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Review Article

MOLECULAR IMAGING IN CANCER: HOW THE HALLMARKS AID IN HUNTING

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ABSTRACT

Diagnosis, therapy response and patient follow up have been significantly benefited by introduction of non-invasive imaging technologies in oncology. In the era of genomics, cancer has been recognised as an organised disease with vast genomic heterogeneity and precise hallmarks as set by Weinberg & Hanahan that define various aspects of tumor biology. Non-invasive imaging of these hallmarks of cancer is important which provides deep understanding of cancer biology and pave the path for future therapy. In this review, we summarize various probes and non-invasive imaging modalities that have been employed in clinics for imaging of some hallmarks of cancer and their impact on disease management. With development of better probes, methodologies and instruments molecular imaging will be going to non-invasively portray each of the hallmarks and fine tune our understanding about this devastating disease.

Keywords: Non-invasive molecular imaging, Hallmarks of cancer.

INTRODUCTION

The overwhelming complexity and heterogeneous nature of cancer make it a challenging disease and has puzzled mankind over a century to reach true depths of its organising principles. Tumors are more than just a mere mass of replicating cells. Countless efforts over the last few decades have shed light on true nature of the disease: a complex tissue like structure where multiple distinct cell types interact with each other and with the surrounding normal tissue/cells (Tumor associated stroma and blood vessels) leading to process of tumorigenesis. Hanahan and Weinberg in 2000 proposed six biological hallmarks that provide a logical framework for organising principles of tumor development and progression. These include sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis and activating invasion and metastasis (Hanahan *et al.*, 2000).

In the year 2011, four new key features of tumorigenesis were introduced by them into the hallmarks of cancer. These are genome instability, tumor promoting inflammation, reprogramming energy metabolism and evading immune destruction (Hanahan *et al.*, 2011). Genomic instability is probably the most prominent characteristic that not only drives tumorigenesis but also leads to tumor heterogeneity while tumor promoting inflammation allows the formation of tumor microenvironment that consists of normal stromal and immune cells. To sustain a never ending need of energy and catabolites to fuel uncontrolled proliferation reprogramming of energy metabolism is important for the cancer cell. The last but not the least is evasion of immune system that act as a key check point to lay an active vigilance on transformed cells and destroy them.

Non-invasive molecular imaging has changed the landscape of cancer management in last three decades. Molecular imaging utilizes a broad range of electromagnetic spectrum, gamma radiation for positron emission tomography (PET) imaging, X-ray for computed tomography (CT), visible to near infra-red

spectrum for optical imaging, sound waves for ultrasound imaging and magnetic resonance imaging (MRI) utilizes intrinsic magnetic properties of molecules to image function and anatomical features. Pre-treatment staging by imaging has significantly helped in designing treatment regime that benefits patients. It has also aided in better monitoring of therapy response and predicts early recurrence of disease. Many probes have been developed for imaging various hallmarks of cancer such as sustained proliferation, apoptosis, limitless replicative potential, angiogenesis, invasion and metastasis, cancer metabolism and immune check points and tested in patient cohorts. Imaging probes for inflammation are still in preclinical levels while probes for genomic instability and evading growth suppressors are yet to be developed and validated. In this review, we aim to provide a combined description of different probes and imaging studies utilized for direct imaging of various hallmarks and their importance in disease management. Elaborated description for individual hallmark and associated imaging studies can be found in other reviews.

HALLMARK 1: IMAGING OF SUSTAINED PROLIFERATIVE SIGNALLING

Sustained proliferative signalling is undoubtedly the most fundamental hallmark of cancer observed consistently across all cancer types. The reason why the cancer cells can multiply constantly, lies in the fact that these cells have deregulated signalling cascades that primarily control cellular proliferation for example EGFR, IGFR, PI3K-mTOR, ras-MAPK mediated signalling (Hanahan *et al.*, 2011). These deregulated cascades could be an outcome of amplification in gene copy number or activation of mutated genes promoting cell proliferation. The cancer cells can also relay sustained proliferative signals by harbouring mutations in the proliferation suppressor genes that render them inactive. Another mechanism exploited for sustained growth stimuli is through autocrine signalling where the growth receptor ligands are produced and sensed by the same cells causing continues firing of the growth axis. For instance, EGFR/Her2 amplification/activation (through mutation) in breast cancer, lung cancer, colon cancer and glioblastoma, PI3K-Akt-mTOR amplification/activation in ovarian and breast cancer are common features and have functional implications in cancer progression (Iqbal *et al.*, 2014 and Martini *et al.*, 2014). Therefore, this hallmark has become an attractive target in precision medicine for diagnostic as well as therapy purpose. A lot of emphasis has been given on the development of molecular imaging probes that will selectively target the biomarkers attributed to this hallmark. Development of molecular probes for detecting activating gene signatures (oncogene) is more feasible than designing probe to identify loss of function or inactivating mutations (tumor suppressors). Majority of these probes are conjugated with antibodies like Zr-89-Labeled trastuzumab, In-111-Labeled trastuzumab, Zr-89 or In-111 labelled R1507 etc. or small molecules like [^{11}C] PD153035 that bind to the whole protein or a specific domain of the target protein (Dijkers *et al.*, 2010, McLarty *et al.*, 2009, Heskamp *et al.*, 2010 and Wang *et al.*, 2007). Out of these, EGFR/Her2 has been widely targeted for molecular imaging and is being actively pursued clinical trials. Imaging studies with markers like IGFR, PI3K are still in the pre-clinical phase and the outcome of few ongoing trials is not out yet. Among all the imaging modalities, PET imaging emerges as a predominant technique for validation and application in both preclinical and clinical studies. A few such important studies haven described below to implicate the role of imaging in deciphering this important hallmark of cancer.

Dijkers *et al.*, 2010 performed PET imaging with humanized Zr-89-trastuzumab in 14 breast cancer patients presented with metastatic lesions. Use of this specific probe enabled them to have better visualization and quantification of HER2-positive metastatic lesions in liver, lung, bone and brain and distinguish between lesions with and without HER2 overexpression. Therefore, this strategy could serve as a very useful tool for monitoring differential response to trastuzumab therapy in patients with advanced

stages with HER2 overexpression. In another study, Memon *et al.*, 2011, evaluated the potential of C-11 labelled erlotinib in visualising non-small cell lung carcinoma (NSCLC) tumor and metastases in comparison to conventional F-18-FDG PET/CT imaging. C-11-erlotinib PET/CT imaging enabled visualization of NSCLC lung tumours, including lymph node metastases which were not identified by F-18-FDG PET/CT. Their data also suggests that this approach could be used to identify differential expression of EGFR between tumours in the same patient which is not always possible with the conventional imaging modalities. In a pilot study by Meng *et al.*, 2011, advanced stage chemo refractory NSCLC patients were administered with erlotinib and the response to this tyrosine kinase inhibitor was predicted using standardized uptake value (SUV) of C-11-PD153035 which is a positron-emitting analog of the EGFR-TKI PD153035 using PET/CT imaging (Figure 1). Monitoring therapy response is another important goal for current precision medicine. In a recent study, anti EGFR monoclonal antibody treatment is administered as monotherapy or in combination with chemotherapy in a small cohort of colorectal carcinoma patients carrying wild type allele of ras oncogene. Zr-89-cetuximab uptake in tumor lesions was detected in 6 out of 10 patient and Zr-89-cetuximab uptake was correlated with the treatment response in 4 patients. Progressive disease was observed in 3 of 4 patients who did not exhibit Zr-89-cetuximab uptake (Houven, *et al.*, 2015). All these and many other studies describe the potential of these imaging probes targeted to proteins responsible for sustained cancer cell proliferation. However, the cost, need of expert radiochemist and availability of specific radioisotope pose limitation to bring them in clinical practice.
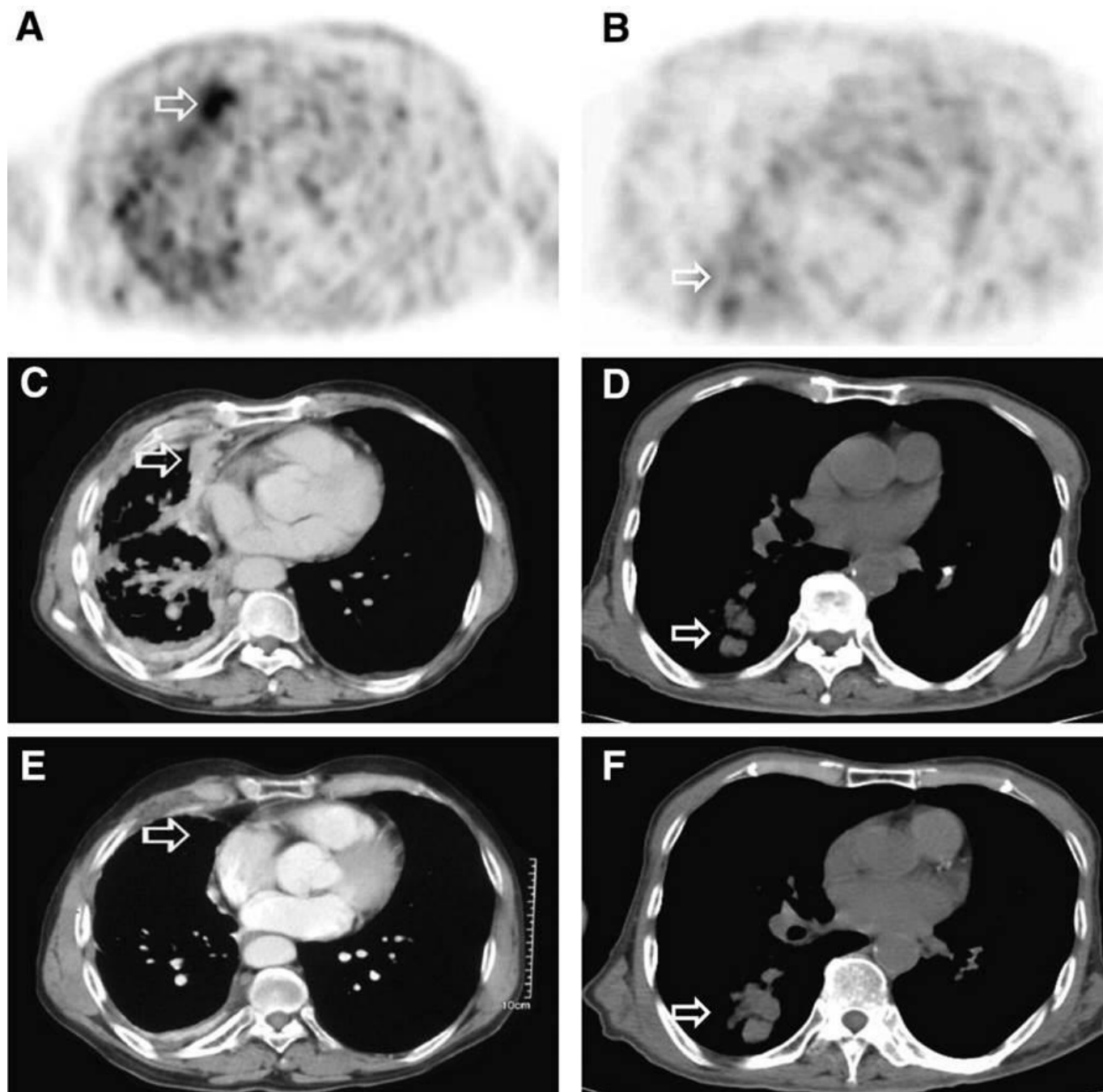
Figure 1: C-11-PD153035 (Analogue of EGFR-TKI) PET imaging after erlotinib treatment in NSCLC patients.

Patient no. 15, had higher baseline C-11-PD153035 PET SUVmax (4.76) as compared to patient no 13 (1.98) shown in figures A and B respectively before initiation of erlotinib treatment. Figures C and D show corresponding CT slices from before treatment and figures E and F correspond to 6 weeks after treatment for both patients. Patient no. 15 had marked radiographic improvement by 6 week after erlotinib was initiated (A, C, and E) and patient remained alive at last follow-up of 11.8 months. However, patient 13, with lower baseline SUVmax, had radiographic progression 6 weeks after erlotinib was initiated (B, D, and F) and died within 2.6 months. (This research was originally published in JNM. Meng, X., et al. J Nucl Med, 2011. 52(10): p. 1573-9. © by the Society of Nuclear Medicine and Molecular Imaging, Inc.)

Till date the most specific probe developed to image cellular proliferation is F-18-fluorothymidine (FLT). FLT is a fluorine modified analogue of thymidine that gets phosphorylated by thymidine kinase-1 during

S-phase of the cell cycle and therefore can act as a marker of proliferation. Good number of studies show significant correlation between FLT-PET and a classical proliferation marker Ki-67 expression (Wool F, *et al.* 2014, Chalkidou, *et al.*, 2012 and Kenny, *et al.* 2005). Buck *et al.* 2003 carried out a comparative study of F-18-FLT PET and F-18-FDG PET on 26 patients with pulmonary chest nodules to determine the superior probe for tumor proliferation. The study revealed that F-18-FLT uptake correlates better with proliferation of lung tumors as compared to the uptake of 18-F-FDG and therefore might serve as a selective biomarker for tumor proliferation. Similarly, a cohort of 25 patients with newly diagnosed or previously treated glioma had undergone PET scan with F-18-FLT and F-18-FDG tracers on consecutive days. F-18-FLT PET scan was found to be more sensitive than F-18-FDG scan for recurrent high-grade tumors, correlated well with Ki-67 index and survival of the patients (Chen, *et al.*, 2005). Therefore owing to its sensitivity and specificity, F-18-FLT is being considered as a powerful predictor of tumor progression and survival.

HALLMARK 2: IMAGING APOPTOSIS

Loss in programed cell death or apoptosis endows cancer cells a limitless potential to expand its population and has been described as a hallmark by Weinberg and Hanahan (Hanahan *et al.*, 2000). Majority of the chemotherapeutic agents as well as radiotherapy aim to induce apoptosis in tumors and efficacy of these therapies can be determined by imaging early events of apoptosis. Several molecular probes aimed to investigate non-invasive imaging of apoptosis have been developed and tested in pre-clinical models. Tc-99m labelled C2A domain of Synaptotagmin-I can bind to phosphatidyl serine in presences of calcium and was used as a SPECT probe for detection of apoptosis in animal model (Zhao, *et al.*, 2006). F-18 labelled non-peptide Isatin bsulfonamide analogs like WC-II-89 targeted against caspases was used to study cyclohexamide induced apoptosis in rat model (Zhou, *et al.*, 2006) Amphiphatic apoptosis markers known as ApoSense tracers like NST-372 and Didansyl-L-cystine have the ability to selectively enter apoptotic cells. These probes were used to image apoptosis in lymphoma and melanoma tumor models respectively. Höglund J *et al.,* 2011 demonstrated the favourable dosimetry, biodistribution, stability, and safety profiles of F-18 labelled 5-fluoropentyl-2-methyl-malonic acid (F-18-ML-10), another ApoSense marker in human subjects. Among all the apoptotic probes, the most widely used is Annexin V which specifically binds to phosphatidylserine (PS) present in the outer membrane of apoptotic cells and had been tested in various clinical trials. PS are the amino phospholipids which reside in the inner leaflet of normal cell membrane. Following the activation of caspase 3, PS is externalised by the apoptotic cells in a calcium dependent process which leads to inactivation of flippase and activation of scramblase resulting in membrane dissociation. Annexin V is a 36 Kda protein which binds specifically to these exposed PS on apoptotic cells (Smith *et al.*, 2012).Thus labelled annexin V with various radioisotopes and suitable imaging modality can provide information on early events of tumor death following therapy (Penberthy, *et al.*, 2016).

More than 20 years back, purified Annexin V was been labelled with fluorescence protein to distinguish between viable and apoptotic cell using microscopy or flow cytometry by Koopman *et al.* 1994. Later, I-123 labeled Anexin-V was used to study dosimetry and biodistribution in human and mice model by Lahorte *et al.,* 2003. Blakenberg *et al.*, 1998 first time demonstrated the expression and imaging of PS through Tc-99m-HYNIC-annexin A5 in cyclophosphamide induced apoptosis in murine lymphoma zenograft model. Mandl *et al.,* 2004 showed that Tc-99m Annexin V imaging can detect the onset of apoptosis induced by doxorubicin in syngeneic orthotopic murine BCL1 lymphoma model. Belhocine T *et al.,* 2002 measured Tc-99m Annexin V uptake pre and post chemotherapy (3 day after first course of chemotherapy) in a cohort of 15 patients. Among these 15 patients, 7 patients who showed higher uptake

of Annexin V had complete or partial response. Van de Wiele *et al.,* 2003 demonstrated the positive correlation between Annexin V uptake and number of apoptotic cell in a cohort of 20 patients suffering from primary and recurrent head and neck carcinoma. Vermeersch *et al.,* 2004 demonstrated the use of Tc-99m Annexin V for identifying primary head neck cancer in a cohort of 18 patients. Tc-99m Annexin V uptake was detected in primary tumors by SPECT tomographic imaging in 17 patients and lymph node involvement in 11 patents. Annexin V imaging were found to be in agreement with CT and histological finding. Hass *et al.,* 2004 demonstrated increased Annexin V uptake after cumulative 4 Gy radiation treatment which correlated with cytological studies indicating apoptosis in follicular lymphoma patients. Karatachova *et al.,* 2007 reported significant correlation between patient's therapeutic outcome and Annexin V uptake after 48 hours of start of chemotherapy in 16 chemotherapy-naive patients with advanced stage non–small-cell lung cancer. Similarly Kartachova *et al.,* 2008 showed positive correlation between Tc-99m-HYNIC-rh-annexin-V tumour uptake (ATU) and therapy outcome in 31 lymphoma, 4 non-small cell lung carcinoma and 3 head and neck squamous cell carcinoma patients. 99mTc EC-annexin V uptake successfully predicted treatment outcome in 9 out of 10 breast cancer patients based on tumor-to-non tumor count density ratios (T/N ratio) of labelled annexin V (Kurihara, *et al.*, 2008).

HALLMARK 3: IMAGING LIMITLESS REPLICATIVE POTENTIAL

The unlimited replicative ability of cancer cells is conferred by enhanced telomerase activity, which acts as a checkmate in normal cells. Thus monitoring telomerase activity *in vivo* would decipher important characteristics of cancer cells and may generate important information of tumorigenesis. Unfortunately, till date a direct biomarker and robust approaches for imaging telomerase activity are not in place. Only few studies in cell line or small animal models had been carried out till now.

Telomerase-specific replication-selective adenovirus carrying green fluorescent protein (GFP) has been used to microscopically image different cancer cells. Kishimoto, *et al.,* 2006 used a dual promoter construct (named OBP-401) which carries an adenoviral E1 gene is cloned under the human telomerase reverse transcriptase (hTERT) promoter and GFP driven by CMV promoter. Cells only with increased hTERT expression allowed the replication of the adenovirus and thus can be detected by GFP expression. This adenoviral construct OBP-401 was successfully used for detection of tumor and lymph node metastasis in subcutaneous human xenograft and orthotopic human rectal cancer mice model. Ren, *et al.,* 2012 developed a Gadolinium based MRI probe using hTERT antisense oligonucleotide and used this sensor for in vivo imaging in mouse xenograft model. A Tc-99m labelled 18-mer antisense oligonucleotide targeting hTERT mRNA was used for SPECT imaging in mice carrying MCF7 breast cancer xenografts (Liu, *et al.*, 2007). The authors reported that the radiolabelled probe is stable at room temperature and tumor can be imaged at 4-8 hours after probe administration.

HALLMARK 4: IMAGING ANGIOGENESIS

Angiogenesis is probably the most critical hallmark for the growth of primary as well as metastatic disease. Cancer cells develop neo vasculature to meet with increasing demands for oxygen and nutrients and to sustain the continuous proliferation. The vasculature is also required to get rid of the metabolic waste and CO_2 that are generated as a result of active metabolism in these cells. The increase in the neo vascularization is usually ascribed to upregulated status of vascular endothelial growth factor and its receptor (VEGF/VEGF-R) signalling in cancer cells. Moreover, during the course of angiogenesis activated endothelial cells express certain integrins like αvb3 that remodels the process of neo vascularization (Hsu *et al.*, 2011). These biomarkers that highlight the various steps of angiogenesis have

been thoroughly studied for their applications for molecular imaging. Currently, molecular probes against VEGF and $\alpha_v b_3$ are in clinical trials and are being considered as detectors to explore tumor biology and monitor therapy response. Some of these probes are discussed below.

The proof of concept study for imaging integrins that are upregulated and over expressed preferentially on activated endothelial cells was first carried out by Gansmo *et al.*, 2006, using breast cancer as model system. In this study, the efficacy and safety of imaging malignant breast tumors using Tc-99m-NC100692, a small peptide with high affinity for integrin receptors were evaluated by scintigraphy. This study showed that this probe can efficiently detect primary tumors along with large lymph node metastases. Axelsson *et al.,* 2010, in a phase 2a clinical study evaluated the feasibility of detecting metastatic lesions in liver, lung, bone, and brain with scintigraphy using the same probe in patients with breast or lung cancer. The outcome of the study suggested that Tc-99m-NC100692 scintigraphy is feasible for detection of lung and brain metastases but not liver and bone lesions. Another promising marker for αvβ3 integrin is the RGD peptide, which has long been identified and through thorough validation reached clinical trials. αvβ3 integrin targeted hybrid Fluorescence Molecular Tomography/X-ray Computed Tomography (FMT-XCT) probe has been used to detect both tumour progression as well as treatment response (Ma *et al.*, 2017). To enhance stability and binding affinity, several modified versions (like cyclic RGD or tetrameric RGD) have been synthesized. A Cu-64 labelled tetrameric RGD peptide was tested in female athymic nude mice bearing the subcutaneous UG87MG glioma xenografts. Non-invasive micro PET studies showed significant tumor uptake and good contrast in the subcutaneous tumor-bearing mice, which agreed well with the bio distribution results (Wu *et al.*, 2005). A novel radiolabelled RGD-based integrin peptide-polymer conjugate, F-18-AH111585 in phase I clinical trial, showed homogeneous distribution kinetics in metastatic breast tumors (Kenny *et al.*, 2008). On the other hand, the αvβ3 along with αvβ5 have been targeted for therapeutic regimen and Cilengitide is the first-of-its-kind cyclized RGD peptide molecule, which acts as an antagonist to integrins has been developed. Although, it has been discontinued in refractory glioblastoma (GBM) patients after phase III (Stupp *et al.*, 2014), it is still being tested in phase I/II trials for non-small cell lung carcinoma (NSCLC) (Manegold *et al.*, 2013) and for head and neck squamous cell carcinoma (HNSCC) (Vermorken *et al.*, 2014).

However, heterogeneous expression of integrins within primary/metastatic tumors, sparse vascularization in tumors and micrometastases (<2-3 mm) pose major limitations to all these approaches. Identification of new biomarkers and molecular imaging strategies for early detection of neo-angiogenesis, kinetics of angiogenesis, vascularization as well as various types of integrin molecules will lead to in depth understanding of this critical hallmark adapted by cancer cells.

HALLMARK 5: IMAGING INVASION AND METASTASIS

From the founding factor of Stephen Paget's "soil & seed" hypothesis (Talmadge *et al.*, 2010) to being touted as one of the hallmarks (Hanahan *et al.*, 2011), metastasis has remained a challenging aspect of cancer both in terms of detection and treatment. It alone causes 90% of the total mortality by cancer (Yilmaz *et al.*, 2007). The pathway leads to the dissemination of micro-metastasis, which ultimately develops; into secondary tumour has orderly and mutually inclusive steps. Broadly speaking, the whole process consists of five episodes, namely, i) growth of primary tumour, ii) invasion into the adjacent tissues and intravasation, iii) carriage by circulatory systems (blood vessels or lymphatic ducts), iv) arrest at the target organ and v) extravasation and proliferation to give rise to secondary tumour mass (Mehlen, *et al.*, 2006).

All the above mentioned individual steps can actually be tapped to assess the development of metastasis through imaging using different tell-tale markers. However, all these steps are interrelated and thus majority of the biomarkers for imaging invasion and metastasis are overlapping with markers for other hallmarks. RGD peptides are used for monitoring angiogenesis as well as epithelial mesenchymal transition (EMT), a crucial step in invasion. Similarly, the gold standard molecular probe for detecting metastasis is F-18-FDG, which is in true sense is a metabolic marker (Youssef, *et al.*, 2012). Till date one direct radioisotope (I-123-MIBG) may be considered as exclusive marker for imaging metastasis. Malignancies of neuroendocrine source bear an inherent challenge of uptake as far as detection and/or prognosis probes are concerned. The best proposition should be some radiolabelled "hot" analogue of naturally accruing agents in neuroendocrine cells like neurotransmitters. C-11-Hydroephidrine (HED) and I-123-MIBG (I-125/I-131 isotopes as well) are two such probes which are analogous to norepinephrine and hence can actively be taken up by the cells and accumulated in the neurosecretory granules (Trampal, *et al.*, 2004 , Rufini, *et al.*, 2006 and Guller, *et al.*, 2006) for both PET and SPECT. A comparative study was done between C-11-HED PET/CT and I-123-MIBG SPECT/CT in 14 patients bearing different tumours of parasympathetic origin, namely, neuroblastomas, pheochromocytomas, ganglioneuroblastoma, and paragangliomas. The study showed better uptake of C-11-HED in osseous lesions and higher accumulation of I-123-MIBG in soft tissue lesions the accumulation is more and in others, it is vice versa. All the metastatic lesions (retrocrural, cervical, and mediastinal) show higher concentration of C-11-HED but in case of I-123-MIBG the uptake is not so significant for retrocrural spread of the malignancy (Figure 2) (Franzius, *et al.*, 2006). Several studies have shown MIBG labelled with either a PET or SPECT tracer can serve as a specific imaging agent for bone metastasis, neuroblastoma (primary and metastatic spread), and other malignant disease. Investigators have also shown that I-131-MIBG has a potential as a drug molecule in refractory neuroblastoma, malignant pheochromocytomas (PHEO) or paragangliomas (PGL) with 67% of the studied cohort displaying drug uptake and retention resulting in either complete remission or partial response or stable disease (Fitzgerald, *et al.*, 2006). Such efficacy of it as a therapeutic candidate as well as a detection probe with high response rate and low nonhematologic toxicity has prompted into pharmaceutical industries to bring about clinical trial for I-123-MIBG as Azedra® by Progenics pharmaceutical® and it has so far progressed into the phase III of the trial (Oh, *et al.*, 2012).
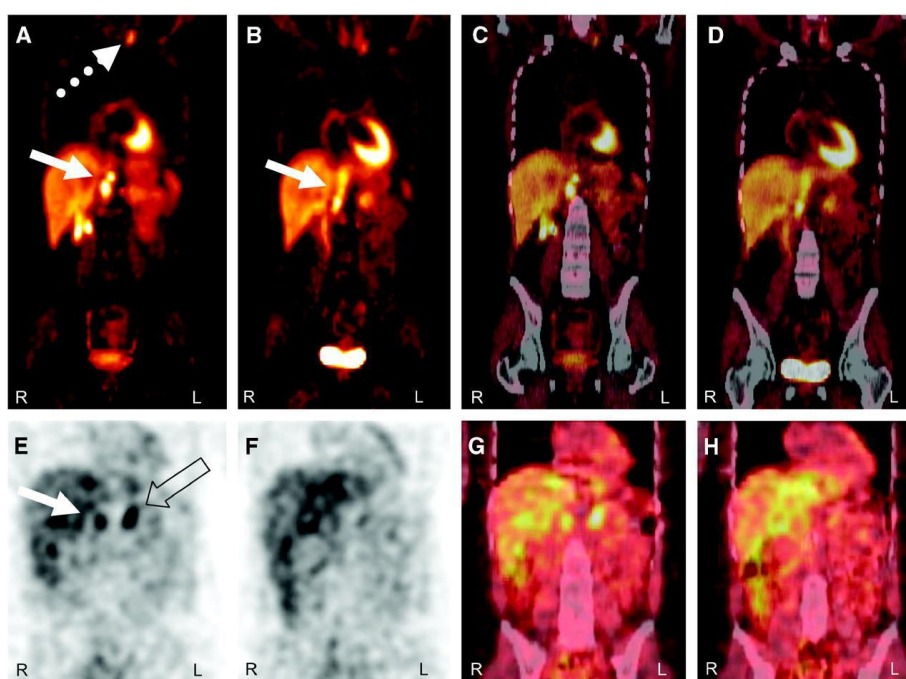
Figure 2: A comparison between I-123-MIBG and C-11-HED probes in pheochromocytoma of adrenal gland.

A 33-y-old woman (patient HA) who had pheochromocytoma of right adrenal gland and resection years ago. C-11-HED PET/CT: (A and B) PET images; (C and D) PET/CT fusion images, 2 levels of coronal slices. There is local relapse (solid arrow) and metastases retrocrural (solid arrow), cervical (dotted arrow), and mediastinal (not shown) with highly increased tracer uptake. C-11-HED uptake of left adrenal gland is very low. I-123-MIBG SPECT/CT: (E and F) SPECT images; (G and H) SPECT/CT fusion images, 2 levels of coronal slices. There is moderately increased tracer uptake in local relapse (solid arrow) and physiologic uptake in left adrenal gland (open arrow). Retrocrural metastases are not visible with increased I-123-MIBG accumulation. Cervical and mediastinal metastases are not within field of view of SPECT. (This research was originally published in JNM. Christiane Franzius et al. J Nucl Med 2006; 47:1635-1642. © by the Society of Nuclear Medicine and Molecular Imaging, Inc).

Another aspect of imaging is the assistance it brings upon during operative procedures which is of immense importance in identifying and mapping metastatic growth in sentinel lymph nodes (SLNs), the primary site next to the tumour for the drainage of lymph. Albeit the standard protocol for such practice is lymphadenectomy and histological evaluation, it lacks precision due to surgical inaccessibility to some regions and manual error (Yang, *et al.*, 2017). Ideally in clinical studies (Mariani, *et al.*, 2002 and Mariani, *et al.*, 2001), the application of technetium-99m radiocolloid followed by a blue stain (for instance, methylene blue) which will make the SLNs appear as blue hot spots in bright field while being detected by gamma probem (Mariani, *et al.*, 2002 and Somasundaram, *et al.*, 2011). However, it carries the inherent problem of poor sensitivity and less spatial resolution. For the achievement of higher spatial and temporal resolution, the application of NIR dyes such as Indocyanine green (ICG) alone or with nanoformulations (e.g. quantum dots) has been used in lymph node navigation intraoperatively for a while now in several cancers like prostate carcinoma, breast carcinoma and bladder cancer to name a few (Mannweiler, *et al.*, 2009, Afshar-Oromieh, *et al.*, 2014, Hirano, *et al.*, 2012 and Koo, *et al.*, 2012). Microbubbles are also very promising candidates in Contrast-enhanced ultrasound imaging (CEUS) technique for preclinical experiments as well as clinical diagnosis (Dewitte, *et al.*, 2015, Sirsi, *et al.*, 2012, Yoon, *et al.*, 2014, Xu, *et al.*, 2014 and Fan, *et al.*, 2014). The advantage of this technique apart from being non-invasiveness, the permeability of microbubble under the field of ultrasound causing perturbation of blood vessel walls imparting better localization and hence resolution giving a possibility of wider application in future.

HALLMARK 6: IMAGING DEREGULATED CANCER CELL METABOLISM

Over the decades, several hallmarks of cancer have been studied, including replicative immortality, resistance to apoptosis, sustained proliferative signalling, surpassing immune check points, metastasis and angiogenesis (Hanahan *et al.*, 2011). However, one of the key aspects of cancer cells remained untouched, "the deregulated cancer cell metabolism" and recently it has been recognized as an emerging hallmark of cancer. To sustain an uncontrolled cell proliferation tumor cells not only require deregulated pathways of cell proliferation but also require and immense battery of cellular metabolism to fuel catabolic and anabolic needs of a constantly proliferating tumor.

As early as in 1930, Otto Warburg described an observation on cancer cell metabolism the "Warburg effect" (Liberti, *et al.*, 2016), which forms the basis of most widely used radio nucleotide imaging modality, F-18-FDG PET, to non-invasively image tumour and metastatic disease and even to follow the

therapy response in many cancers. Recent studies have demonstrated that not only aerobic glycolysis but also other metabolic pathways being deregulated in cancer and are intimately related with genetic heterogeneity as well as tumor microenvironment. The enormous metabolic differences between healthy and tumor cells not only provides a window for therapeutic approach but also an excellent opportunity to develop probes other than F-18-FDG to image cancer progression. Choline has been found to accumulate in certain cancers due to upregulation of the production of choline kinase. ^{11}C acetate demonstrated marked uptake in prostate cancer as opposed to F-18-FDG and is used to image prostate cancer, including metastatic lessions. ^{18}F-fluoroethyltyrosine (F-18-FET) and ^{18}F-dihydroxy-phenyl-alanine (F-18-DOPA), are being used for cancer diagnosis and for monitoring treatment response in tumors that are difficult to image with F-18-FDG, specifically brain tumors. However, the way F-8-FDG received FDA approval and entered into clinical practice, none of the other metabolic tracer achieved that pace. Here we highlight F-18-FDG PET as an important non-invasive imaging modality that utilised cancer metabolism, for better disease management of various cancer types with few examples.

Upregulated aerobic glycolysis is a key feature of many cancers and it fuels the never-ending demand of energy and catabolites to tumor cells. Cancer cells upregulate the expression of glucose transporters GLUT1 and GLUT3 and are known to have significantly higher uptake of glucose compared to normal tissue (Krzeslak, *et al.*, 2012 and Wang, *et al.*, 2016). This forms the basis of F-18-FDG PET. Since 1980s F-18-FDG-PET has evolved as an invaluable tool for tumor diagnosis, staging and therapy response in breast cancer, lung cancer, head and neck cancer, sarcoma, ovarian cancer and many other malignancies. F-18-FDG-PET has high sensitivity but PET imaging lacks the anatomical information. Hence, PET is often combined with CT to have complete visualization of tumor with respect to anatomy. A literature based summarization of 419 articles in year 2001 shows that since the use of FDG PET, the average management change across all applications (Oncology, Neurology and Cardiology) is estimated to be 30%. The average F-18-FDG PET sensitivity and specificity across all oncology applications are estimated to be at 84% and 88% respectively. According to this data analysis, accuracy of F-18-FDG PET ranged 87%–90% (Gambhir, *et al.*, 2001). F-18-FDG PET allows absolute quantification of radioisotope uptake in tumor tissue and hence makes a valuable tool to form a predictive value for overall and disease free survival of patients. Standard uptake value (SUV), SUV mean, SUV_{max}, metabolic tumor volume (MTV) and total lesion glycolysis (TLG) are used as parameters for predictive value of F-18-FDG PET. In a meta-analysis of forty-five head and neck cancer (HNCC) studies, for a total of 2928 patients, quantitative parameters from PET/CT performed at the diagnosis, during treatment and during follow were analysed to predict overall and disease free survival in head and neck cancer. It was observed that in HNCC, MTV was well correlated with overall survival and disease free survival, with a higher predictive value than the SUV. In the same study, they evaluated the predictive value of MTV in 26 studies, with 21 of them also evaluating SUV_{Max} (for a total of 1464 patients). They found that all these studies clearly indicate that MTV/TLG were predictive for clinical outcome, with a higher predictive value than SUV_{max} (Castelli, *et al.*, 2016). In a study of 287 patients with locally advanced HNC treated with radiotherapy with/without chemotherapy, SUV_{max} showed a higher predictive value for recurrence than T stage, N stage, and age. In another meta-analysis of literature comprised of endometrial cancer, consisting of 21 studies, 13 for lymph node metastasis (LNM) and 8 for endometrial cancer recurrence (ECR), it was found that F-18-FDG PET has an overall diagnostic accuracy of 0.88 and 0.93 for LNM and ECR respectively. The study concludes that F-18-FDG PET/CT has an excellent diagnostic value for detecting LNM preoperatively and disease recurrence postoperatively in endometrial cancer patients (Rasmussen, *et al.*, 2015). F-18-FDG PET combined with CT has changed the landscape in lung cancer management. F-18-FDG PET is routinely used to detect nodal and distal metastasis in lung cancer. F-18-FDG PET has improved early detection of lung cancer and SUV_{max} is proven to be good pre-treatment prognostic marker for early stage non-small cell lung carcinoma (NSCLC) (Horne, *et al.*, 2014). However, it is not a

good pre-treatment prognostic marker for stage IV NSCLC (Clausen, *et al.*, 2016). FDG-PET has been proven to be more sensitive in detection of bone metastasis than Tc^{99} bone scintigraphy. A study consisting of 121 patients, F-18-FDG PET detected 42/43 bone metastasis with 97.7% sensitivity, while bone scintigraphy could detect 38/43 bone metastasis with 87.8 sensitivity (Rodrigues, *et al.*, 2016). F-18-FDG PET has become an important tool to detect locally invasive and distal breast metastasis including bone metastases (Walker, *et al.*, 2012, Hoeven, *et al.*, 2004 and Groheux, *et al.*, 2013). However, it has a much lower detection level for early stage disease (below clinical stage IIa) with 2cm or smaller tumors with no palpable nodules. Axillary lymph node imaging with F-18-FDG PET has lower sensitivity than sentinel node imaging (Jeong, *et al.*, 2014 and Cooper, *et al.*, 2011). However in a retrospective study of 240,012 breast cancer patients it was found that women with stage I/II breast cancer who were younger than 40 years were more likely to die of breast cancer than older women (Gnerlich, *et al.*, 2009). In a study of 134 patients, who were staged between I to IIIC (met included) by conventional staging method, it was found that F18-FDG PET/CT detected distant metastases in 17% of asymptomatic stage IIB breast cancer patients younger than 40 years. Upstaging to stage III or IV was observed in 21% patients and unexpected extra-axillary regional nodes were detected in 11% patients (Figure 3) (Riedl, *et al.*, 2014). However, F-18-FDG-avidity, which is uptake of FDG is a characteristic of the individual tumor. Different tumors as well as same tumor exhibit differential FDG uptake, which depends on tumor cell metabolic rate. Prostate and neuroendocrine tumors specifically show low uptake of FDG. Therefore, it is important to evaluate optimal sensitivity of FDG-PET in each cancer type. In a study of 1,093 patients with newly diagnosed Hodgkin disease and non-Hodgkin lymphoma it was found that F-18-FDG avidity was 97% for patients with diffuse large B-cell lymphoma and 95% for patients with follicular lymphoma. However, uptake was less than 83% for small lymphocytic lymphoma and 55% for extra-nodal marginal zone lymphoma (Weiler-Sagie, *et al.*, 2010). In a study of 132 patients with colorectal cancer, correlation between F-18-FDG uptake and presence of EGFR receptor was evaluated in primary lesions. It was found that SUV_{max} was significantly lower in EGFR-negative tumors than in EGFR-expressing tumors (10.0 ± 4.2 *vs.* 12.1 ± 2.1; p=0.012). They also showed that at SUV_{max} threshold of 7.5, the sensitivity and specificity for predicting EGFR expression were 84.9% and 40.4%, respectively (Choi, *et al.*, 2016). FDG-PET imaging continues to benefit understanding of cancer biology and better disease management in field of cancer biology.
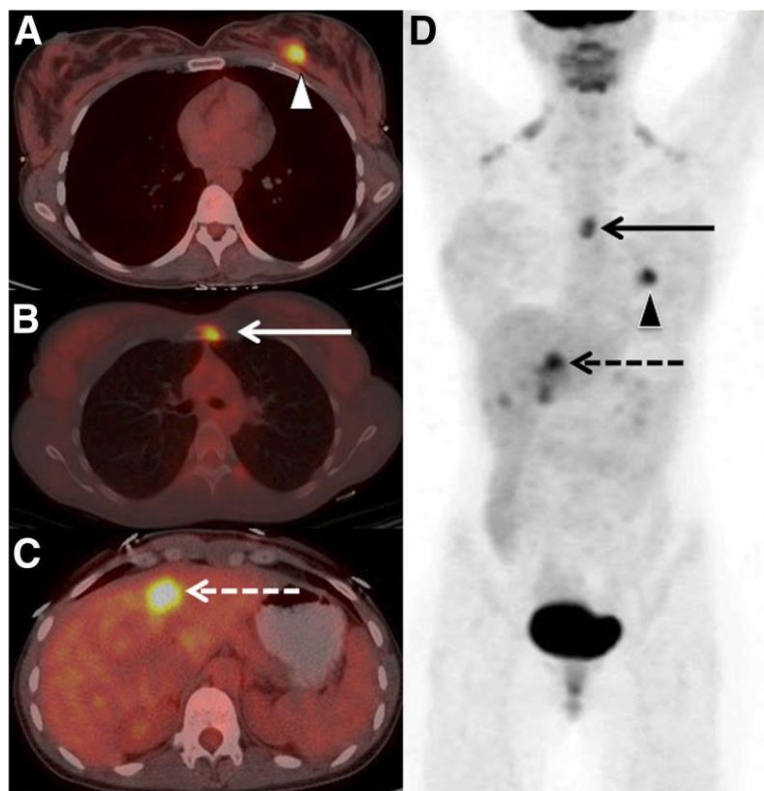
Figure 3: A 29-y-old woman with clinical stage IIA breast cancer was upstaged to stage IV by F-18-FDG PET/CT.

Axial PET/CT images showed a known primary left breast cancer (arrowhead) (A), previously unknown osseous metastasis (solid arrow) (B), and previously unknown liver metastasis (dashed arrow) (C) proven by biopsy. (D) Maximum-intensity-projection PET gives overview of all lesions. (This research was originally published in JNM. Riedl C.C. et al. J Nucl Med, 2014. 55(10): p. 1578-83. © by the Society of Nuclear Medicine and Molecular Imaging, Inc.).

HALLMARK 7: IMAGING IMMUNOTHERAPY

Immune surveillance protects our body from foreign intruders such as bacteria and viruses and domestic miscreants like cancer or precancerous cells. Though this scrutiny by immune cells helps to reduce cancer cell burden, tumors still grow by editing the immune system and utilizing cytokines for proliferation. Thus, immune evasion has been recognized as a crucial hallmark of cancer. Distinct mechanisms towards fighting the immune attacks adapted by the cancer cells have been identified. The major ones are T-cell immune checkpoint inhibition, downregulation of MHC I complex, secreting immunosuppressive factors (TGF-b, IL-10) and presentation of antigens which are less likely to be recognized by immune cells (Juergens, *et al.*, 2016). These approaches actively modulate immune cell response, immune cell behaviour allowing the cancer cells to proliferate in disguise.

Since 2000, several strategies have been developed to actively modulate the adaptive response of immune system to kill the cancer cells, which attempt to evade immune attacks. These strategies collectively known as Active cancer Immunotherapy showed promising results in melanoma, pancreatic, glioblastoma. Active immunotherapy is different from passive immunotherapy where introduction of specific antibody (VEGF-R mAb or Her2-Neu mAb) leads to antitumor effects and does not require

activation of immune system. Currently used active immunotherapy involves recombinant cytokines, bio chemotherapy, vaccinations, and immunomodulatory monoclonal antibodies (Aarntzen, *et al.*, 2013).

The classic examples of recombinant cytokines are FDA approved IL-2 and IFN-gamma which are being used for treatment of melanoma, renal cell carcinoma, lymphoma, chronic myelogenous leukemia, and Kaposi sarcoma (Aarntzen, *et al.*, 2013 and Liu, *et al.*, 2014). IL-2 and IFN-gamma stimulate T-cell proliferation and function, augment natural killer cell activity, and trigger the release of additional pro-inflammatory cytokines from activated lymphocytes. Biochemotherapy includes combinatorial treatment of cytotoxic drugs (such as Temozolomide, cisplatin etc) and recombinant cytokines (IL-2 or IFN-a). Cancer cell damage by cytotoxic agents exposes larger number of tumor antigens for detection by immune system. These along with immune response generated by cytokines improve the effectivity of treatment. Cancer vaccines are used to trigger an active immune response against the cancer cells. Other than FDA approved cancer vaccine for metastatic prostate cancer, such vaccines are still in testing phase for glioblastoma, breast cancer, melanoma and few other cancer types. The last but the most promising one is immunomodulatory therapy, which acts on the immune checkpoints and thereby regulates T-cell activation. Ipilimumab (CTAL-4 MaB), Nivolumab (PD1/PD1-LIGAND) already showed promising effect in metastatic and unresectable melanoma and are under clinical trial with non-small cell lung cancer & gastric cancer where standard chemotherapy failed (Liu, *et al.*, 2014).

As more and more evidence of effective immunotherapy treatment in recurrent or metastatic cancers are emerging, the need of imaging evaluation of response is gaining importance. Response to traditional cytotoxic, radiation or surgical resection treatment is usually measured by reduction in tumor size and absence of new tumor emergence as per WHO guidelines or RECIST criteria. In contrast, immunotherapy shows a delayed response with disease stability, transient enlargement of tumor volume and sometimes with emergence of new tumors followed by tumor shrinkage and/or long term stability of tumor size (Kwak, *et al.*, 2015).Thus appropriate follow-up imaging is important to identify potential treatment benefits of cancer immunotherapy. It is recommended that imaging assessment of treatment response or disease progression after completion of treatment should be made with two consecutive follow-up imaging studies performed at least 4 weeks apart.

Majority of clinical imaging techniques like PET, SPECT, MR in combination with CT can be used for imaging immunotherapy. 18F-FDG has been extensively used for measuring efficacy of immunotherapy. However, 18F-FDG is a metabolic probe and care should be taken while scheduling the image sequence and during interpretation as inflammation caused by biochemotherapy or the other immunotherapy leads to high uptake of 18F-FDG. In a true sense, probes exclusive to immunotherapy are still in preclinical phase. These emerging probes are antibodies generated against CD4, CD8, CD11b, PD-1/PD-L1, CTAL-4, B7-H3 (CD276), which are radiolabelled and validated in tumor xenograft models. However, majority of these probes are yet to be validated in a proper mouse model of immunotherapy. Such models are important to develop for assessing toxicity of the probes, percent uptake in other organs as well as efficacy of the treatments. Immune checkpoint inhibitors PD-1/PD-L1 and CTAL-4 have shown promising response for several tumors such as melanoma, renal cancer, glioblastoma and hepatocellular carcinoma where standard cytotoxic or radiation therapy failed or showed minimal effect (Aarntzen, *et al.*, 2013). Multicentre clinical trials that use anti-PD-L1 antibody for treatment of non–small cell lung cancer, melanoma, and colorectal, renal cell, ovarian, breast, pancreatic, and gastric cancers are under way (Chen, *et al.*, 2015).

Recently, Natarajan, *et al.*, 2015 labelled an anti-mouse PD-1 mAb with 64-Copper and successfully validated in a Treg+-transgenic mouse model (Tregs express bioluminescence reporter) bearing

melanoma tumors. Specific uptake of labelled probe was seen in tumor and spleen by microPET imaging along with homing of Tregs to tumor by optical imaging. This proof of principle study showed non-invasive imaging of PD-1 expression is possible and associated with localized Treg cells (Natarajan, *et al.*, 2015). The high background signal post 48 hrs injection of the tracer limits the use of the full length antibody developed against PD-1. To circumvent the issue, Motou *et al.,* 2015 developed an engineered PD-1 ectodomain (HAC-PD1) as a high affinity competitive binder of PD-L1 through yeast surface display technique. This probe showed superior and specific uptake in PD-L1 expressing tumors by microPET imaging and resulted excellent anti-tumor efficacy in syngeneic CT26 tumor model (Rashidian, *et al.*, 2015).

Immune checkpoint inhibitors are showing excellent translational promise in cancer treatment. However, successful targeting of these blockers (likePD-1 or CTAL-4) requires high or optimal expression of PD-1 ligands by the tumor cells. It is therefore crucial to evaluate expression of PD-L1 in tumor tissues to identify patients who would be beneficial availing these therapies. Currently immunohistochemistry is the only tool to determine the level of PD-1/PD-L1 that requires invasive biopsy, inadequate sampling of the highly heterogeneous primary tumor and lack of information about expression profiles of metastatic lesions. Molecular imaging provides an ideal alternative that allows for non-invasive, dynamic monitoring and overall evaluation of checkpoint receptor expression.

CONCLUDING REMARKS

Non-invasive imaging of cancer either using inherent properties of tissue or by use of radiolabelled probes such as F-18-FDG, small peptides and antibodies is having a huge impact on disease management. Though initially started as tumor detection and staging method, non-invasive imaging has evolved beyond and is playing key role in enabling molecular imaging of various hallmarks of cancer. Imaging metabolic output, apoptosis, angiogenesis, metastasis and proliferation has improved our understating of therapy response in patients and has further helped in categorizing patients for different therapeutic regimes leading to better disease management. While most imaging probes enable imaging of single hallmark of cancer, F-18-FDG PET imaging enables detection of both primary and metastatic disease making it an important probe for both metabolic and metastasis imaging. Similar is the radiolabelled RGD-peptide which is often used to image both tumor angiogenesis and invasion. More research is needed to develop new probes for imaging one or more hallmarks of cancer simultaneously and more precisely for better understanding of tumor biology. Full potential of non-invasive modalities is yet to be achieved, with improvement in probes, algorithms, instrument sensitivity, and simplicity in use. Imaging cancer hallmarks using non-invasive modalities continues to fill link between genetic information to phenotypic observation of disease and has emerged an invaluable tool in era of precision medicine in fight against cancer.

REFERENCES

1. Aarntzen, EH; Srinivas, M; Radu, CG; Punt, CJ; Boerman, OC; Figdor, CG; Oyen, JG and de Vries, IJM (2013) "In vivo imaging of therapy-induced anti-cancer immune responses in humans", *Cell Mol Life Sci*, 70(13): 2237-2257.
2. Afshar-Oromieh, A; Zechmann, CM; Malcher, A; Eder, M; Eisenhut, M; Linhart, HG; Holland-Letz, T and Haberkorn, U (2014) "Comparison of PET imaging with a 68Ga-labelled PSMA ligand and 18F-choline-based PET/CT for the diagnosis of recurrent prostate cancer", *Eur J Nucl Med Mol Imaging*, 41(1): 11-20.

3. Axelsson, R; Bach-Gansmo, T; Castell-Conesa, J and McParland, BJ (2010) "An open-label, multicenter, phase 2a study to assess the feasibility of imaging metastases in late-stage cancer patients with the αv β 3-selective angiogenesis imaging agent 99mTc-NC100692", *Acta Radiologica*, 51(1): 40-46.

4. Bach-Gansmo, T; Danielsson, R; Saracco, A; Wilczek, B; Bogsrud, TV; Fangberget, A and Tobin, D (2006) "Integrin receptor imaging of breast cancer: a proof-of-concept study to evaluate 99mTc-NC100692", *J Nucl Med*, 47(9): 1434-1439.

5. Belhocine, T; Steinmetz, N; Hustinx, R; Bartsch, P; Jerusalem, G; Seidel, L and Green, A (2002) "Increased uptake of the apoptosis-imaging agent (99m) Tc recombinant human Annexin V in human tumors after one course of chemotherapy as a predictor of tumor response and patient prognosis", *Clin Cancer Res*, 8(9): 2766-2774.

6. Blankenberg, FG; Katsikis, PD; Tait, JF; Davis, RE; Naumovski, L; Ohtsuki, K and Maecker, HT (1998) "In vivo detection and imaging of phosphatidylserine expression during programmed cell death", *Proc Natl Acad Sci*, 95(11): 6349-6354.

7. Buck, AK; Halter, G; Schirrmeister, H; Kotzerke, J; Wurziger, I; Glatting, G and Hetzel, M (2003) "Imaging proliferation in lung tumors with PET: 18F-FLT versus 18F-FDG", *J Nucl Med*, 44(9): 1426-1431.

8. Castelli, J; De Bari, B; Depeursinge, A; Simon, A; Devillers, A; Jimenez, GR and Bourhis, J (2016) "Overview of the predictive value of quantitative 18 FDG PET in head and neck cancer treated with chemoradiotherapy", *Crit Rev Oncol Hematol*, 108: 40-51.

9. Chalkidou, A; Landau, DB; Odell, EW; Cornelius, VR; O'Doherty, MJ and Marsden, PK (2012) "Correlation between Ki-67 immunohistochemistry and 18F-fluorothymidine uptake in patients with cancer: A systematic review and meta-analysis", *Eur J Cancer*, 48(18): 3499-3513.

10. Chen, L and Han, X (2015) "Anti-PD-1/PD-L1 therapy of human cancer: past, present, and future*", J Clin Invest*, 125(9): 3384-3391.

11. Chen, W; Cloughesy, T; Kamdar, N; Satyamurthy, N; Bergsneider, M; Liau, L and Silverman, DH (2005) "Imaging proliferation in brain tumors with 18F-FLT PET: comparison with 18F-FDG", *J Nucl Med*, 46(6): 945-952.

12. Choi, YJ; Kim, MJ; Lee, BH; Kwon, MJ and Hwang, HS (2016) "Relationship between Preoperative 18F-Fluorodeoxyglucose Uptake and Epidermal Growth Factor Receptor Status in Primary Colorectal Cancer", *Yonsei Med J*, 57(1): 232-237.

13. Clausen, M; Brittain, J; Loft, A; Langer, S and Kjaer, A (2016) "Pretreatment FDG-PET SUVmax is not a prognostic marker in stage IV non-small cell lung cancer", *J Nucl Med*, 57(supplement 2): 191-191.

14. Cooper, KL; Harnan, S; Meng, Y; Ward, SE; Fitzgerald, P; Papaioannou, D and Lorenz, E (2011) "Positron emission tomography (PET) for assessment of axillary lymph node status in early breast cancer: A systematic review and meta-analysis", *Eur J Surg Oncol*, 37(3): 187-198.

15. Dewitte, H; Vanderperren, K; Haers, H; Stock, E; Duchateau, L; Hesta, M and Lentacker, I (2015) "Theranostic mRNA-loaded microbubbles in the lymphatics of dogs: implications for drug delivery", *Theranostics*, 5(1): 97-109.

16. Dijkers, EC; Oude Munnink, TH; Kosterink, JG; Brouwers, AH; Jager, PL; de Jong, JR; van Dongen, GA; Schröder, CP; Lub-de Hooge, MN and Vries EG (2010) "Biodistribution of 89Zr-trastuzumab and PET imaging of HER2-positive lesions in patients with metastatic breast cancer", *Clin Pharmacol Ther*, 87, 586-592.

17. Fan, CH; Lin, WH; Ting, CY; Chai, WY; Yen, TC; Liu, HL and Yeh, CK (2014) "Contrast-enhanced ultrasound imaging for the detection of focused ultrasound-induced blood-brain barrier opening", *Theranostics*, 4(10): 1014-1025.

18. Fitzgerald, PA; Goldsby, RE; Huberty, JP; Price, DC; Hawkins, RA; Veatch, JJ and Matthay, KK (2006) "Malignant Pheochromocytomas and Paragangliomas", *Ann N Y Acad Sci*, 1073(1): 465-490.

19. Franzius, C; Hermann, K; Weckesser, M; Kopka, K; Juergens, KU; Vormoor, J and Schober, O (2006) "Whole-body PET/CT with 11C-meta-hydroxyephedrine in tumors of the sympathetic nervous system: feasibility study and comparison with 123I-MIBG SPECT/CT", *J Nucl Med*, 47(10): 1635-1642.

20. Gambhir, SS; Czernin, J; Schwimmer, J; Silverman, DH; Coleman, RE and Phelps, ME (2001) "A tabulated summary of the FDG PET literature", *J Nucl Med*, 42(5 Suppl): 1S-93S.

21. Gnerlich, JL; Deshpande, AD; Jeffe, DB; Sweet, A; White, N and Margenthaler, JA (2009) "Elevated breast cancer mortality in women younger than age 40 years compared with older women is attributed to poorer survival in early-stage disease", *J Am Coll Surg*, 208(3): 341-347.

22. Groheux, D; Giacchetti, S; Delord, M; Hindié, E; Vercellino, L; Cuvier, C and Espié, M (2013) "18F-FDG PET/CT in staging patients with locally advanced or inflammatory breast cancer: comparison to conventional staging", *J Nucl Med*, 54(1): 5-11.

23. Guller, U; Turek, J; Eubanks, S; Delong, ER; Oertli, D and Feldman, JM (2006) "Detecting pheochromocytoma: defining the most sensitive test", *Ann Surg*, 243(1): 102-107.

24. Haas, RL; de Jong, D; Olmos, RAV; Hoefnagel, CA; van Den Heuvel, I; Zerp, SF and Verheij, M (2004) "In vivo imaging of radiation-induced apoptosis in follicular lymphoma patients*", Int J Radiat Oncol Biol Phys*, 59(3): 782-787.

25. Hanahan, D and Weinberg, RA (2000), "The hallmarks of cancer", *Cell*, 100, 57-70.

26. Hanahan, D and Weinberg, RA (2011), "Hallmarks of cancer: the next generation", *Cell*, 144, 646-674.

27. Hanahan, D and Weinberg, RA (2011) "Hallmarks of cancer: the next generation", *Cell*, 144(5): 646-674.

28. Heskamp, S; van Laarhoven, H.W; Molkenboer-Kuenen, J.D; Franssen, G.M; Versleijen-Jonkers, Y.M; Oyen, W.J; van der Graaf, W.Tand Boerman, O.C; (2010), "ImmunoSPECT and immunoPET of IGF-1R expression with the radiolabeled antibody R1507 in a triple-negative breast cancer model", *J Nucl Med*, 51: 1565-1572.

29. Hirano, A; Kamimura, M; Ogura, K; Kim, N; Hattori, A; Setoguchi, Y and Fujibayashi, M (2012) "A comparison of indocyanine green fluorescence imaging plus blue dye and blue dye alone for sentinel node navigation surgery in breast cancer patients", **Ann surg oncol**, 19(13): 4112-4116.

30. Van Der Hoeven, JJ; Krak, NC; Hoekstra, OS; Comans, EF; Boom, RP; Van Geldere, D and Teule, GJ (2004) "18F-2-Fluoro-2-Deoxy-d-Glucose Positron Emission Tomography in Staging of Locally Advanced Breast Cancer", J Clin Oncol, 22(7): 1253-1259.

31. Höglund, J; Shirvan, A; Antoni, G; Gustavsson, SÅ; Långström, B; Ringheim, A and Ziv, I (2011) "18F-ML-10, a PET tracer for apoptosis: first human study", *J Nucl Med*, 52(5): 720-725.

32. Horne, ZD; Clump, DA; Vargo, JA; Shah, S; Beriwal, S; Burton, SA and Luketich, JD (2014) "Pretreatment SUVmax predicts progression-free survival in early-stage non-small cell lung cancer treated with stereotactic body radiation therapy", *Radiat Oncol*, 9: 41.

33. Hsu, A.R; et al; "Integrin αvβ3 antagonists for anti-angiogenic cancer treatment", Recent patents on anti-cancer drug discovery, 20072(2): 143-158.

34. Iqbal, N and Iqbal, N (2014), "Human Epidermal Growth Factor Receptor 2 (HER2) in Cancers: Overexpression and Therapeutic Implications", *Mol Biol Int*, 852748.

35. Jeong, YJ; Kang, DY; Yoon, HJ and Son, HJ (2014) "Additional value of F-18 FDG PET/CT for initial staging in breast cancer with clinically negative axillary nodes", *Breast Cancer Res Treat*, 145(1): 137-142.

36. Juergens, RA; Zukotynski, KA; Singnurkar, A; Snider, DP; Valliant, JF and Gulenchyn, KY (2016) "Imaging Biomarkers in Immunotherapy", ***Biomark Cancer***, 8(Suppl 2): 1-13.

37. Kartachova, MS; Olmos, RAV; Haas, RL; Hoebers, FJ; van Herk, M and Verheij, M (2008) "99mTc-HYNIC-rh-annexin-V scintigraphy: visual and quantitative evaluation of early treatment-induced apoptosis to predict treatment outcome", ***Nucl Med Commun***, 29(1): 39-44.

38. Kartachova, M; van Zandwijk, N; Burgers, S; van Tinteren, H; Verheij, M and Valdés Olmos, RA (2007) "Prognostic significance of 99mTc Hynic-rh-annexin V scintigraphy during platinum-based chemotherapy in advanced lung cancer", ***J Clin Oncol***, 25(18): 2534-2539.

39. Kenny, LM; Coombes, RC; Oulie, I; Contractor, KB; Miller, M; Spinks, TJ and Osman, S (2008) "Phase I trial of the positron-emitting Arg-Gly-Asp (RGD) peptide radioligand 18F-AH111585 in breast cancer patients", ***J Nucl Med***, 49(6): 879-886.

40. Kenny, LM; Vigushin, DM; Al-Nahhas, A; Osman, S; Luthra, SK; Shousha, S and Aboagye, EO (2005) "Quantification of cellular proliferation in tumor and normal tissues of patients with breast cancer by [18F] fluorothymidine-positron emission tomography imaging: evaluation of analytical methods", ***Cancer Res***, 65(21): 10104-10112.

41. Kishimoto, H; Kojima, T; Watanabe, Y; Kagawa, S; Fujiwara, T; Uno, F and Urata, Y (2006) "In vivo imaging of lymph node metastasis with telomerase-specific replication-selective adenovirus", ***Nat Med***, 12(10): 1213-9.

42. Koo, J; Jeon, M; Oh, Y; Kang, HW; Kim, J; Kim, C and Oh, J (2012) "In vivo non-ionizing photoacoustic mapping of sentinel lymph nodes and bladders with ICG-enhanced carbon nanotubes", ***Phys Med Biol***, 57(23): 7853.

43. Koopman, G; Reutelingsperger, CP; Kuijten, GA; Keehnen, RM; Pals, ST and Van Oers, MH (1994) "Annexin V for flow cytometric detection of phosphatidylserine expression on B cells undergoing apoptosis", ***Blood***, 84(5): 1415-1420.

44. Krzeslak, A; Wojcik-Krowiranda, K; Forma, E; Jozwiak, P; Romanowicz, H; Bienkiewicz, A and Brys, M (2012) "Expression of GLUT1 and GLUT3 glucose transporters in endometrial and breast cancers", ***Pathol Oncol Res***, 18(3): 721-728.

45. Kurihara, H; Yang, DJ; Cristofanilli, M; Erwin, WD; Yu, DF; Kohanim, S and Kim, EE (2008) "Imaging and dosimetry of 99mTc EC annexin V: preliminary clinical study targeting apoptosis in breast tumors", ***Appl Radiat Isot***, 66(9): 1175-1182.

46. Kurihara, H; Yang, DJ; Cristofanilli, M; Erwin, WD; Yu, DF; Kohanim, S and Kim, EE (2008) "Cancer immunotherapy: imaging assessment of novel treatment response patterns and immune-related adverse events", ***Radiographics***, 35(2): 424-437.

47. Lahorte, CMM; Van De Wiele, C; Bacher, K; Van Den Bossche, B; Thierens, H; Van Belle, S and Dierckx, RA (2003) "Biodistribution and dosimetry study of 123I-rh-annexin V in mice and humans", ***Nucl Med Commun***, 24(8): 871-880.

48. Liberti, M.Vand Locasale JW (2016) "The Warburg Effect: How Does it Benefit Cancer Cells?",***Trends Biochem Sci***, 41(3): 211-218.

49. Liu, M; Wang, RF; Zhang, CL; Yan, P; Yu, MM; Di, LJ and Guo, FQ (2007) "Noninvasive imaging of human telomerase reverse transcriptase (hTERT) messenger RNA with 99mTc-radiolabeled antisense probes in malignant tumors", ***J Nucl Med***, 48(12): 2028-2036.

50. Liu, Z and Li, Z (2014) "Molecular imaging in tracking tumor-specific cytotoxic T lymphocytes (CTLs)", ***Theranostics***, 4(10): 990-1001.

51. Ma, X; Van, VP; Kimm, MA; Prakash, J; Kessler, H; Kosanke, K and Eisenblätter, M (2017) "Integrin-Targeted hybrid Fluorescence Molecular Tomography/X-ray Computed Tomography for Imaging Tumor Progression and Early Response in Non-Small Cell Lung Cancer", ***Neoplasia***, 19(1): 8-16.

52. Mandl, SJ; Mari, C; Edinger, M; Negrin, RS; Tait, JF; Contag, CH and Blankenberg, FG (2004) "Multi-modality imaging identifies key times for annexin V imaging as an early predictor of therapeutic outcome", *Mol Imaging*, 3(1): 1-8.

53. Manegold, C; Vansteenkiste, J; Cardenal, F; Schuette, W; Woll, PJ; Ulsperger, E and von Pawel, J (2013) "Randomized phase II study of three doses of the integrin inhibitor cilengitide versus docetaxel as second-line treatment for patients with advanced non-small-cell lung cancer", *Invest New Drugs*, 31(1): 175-182.

54. Mannweiler, S; Amersdorfer, P; Trajanoski, S; Terrett, JA; King, D and Mehes, G (2009) "Heterogeneity of prostate-specific membrane antigen (PSMA) expression in prostate carcinoma with distant metastasis", *Path Oncol Res*, 15(2): 167-172.

55. Mariani, G; Moresco, L; Viale, G; Villa, G; Bagnasco, M; Canavese, G and Paganelli, G (2001) "Radioguided sentinel lymph node biopsy in breast cancer surgery", *J Nucl Med*, 42(8): 1198-1215.

56. Mariani, G; Gipponi, M; Moresco, L; Villa, G; Bartolomei, M; Mazzarol, G and Strauss, HW (2002) "Radioguided sentinel lymph node biopsy in malignant cutaneous melanoma", *J Nucl Med*, 200243(6): 811-827.

57. Martini, M; De Santis, MC; Braccini, L; Gulluni, F and Hirsch, E (2014), "PI3K/AKT signaling pathway and cancer: an updated review", *Ann Med*, 46, 372-383.

58. McLarty, K, Cornelissen, B, Scollard, DA, Done, SJ, Chun, K, and Reilly, RM (2009), "Associations between the uptake of 111In-DTPA-trastuzumab, HER2 density and response to trastuzumab (Herceptin) in athymic mice bearing subcutaneous human tumour xenografts", *Eur J Nucl Med Mol Imaging*, 36, 81-93.

59. Mehlen, P and Puisieux, A (2006) "Metastasis: a question of life or death", *Nat Rev Cancer*, 6(6): 449-458.

60. Memon, AA; Weber, B; Winterdahl, M; Jakobsen, S; Meldgaard, P; Madsen, HHT and Sorensen, BS (2011) "PET imaging of patients with non-small cell lung cancer employing an EGF receptor targeting drug as tracer", *Br J Cancer*, 105(12): 1850-1855.

61. Meng, X; Loo, BW; Ma, L; Murphy, JD; Sun, X and Yu, J (2011) "Molecular imaging with 11C-PD153035 PET/CT predicts survival in non-small cell lung cancer treated with EGFR-TKI: a pilot study", *J Nucl Med*, 52(10): 1573-1579.

62. Menke-van der Houven, C.W; et al; "89Zr-cetuximab PET imaging in patients with advanced colorectal cancer", *Oncotarget*, 6(30): 30384.

63. Natarajan, A; Mayer, AT; Xu, L; Reeves, RE; Gano, J and Gambhir, SS (2015) "Novel Radiotracer for ImmunoPET Imaging of PD-1 Checkpoint Expression on Tumor Infiltrating Lymphocytes", *Bioconjug Chem*, 26(10): 2062-2069.

64. Oh, DY; Kim, TW; Park, YS; Shin, SJ; Shin, SH; Song, EK and Bang, YJ (2012) "Phase 2 study of everolimus monotherapy in patients with nonfunctioning neuroendocrine tumors or pheochromocytomas/paragangliomas", *Cancer*, 118(24): 6162-6170.

65. Penberthy, KK and Ravichandran, KS (2016) "Apoptotic cell recognition receptors and scavenger receptors", *Immunol Rev*, 269(1): 44-59.

66. Rashidian, M; Keliher, EJ; Bilate, AM; Duarte, JN; Wojtkiewicz, GR; Jacobsen, JT and Ploegh, HL (2015) "Noninvasive imaging of immune responses", *Proc Natl Acad Sci*, 112(19): 6146-6151.

67. Rasmussen, JH; Vogelius, IR; Fischer, BM; Friborg, J; Aznar, MC; Persson, GF and Specht, L (2015) "Prognostic value of 18F-fludeoxyglucose uptake in 287 patients with head and neck squamous cell carcinoma", *Head Neck*, 37(9): 1274-1281.

68. Ren, BX; Yang, F; Zhu, GH; Huang, ZX; Ai, H; Xia, R and Song, B (2012) "Magnetic resonance tumor targeting imaging using gadolinium labeled human telomerase reverse transcriptase antisense probes", *Cancer Sci*, 103(8): 1434-1439.

69. Riedl, CC; Slobod, E; Jochelson, M; Morrow, M; Goldman, DA; Gonen, M and Ulaner, GA (2014) "Retrospective analysis of 18F-FDG PET/CT for staging asymptomatic breast cancer patients younger than 40 years*", J Nucl Med*, 55(10): 1578-1583.

70. Rodrigues, M; Stark, H; Rendl, G; Rettenbacher, L; Datz, L; Studnicka, M and Pirich, C (2016) "Diagnostic performance of [18F] FDG PET-CT compared to bone scintigraphy for the detection of bone metastases in lung cancer patients", *Q J Nucl Med Mol Imaging*, 60(1): 62-68.

71. Rufini, V; M.LCalcagni, and R.PBaum (2006) "Imaging of neuroendocrine tumors in Seminars in nuclear medicine", *Elsevier*.

72. Sirsi, SR and Borden, MA (2012) "Advances in ultrasound mediated gene therapy using microbubble contrast agents", *Theranostics*, 2(12): 1208-1222.

73. Smith, BA and Smith, BD (2012) "Biomarkers and molecular probes for cell death imaging and targeted therapeutics", *Bioconjug Chem,* 23(10): 1989-2006.

74. Somasundaram, SK; Ironside, A; McCarthy, R; Davison, S; Davidson, T; McDermott, N and Keshtgar, M (2011) "RT–PCR technique for the intra-operative assessment of breast sentinel lymph nodes–Is this the way forward? ", *J Solid Tumors*, 1(2): 56.

75. Stupp, R; Hegi, ME; Gorlia, T; Erridge, SC; Perry, J; Hong, YK and Steinbach, JP (2014) "Cilengitide combined with standard treatment for patients with newly diagnosed glioblastoma with methylated MGMT promoter (CENTRIC EORTC 26071-22072 study): a multicentre, randomised, open-label, phase 3 trial", *The lancet oncology*, 15(10): 1100-1108.

76. Talmadge, JE and Fidler, IJ (2010) "AACR Centennial Series: The Biology of Cancer Metastasis: Historical Perspective", *Cancer Res*, 70(14): 5649-5669.

77. Trampal, C; Engler, H; Juhlin, C; Bergström, M and Långström, B (2004) "Pheochromocytomas: Detection with 11C Hydroxyephedrine PET 1", *Radiology*, 230(2): 423-428.

78. Van de Wiele, C; Lahorte, C; Vermeersch, H; Loose, D; Mervillie, K; Steinmetz, ND and Dierck, RA (2003) "Quantitative tumor apoptosis imaging using technetium-99m-HYNIC annexin V single photon emission computed tomography", *J Clin Oncol*, 21(18): 3483-3487.

79. Vermeersch, H; Loose, D; Lahorte, C; Mervillie, K; Dierckx, R; Steinmetz, N and Van de Wiele, C (2004) "99mTc-HYNIC Annexin-V imaging of primary head and neck carcinoma", *Nucl Med Commun*, 25(3): 259-263.

80. Vermorken, JB; Peyrade, F; Krauss, J; Mesia, R; Remenar, E; Gauler, TC and Brümmendorf, TH (2014) "Cisplatin, 5-fluorouracil, and cetuximab (PFE) with or without cilengitide in recurrent/metastatic squamous cell carcinoma of the head and neck: results of the randomized phase I/II ADVANTAGE trial (phase II part) ", *Ann Ooncology*, 25(3): 682-688.

81. Walker, GV; Niikura, N; Yang, W; Rohren, E; Valero, V; Woodward, WA and Buchholz, TA (2012) "Pretreatment Staging Positron Emission Tomography/Computed Tomography in Patients With Inflammatory Breast Cancer Influences Radiation Treatment Field Designs", *Int J Radiat Oncol Biol Phys*, 83(5): 1381-1386.

82. Wang, H; Yu, J; Yang, G; Song, X; Sun, X; Zhao, S and Mu, D (2007), "Assessment of 11C-labeled- 4- N- (3- bromoanilino)- 6, 7- dimethoxyquinazoline as a positron emission tomography agent to monitor epidermal growth factor receptor expression", *Cancer Sci*, 98(9): 1413-1416.

83. Wang, ZG; Yu, MM; Han, Y; Wu, FY; Yang, GJ; Li, DC and Liu, SM (2016) "Correlation of Glut-1 and Glut-3 expression with F-18 FDG uptake in pulmonary inflammatory lesions", *Medicine (Baltimore),* 95(48): e5462.

84. Weiler-Sagie, M; Bushelev, O; Epelbaum, R; Dann, EJ; Haim, N; Avivi, I and Israel, O (2010) "(18)F-FDG avidity in lymphoma readdressed: a study of 766 patients", *J Nucl Med*, 201051(1): 25-30.

85. Woolf, DK; Beresford, M; Li, SP; Dowsett, M; Sanghera, B; Wong, WL and Miles, D (2014) "Evaluation of FLT-PET-CT as an imaging biomarker of proliferation in primary breast cancer", *Br J Cancer*, 110(12): 2847-2854.

86. Wu, Y; Zhang, X; Xiong, Z; Cheng, Z; Fisher, DR; Liu, S and Chen, X (2005) "MicroPET imaging of glioma integrin αvβ3 expression using 64Cu-labeled tetrameric RGD peptide", *J Nucl Med*, 46(10): 1707-1718.

87. Xu, PL; Zheng, Y; Song, L and Zhou, X (2014) "India ink incorporated multifunctional phase-transition nanodroplets for photoacoustic/ultrasound dual-modality imaging and photoacoustic effect based tumor therapy".

88. Yang, X; Wang, Z; Zhang, F; Zhu, G; Song, J; Teng, GJ and Chen, X (2017) "Mapping Sentinel Lymph Node Metastasis by Dual-probe Optical Imaging", *Theranostics*, 7(1): 153.

89. Yilmaz, M; Christofori, G and Lehembre, F (2007) "Distinct mechanisms of tumor invasion and metastasis", *Trends Mol Med*, 13(12): 535-541.

90. Yoon, YI; Kwon, YS; Cho, HS; Heo, SH; Park, KS; Park, SG and Ahn, GJ (2014) "Ultrasound-mediated gene and drug delivery using a microbubble-liposome particle system", *Theranostics*, 20144(11): 1133-44.

91. Youssef, G; Leung, E; Mylonas, I; Nery, P; Williams, K; Wisenberg, G and Beanlands, RS (2012) "The use of 18F-FDG PET in the diagnosis of cardiac sarcoidosis: a systematic review and metaanalysis including the Ontario experience", *J Nucl Med*, 53(2): 241-248.

92. Zhao, M; Zhu, X; Ji, S; Zhou, J; Ozker, KS; Fang, W and Hellman, RS (2006) "99mTc-labeled C2A domain of synaptotagmin I as a target-specific molecular probe for noninvasive imaging of acute myocardial infarction", *J Nucl Med*, 47(8): 1367-1374.

93. Zhou, D; Chu, W; Rothfuss, J; Zeng, C; Xu, J; Jones, L and Mach, RH (2006) "Synthesis, radiolabeling, and in vivo evaluation of an 18F-labeled isatin analog for imaging caspase-3 activation in apoptosis", *Bioorg Med Chem Lett*, 16(19): 5041-5046.

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