 18FDG-PET CT is superior to RECIST in predicting outcome following EGFR TKIs [6, 85, 105-107], although its specific role in this setting of pseudo-progression and slow growing lesions while on therapy has not yet been investigated. The fact that disparate study protocols have consistently shown metabolic response evaluation with 18FDG PET (performed as early as 48 hours after chemotherapy) to predict outcome following a range of therapeutic agents demonstrates the robustness of this biomarker.

Conflicting reports exist of SUV change on 18FLT-PET CT following EGFR-TKI therapy. In one recent study, 18FLT-PET SUVmax measured one week post gefitinib was significantly different between responders and non-responders (assessed by later WHO size-criteria) [108], while in another study, although a reduction of ≥ 30% in 18FLT-PET SUVpeak following gefitinib predicted significantly longer PFS, it did not predict OS or RECIST non progression at 6 weeks [109].

Bevacizumab and ramucirumab are antiangiogenic monoclonal antibodies, licensed in NSCLC, that target VEGF (vascular endothelial growth factor) and its receptor, leading to the inhibition of tumour vessel growth [110], while small molecules such as Cediranib interact with multiple intracellular kinases, including VEGFR kinase [111]. Response to these agents may be interrogated using dynamic contrast techniques that evaluate tumour perfusion [47, 112-117]. However, small patient cohorts receiving disparate treatment regimens and inconsistent imaging protocols in these studies highlight the need for standardisation and validation before widespread clinical application.

Immunotherapies inhibit tumour evasion of the host immune response, an evasion that is mediated in some tumours by PD-1 (programmed cell death-1) T-cell deactivation, with up to 20% of NSCLC responding to immunotherapy [118, 119]. Antitumor biological activity and radiological response patterns with these agents can be different from those seen with cytotoxic agents, although RECIST size-based response criteria still apply [5, 8, 120-122]. Following PD-1 blockade of NSCLC, neither the tumour infiltrating lymphocyte population nor suitable imaging biomarkers for the variation in anti-tumour responses have been investigated with functional imaging. However, as both 18FDG-PET uptake and CT are unable to differentiate between viable tumour and inflammation [123-127], it is expected that immune cell infiltration following response will cause in some patients a paradoxical increase in both tumour size and 18FDG uptake (‘pseudoprogression’) [5, 122]. An imaging strategy is required to differentiate inflammation from viable tumour and multi-parametric imaging including 18FDG PET-CT and ADC is a potential candidate.

*4. Local ablative therapy:* Treatment of pulmonary tumours by microwave ablation (MA) or radiofrequency ablation (RFA) is performed percutaneously under CT guidance. Guidelines vary regionally, with some centres considering patients with tumours < 3-3.5cm in diameter [128]. Following the procedure, the CT and 18FDG-PET CT imaging features of the ablation defect and tract vary both with time and between patients [128, 129]. Pneumothorax occurs in up to 40% of patients and immediately post treatment CT typically shows a halo of ground glass surrounding the lesion and electrode tract. This corresponds in animal models to cell death, haemorrhage and neutrophil infiltration [130]. On CT, the overall size of the ablation zone reaches a maximum at around 1 week, followed by involution [130]. Between around one to four weeks, an enhancing rim of inflammatory infiltration and granulation tissue develops. The central ablation zone appears as a dense nodule at 1-3 months, typically involuting by 12 months to leave a scar. The features on 18FDG-PET CT follow a similar course, with peripheral rim of 18FDG uptake developing between 1 and 6 months for around 68% of ablations, which is often replaced by increasing activity centrally between 6 months and a year. Standard of care imaging therefore fails to differentiate treatment effect from local recurrence in the first year [129]. DW-MRI has potential in this context, as an increasing ADC has been demonstrated following therapy and favours treatment response [131].

**Conclusion and Future Perspectives**

In addition to the existing functional parameters discussed, a range of combined imaging agents are under investigation, many of which are aimed at increasing specificity for the biological process targeted by anti-cancer agents. 89Zirconium PET tracer labelling of therapeutic agents (eg anti PDL-1 antibody), has potential to quantify the pharmacokinetics of drug delivery to target [132], as well as to track T-cells for immunotherapy planning and response evaluation [133]. These studies are however investigational and available at specialist centres only.

Currently, size based evaluation remains the cornerstone in monitoring treatment response. 18FDG-PET CT based functional metabolic evaluation offers both an improvement in prognostication and an earlier response evaluation compared with CT for cytotoxic agents and EGFR inhibitors, although is limited where inflammatory uptake confounds tumour cell death. The latter scenario is common following immunotherapy, radiotherapy or local ablative therapies. DW-MRI derived ADC, in combination with standard T1W and T2W anatomical sequences, shows greatest promise in terms of repeatability and potential additional value in these situations. The multimodality capacity of MRI, combined with emergence of PET-MRI scanners offers a vast array of potential imaging probes; each combination will require systematic evaluation with both existing and emerging therapeutic regimes. Knowledge of the pathological processes driving treatment response and of the biological processes to which each imaging modality relates should in the future inform which techniques are best suited to any specific targeted anti-cancer therapy, so that rationally constructed multi-modality imaging evaluation is undertaken.

**Figure legends**

**Figure 1)** Correlation between imaging modalities and the mechanistic steps involved in tumour growth, vascularisation, stromal interaction and metastasis. The process interrogated and the resolution (spatial and temporal) afforded by each imaging modality is required for rational selection of imaging biomarkers.

**Figure 2)** Critical timing of assessment using size based response criteria following immunotherapy. Axial CT images through a PDL1 positive left upper lobe primary NSCLC and the liver: (a) At baseline; (b) 8 weeks after commencing Pembrolizumab (anti-PDL1), showing decrease in size of the left upper lobe tumour (arrow-head) but with developing liver lesions reported as new metastases (arrows). (c) 12 weeks after commencing therapy both the primary lesion and the liver lesions show overall RECIST partial response from baseline. ‘RECIST atypical’ response patterns early (less than 12 weeks) following immunotherapy and targeted agents (eg TKI) must be recognised prior to changing treatment strategies.

**Figure 3)** Metabolic response to cytotoxic chemotherapy. Axial CT and 18FDG PET CT images through a right lower lobe primary NSCLC: (a and b) Before and; (c and d) after 4 cycles of platinum based cytotoxic chemotherapy. This confirms that 18FDG-PET metabolic response precedes reduction in size.

**Figure 4)** A case in which metabolic response is seen following curative radiotherapy. Axial CT and 18FDG PET CT images through a right upper lobe primary NSCLC: (a and b) Before and; (c and d) 12 months after curative radiotherapy (64Gy in 32#). Complete metabolic response within the residuum at the primary tumour site confirms that this remains clear of macroscopic tumour recurrence, despite residual post therapy scarring on CT. However, microscopic disease below 0.5-1cm below the resolution of PET imaging and may yield false negative results and the timing of response can vary between individuals.

**Figure 5)** Limitation of CT and 18FDG PET evaluation post stereotactic ablative radiotherapy (SABR). Axial CT and 18FDG PET CT images through a left lower lobe NSCLC: (a and b) 6 months and (c) 12 months after stereotactic ablative radiotherapy (SABR). (a and b) 6 months post SABR: (a) A mass within the high dose treatment field on CT; (b) has residual metabolic activity on 18FDG PET, within which tumour residuum is not differentiated from inflammation. (c) Lack of growth on CT 11 months post SABR confirms that the soft tissue residuum is in keeping with radiotherapy induced inflammatory infiltrate. This was not characterised on FDG PET at 6 months.

**Figure 6)** DW-MRI: (a and b) pre-treatment and; (c and d) 3 weeks after cytotoxic chemotherapy for a right lower lobe primary NSCLC. (a) On the pre-treatment axial ADC map, the tumour has a mean ADC value of 57 x10-5mm2s-1; (b) tumour size and anatomic extent are better assessed on the fused high b-value (b750) and anatomic T1W images. (c) 3 weeks after commencing chemotherapy, the mean tumour ADC is increased to 92 x10-5mm2s-1, demonstrating the potential of ADC for quantifying treatment response; (d) this treatment response is also evaluable qualitatively on the fused high-b (b750) and T1W images.

**Conflict of Interest Statement:**

A Weller1  No Conflict of Interest

MER O’Brien2  Editor of EJC: Otherwise, no Conflict of Interest.

M Ahmed3  No Conflict of Interest

S Popat2  SP reports institutional research grant funding from Pierre-Fabre, Otsuka, and Boehringer-Ingelheim, honoraria from Eli-Lilly, and is non-reimbursed consultant to AstraZeneca, Boehringer-Ingelheim, BMS, Novartis, MSD, Pfizer, Roche.

J Bhosle2  No Conflict of Interest

F MacDonald3  No Conflict of Interest

TA Yap2  No Conflict of Interest

Y Du4  No Conflict of Interest

I Vlahos5  No Conflict of Interest

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**Acknowledgements:**

1. We acknowledge CRUK and EPSRC support to the Cancer Imaging Centre at ICR and RMH inassociation with MRC & Dept of Health C1060/A10334, C1060/A16464 and NHS funding to theNIHR Biomedical Research Centre and the Clinical Research Facility in Imaging.
2. AW wasfunded by EORTC Innovative Medicines Initiative Joint Undertaking, under grant agreement number115151.

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