

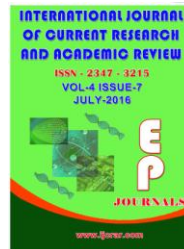


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### Magical Bullets: A New Era of Molecular Imaging

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#### A B S T R A C T

Antibody Imaging-A Star In Oral Cancer Diagnosis Over the past two decades, the role of radiolabeled monoclonal antibodies has been steadily growing in clinical practice, particularly for staging and locating variety of oral cancer. This due largely to the availability of these products in pharmaceutical quantities as well as the relatively low incidence of adverse reactions following i.v. administration of antibodies to patients. The applications for use of antibodies, their derivatives and fragments continue to hold even more potential. The route of this biotechnology establishes, targeting the specific site with radiolabeled diagnostic and therapeutic viable with minimum decreased adverse effects, toxicities. Antibody imaging has proven to be useful in localization and assessment of metastatic diseases. thus, this paper tends to highlight the importance of antibody imaging as an aid in detection of oral cancer.

### Introduction

Molecular imaging is one of the most rapidly growing areas of science. Recent developments facilitated the detection of novel molecular targets for tumor cells, principally involved in differentiation, signal transduction, protein interaction networks, cell death and apoptosis, angiogenesis, immune recognition, invasion, proliferation and metastasis plays a central role in clinical oncology (Salsano *et al.*, 2013). The antibody revolution started in 1975 with the introduction of the hybrid technology by Kohler and Milstein. Molecular imaging aims to visualize, characterize and measure

processes on molecular and cellular levels in living systems in a noninvasive way (Salsano *et al.*, 2013). Consequently, there is a growing demand for appropriate PET tracers which allows for a specific accumulation in the target structure as well as its visualization and exhibit decay characteristics matching their in vivo pharmacokinetics (Boerman *et al.*, 2011). The glucose analog, fluorodeoxyglucose (FDG), marked with the radioisotope, fluorine-18 (18F), is the most widely used (Salsano *et al.*, 2013).

The hope is that clinical molecular imaging will one day be used to achieve the following: (i) the detection of molecular or physiological alterations that signal the presence of cancer when it is still at a curable stage (ii) the ability to evaluate and adjust treatment protocols in real time (iii) the ability to streamline the cancer drug development process (iv) Determine the extent or severity of the disease, including whether it has spread elsewhere in the body.

There is tremendous incentive for developing technologies that detect cancer at its earliest stages (Scott *et al.*, 2012).

Today, imaging is at crossroads, with molecularly targeted imaging agents expected to broadly expand the capabilities of conventional anatomical imaging methods. The most commonly used molecular imaging procedure for diagnosing or guiding treatment of head and neck cancer is Positron Emission Tomography (PET) scanning. (PET) Positron emission.

Tomography (PET) imaging has emerged as a clinical cornerstone in cancer staging and restaging for a number of malignancies and is one of the few molecular imaging technologies approved by the Food and Drug Administration (FDA, USA). PET is an analytical nuclear medicine imaging technology that uses positron-labeled molecules in very low mass amounts to image and measure the function of biological processes with minimal disturbance. PET involves the use of an imaging device (PET scanner) and a radiotracer (Salsano *et al.*, 2013). A frequently used PET radiotracer is 18F-fluorodeoxyglucose (FDG), a compound derived from a simple sugar and a small amount of radioactive fluorine. 18F-FDG is injected intravenously and transported into cells by glucose transporter proteins (Salsano *et al.*, 2013).

Where it is phosphorylated by hexokinase to form 18F-FDG-6-phosphate. The cell membrane is impermeable to both glucose-6-phosphate and 18F-FDG-6-phosphate. 18F-FDG-6-phosphate remains trapped within the cell in a concentration correlated to cell uptake. Once the FDG radiotracer accumulates in the body's tissues and organs, its natural decay includes emission of tiny particles called positrons that react with electrons in the body. This reaction, known as annihilation, produces energy in the form of a pair of photons. FDG are nonspecific and elevated uptake in infectious or inflammatory lesions variable physiological uptake in normal tissues/organs that may mask the presence of malignant neoplasm, and the lack of uptake in metabolically inactive malignancy (Salsano *et al.*, 2013).

The PET scanner, which is able to detect these photons, creates three-dimensional images that show how the FDG is distributed in the organs of the body. Because highly active cancer cells absorb more glucose than normal cells, they appear brighter on PET scans. So, areas where a large amount of FDG accumulates, called "hot spots" because they appear more intense than surrounding tissue, indicating that a high level of chemical activity or metabolism is occurring there. Areas of low metabolic activity appear less intense and are sometimes referred to as "cold spots." Using these images and the information they provide, physicians are able to evaluate how well organs and tissues are working and to detect abnormalities. FDG PET detects abnormal tumor metabolism before anatomic change appears and allows differentiation of malignant from benign anatomic abnormalities. PET imaging and offers, visualization of physiological and biochemical changes. Conventional imaging modalities like radiography,

ultrasonography, computed tomography (CT), in contrast to PET, only offer visualization of nonspecific changes related to morphology. PET is a highly sensitive functional imaging modality and enables quantitative imaging. The physical properties of a specific PET isotope radionuclide, including its half-life, positron yield, positron range, and overall decay scheme (including additional emissions) will determine suitability for an antibody imaging application (Salsano *et al.*, 2013; Zhao *et al.*, 2008; Kaur *et al.*, 2012)

The mAb (Monoclonal Antibodies) with its best characteristics in binding affinity, blood clearance, and tumor penetration improves the imaging performance (Figure 1). Alternative ligands have also been developed, among which antibody fragments and engineered variants such as antigen-binding F(ab')<sub>2</sub> and F(ab'), single-chain variable fragments (scFv), diabodies, and minibodies (molecular weights ranging from 25-100 kDa) most commonly used. These fragments achieve optimal ratio typically in 1-6 hours after injection, different from intact mAbs that achieve tumor-to-nontumor ratio in 2-4 days after injection. Affibodies in particular are very stable and highly water-soluble  $\alpha$ -helical proteins, with facilitated conjugation chemistry. In particular, the small size, resulting in rapid blood clearance, good tumor penetration, and high binding affinity to, selected targets, makes affibody molecules ideal candidates for imaging purposes (Kaur, 2012; Olafsen *et al.*, 2010).

### **Mechanisms of tumour cell killing**

This cell killing can be summarized as being due to several mechanisms (Figure 2); direct action of the antibody (through receptor blockade or agonist activity, induction of apoptosis, or delivery of a drug or cytotoxic

agent), immune-mediated cell killing mechanisms (including, complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC) and regulation of T cell function) and specific effects of an antibody on tumour vasculature and stroma. The Fc function of antibodies is particularly important for mediating tumour cell killing through CDC3 and ADCC. All of these approaches have been successfully applied in the clinics. The abrogation of tumour cell signalling (for example, by cetuximab and trastuzumab) the induction of effector function primarily through ADCC (for example, by rituximab) and the immune modulation of T cell function (for example, by ipilimumab) are the approaches that have been most successful and that have led to the approval of antibodies using these mechanisms (Scott *et al.*, 2012).

**Direct tumour cell killing** can be elicited by receptor agonist activity, such as an antibody binding to a tumour cell surface receptor and activating it, leading to apoptosis (represented by the mitochondrion). It can also be mediated by receptor antagonist activity, such as an antibody binding to a cell surface receptor and blocking dimerization, kinase activation and downstream signalling, leading to reduced proliferation and apoptosis. An antibody binding to an enzyme can lead to neutralization, signalling abrogation and cell death, and conjugated antibodies can be used to deliver a payload (such as a drug, toxin, small interfering RNA or radioisotope) to a tumour cell (Scott *et al.*, 2012).

**Immune-mediated tumour cell killing** can be carried out by the induction of phagocytosis, complement activation, antibody-dependent cellular cytotoxicity (ADCC), genetically modified T cells being targeted to the tumour by single-chain

variable fragment (scFv), T cells being activated by antibody-mediated cross-presentation of antigen to dendritic cells; and inhibition of T cell inhibitory receptors, such as cytotoxic T lymphocyte-associated antigen 4 (CTLA4) (Scott *et al.*, 2012).

**Vascular and stromal cell ablation** can be induced by vasculature receptor antagonism or ligand trapping (not shown); stromal cell inhibition; delivery of a toxin to stromal cells; and delivery of a toxin to the vasculature (MAC) membrane attack complex, (MHC) major histocompatibility complex, (NK) natural killer (Scott *et al.*, 2012).

### **Tumour antigens as antibody targets**

The safety and efficacy of therapeutic mAbs in oncology vary depending on the nature of the target antigen. Ideally, the target antigen should be abundant and accessible and should be expressed homogeneously, consistently and exclusively on the surface of cancer cells (Scott *et al.*, 2012). Antigen secretion should be minimal, as secreted antigens can bind the antibody in the circulation and could prevent sufficient antibody from binding to the tumour. The desired mechanism of action is ADCC or CDC, then it is desirable that the antigen-mAb complex should not be rapidly internalized so as to maximize the availability of the Fc region to immune effector cells and complement proteins, respectively. By contrast, good internalization is desirable for antibodies or proteins that deliver toxins into the cancer cell and for antibodies the action of which is primarily based on the down regulation of cell surface receptors. Tumour-associated antigens recognized by therapeutic mAbs fall into several different categories. Haematopoietic differentiation antigens are glycoproteins that are usually associated

with cluster of differentiation (CD) groupings and include CD20, CD30, CD33 and CD52. Cell surface differentiation antigens are a diverse group of glycoproteins and carbohydrates that are found on the surface of both normal and tumour cells. Antigens that are involved in growth and differentiation signalling are often growth factors and growth factor receptors. Growth factors that are targets for antibodies in cancer patients include CEA2, epidermal growth factor receptor (EGFR; also known as ERBB1), ERBB2 (also known as HER2), ERBB3, MET (also known as HGFR), insulin-like growth factor 1 receptor (IGF1R)20, ephrin receptor A3 (EPHA3)21, tumour necrosis factor (TNF)-related apoptosis-inducing ligand receptor 1 (TRAILR1; also known as TNFRSF10A), TRAILR2 (also known as TNFRSF10B) and receptor activator of nuclear factor- $\kappa$ B ligand (RANKL; also known as TNFSF11). Antigens involved in angiogenesis are usually proteins or growth factors that support the formation of new microvasculature, including vascular endothelial growth factor (VEGF), VEGF receptor (VEGFR), integrin  $\alpha$ V133 and integrin  $\alpha$ 5131. Tumour stroma and the extracellular matrix are indispensable support structures for a tumour. Stromal and extracellular matrix antigens that are therapeutic targets include fibroblast activation protein (FAP) and tenascin (Scott *et al.*, 2012).

Considerable effort has recently been invested in identifying new antigen targets that are suitable for antibody-based therapies in cancer. Serological, genomic, proteomic and bioinformatic databases have been used to identify antigens and receptors that are overexpressed in tumour cell populations or that are linked to gene mutations identified as driving cancer cell proliferation. Examples of antigens that have been

identified as suitable targets for antibody therapy with these approaches include EGFRVIII, MET, cytotoxic T lymphocyte-associated antigen 4 (CTLA4) and FAP (Scott *et al.*, 2012).

### **Targeting CD44**

The cell-surface glycoprotein, CD44, is involved in many biological processes including adhesion of cells to extracellular matrix proteins, lymphocyte-endothelial cell interactions, metastasis formation, migration of cells, and I cell activation/adherence (Watering *et al.*, 2014).

### **Targeting EGFR**

The epidermal growth factor receptor (EGFR) is a member of the ErbB family. It plays a crucial role in differentiation, proliferation and survival of many different tumor types, including breast, lung, bladder, and colon carcinoma. The overexpression of EGFR is associated with more aggressive tumors and poor prognosis due to the resistance of treatment. Many mAbs have been developed to inhibit the EGFR activation for example: cetuximab (Erbix), a chimeric IgG, which upon binding to the ligand-binding domain induces internalization of EGFR and thereby blocking downstream signaling. Another approved mAb to inhibit the EGFR signaling is panitumumab.

### **Targeting HER267**

Human epidermal growth factor receptor 2 (HER2) is another member of the ErbB family. It is involved in angiogenesis, differentiation, metastasis, proliferation and cell survival upon heterodimerization with other members of the EGF receptor family. HER2 overexpression found in many types of tumors including breast and ovarian cancer. The FDA approved anti-HER2 mAb trastuzumab (Herceptin, Genentech, CA,USA) to be used for the treatment of

HER2 positive breast tumors, since it blocks the HER2 activation. In addition, the HER2 expression is found to be different between the primary lesion and the distant metastatic lesions in the same patient. Noninvasive in vivo imaging to visualize HER2 expression using radiolabeled trastuzumab has been extensively investigated (Watering *et al.*, 2014).

### **Targeting VEGF**

Vascular endothelial growth factor (VEGF) is a proangiogenic factor in both normal tissues and in tumors. The overexpression of VEGF and its receptors (VEGFR) are associated with poor prognosis. The humanized anti-VEGF mAb, vacizumab, is capable of blocking angiogenesis by depleting VEGF and thereby preventing its binding to the VEGFR. This neutralizes VEGF actions (Watering *et al.*, 2014).

### **Targeting PIGF**

The clinical benefits of angiogenesis inhibitors can be compromised by the upregulation of proangiogenic factors such as the placental growth factor (PIGF). PIGF, a VEGF homolog, is expressed in low levels in normal tissue and can be overexpressed in tumor cells. PIGF contributes to angiogenesis in pregnancy, wound healing, ischemic conditions, and tumor growth. PIGF inhibitors are able to reduce the angiogenesis and tumor cell motility. The antitumor activity of a humanized mAb directed against PIGF-1 and PIGF-2 (Watering *et al.*, 2014).

### **Targeting PSMA**

Prostate-specific membrane antigen, (PSMA) is a transmembrane glycoprotein which is associated with increased tumor progression, development of castration resistance, and/or resistance to hormone-based treatments.



PSMA is expressed in a limited range of normal tissues including benign prostatic epithelium, renal proximal tubule, small bowel, and the brain; however, the expression level is 2 to 3 times lower than in prostate cancer specimens. Anti-PSMA mAb, was able to differentiate between subcutaneous PSMA positive and negative tumors in athymic nude mice, making it a potential target for clinical noninvasive identification and quantification of PSMA-positive tumors (Watering *et al.*, 2014).

### **Targeting CD147**

CD147, a member of the immunoglobulin superfamily, is involved in many physiological functions including embryo implantation, early, stage neural network formation, and spermatogenesis. Overexpression of CD 147 is found in many types of cancer including pancreatic cancer and induces expression of Matrix MetalloProteinases (MMPs) and VEGF (Watering *et al.*, 2014).

### **Targeting CAIX**

Hypoxia in tumors is associated with a poor prognosis in many tumor types since it is associated with resistance to radiotherapy and chemotherapy. All antibodies directed against CAIX it is possible to select patients for hypoxia-targeting or modifying treatment combined with radiotherapy (Watering *et al.*, 2014).

### **Targeting IGF-1R**

The insulin like growth factor 1 receptor (IGF-1R) is a transmembrane receptor expressed in many human cancers, including 35% of all triple-negative breast carcinomas. It is involved in the proliferation, apoptosis, angiogenesis and tumor invasion (Watering *et al.*, 2014).

### **Targeting Met**

The expression of hepatocyte growth factor receptor tyrosine kinase (Met) was measured by PET, using onartuzumab, a mAb against Met (Watering *et al.*, 2014).

### **Targeting GPC3**

The glypican-3 (GPC3) is a hepatocellular-specific cell surface proteoglycan overexpressed in most hepatocellular carcinomas (Watering *et al.*, 2014).

### **Applications**

A major impetus behind recent interest in molecular imaging has been the development and maturation of molecularly-targeted therapeutics, spanning small molecules, biologicals, vaccines, and cell-based therapies. In order to tailor treatments specifically to a patient's individual disease, equally sophisticated diagnostics are needed. The power of immunoPET is vested in the multitude of applications that can be addressed. For example, the availability of more sensitive and specific imaging agents will enhance the determination of whether tumors are present or have spread, providing essential information to inform decisions between local and systemic therapies.

Moreover, phenotypic information supplied by immunoPET can be exploited to provide information on the tissue of origin and expression of a variety of biological markers including receptors, adhesion molecules, activation markers, etc. If there is a corresponding targeted therapeutic (which can be a biomolecule, small molecule, or other agent), target expression can play a central role in therapy selection (Wu, 2014; Salsano *et al.*, 2013; Scott *et al.*, 2012).

During the development of therapeutic antibodies (including antibody-drug conjugates), radiolabeling and PET imaging

of the agent itself can provide quantitative information on targeted delivery, pharmacokinetics, and normal tissue retention. It also opens the way for modeled on extensive use of PET imaging in neurosciences for assessing receptor occupancy. Importantly, quantitative imaging may provide an important approach for antibody dose determination through traditional biodistribution and competitive-binding assays (Wu, 2014; Salsano *et al.*, 2013; Scott *et al.*, 2012).

Another practical application will be the ability to assess the rate and extent of internalization and catabolism of an antibody: antigen complex *in vivo*. Internalization and metabolism are key process that must be understood in order to develop effective therapeutics such as antibody-drug conjugates (Wu, 2014; Salsano *et al.*, 2013; Scott *et al.*, 2012).

Response to therapy can be assessed using antibody-targeted imaging, which can be employed whether the drug is a small molecule, antibody, or cell-based or immunological agent. Antibody-directed imaging can provide measure of decrease in tumor size, complementary to anatomic imaging such as CT or MRI. Loss of antigen-positive cells and/or target downregulation can also be imaged. If the therapeutic itself is an antibody, one can also employ antibody agents with non-overlapping epitope specificity to image in the presence of blocking or saturating doses of the therapeutic (Wu, 2014; Salsano *et al.*, 2013; Scott *et al.*, 2012).

These guidelines were approved by the Board of Directors of the Society of Nuclear Medicine on February 11, 2006)

### **Patient Preparation**

The optimum preparation for patients about to undergo PET/CT is evolving. The major

goals of preparation are to minimize tracer uptake in normal tissues, such as the myocardium and skeletal muscle, while maintaining uptake in target tissues (neoplastic disease). The following is a commonly used protocol.

### **Before arrival**

Patients should be instructed to fast and not to consume beverages, except for water, for at least 4-6 hrs before the administration of 18F-FDG to decrease physiologic glucose levels and to reduce serum insulin levels to near basal levels. Oral hydration with water is encouraged. Intravenous fluids containing dextrose or parenteral feedings also should be withheld for 4-6 hrs. When intravenous contrast material is to be used, patients should be screened for a history of iodinated contrast material allergy, use of Metformin for the treatment of diabetes mellitus, and renal disease. Intravenous contrast material should not be administered when the serum creatinine level is above 2.0 mg/dL.

### **Before injection**

- a) For brain imaging, the patient should be in a quiet and dimly lit room for 1 8F-FDG administration and the subsequent uptake phase.
- b) For body imaging, the patient should remain seated or recumbent for 18F-FDG administration and the subsequent uptake phase to avoid muscular uptake.<sup>11</sup>
- c) The blood glucose level should be checked before 18F-FDG administration. Tumor uptake of 18F-FDG is reduced in hyperglycemic states. If the blood glucose level is greater than 150-200 mg/dL. Reducing the serum glucose level by administering insulin can be considered, but the administration of 18F-FDG should be delayed after insulin administration.

### Information Pertinent to Performing Procedure

Focused history, including the type and site of malignancy, dates of diagnosis and treatment (biopsy result, surgery, radiation, chemotherapy and administration of bone marrow stimulants and steroids) and current medications. 2. History of diabetes, fasting state, and recent infection 3. Patient's ability to lie still for the duration of the acquisition (15-45 min) 4. History of claustrophobia 5. Patient's ability to put his or her arms overhead (Delbeke).

### Conclusion

Molecular imaging could therefore become relevant as a tool to guide rational drug development and treatment. Progress in tracer development and imaging platforms will enhance the potential of molecular imaging in this setting. With innovative technologies, these engineered antibody fragments retain the targeting specificity of whole mAbs, but are more economically available. In addition, by forging them into multivalent and multispecific reagents or linking them to therapeutic payloads for many diagnostic and therapeutic applications, they can possess other superior properties. As these properties allow them to be used for real-time imaging with higher specificity, they doubtless will become very important in this field and, in years to come, will be indispensable as both clinical and research reagents.

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