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Antibody Based Imaging Strategies of Cancer

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Abstract

Although mainly developed for preclinical research and therapeutic use, antibodies have high antigen specificity, which can be used as a courier to selectively deliver a diagnostic probe or therapeutic agent to cancer. It is generally accepted that the optimal antigen for imaging will depend on both the expression in the tumor relative to normal tissue and the homogeneity of expression throughout the tumor mass and between patients. For the purpose of diagnostic imaging, novel antibodies can be developed to target antigens for disease detection, or current FDA-approved antibodies can be repurposed with the covalent addition of an imaging probe. Reuse of therapeutic antibodies for diagnostic purposes reduces translational costs since the safety profile of the antibody is well defined and the agent is already available under conditions suitable for human use. In this review, we will explore a wide range of antibodies and imaging modalities that are being translated to the clinic for cancer identification and surgical treatment.

Keywords

antibody; imaging; cancer

Overview of antibody based imaging

The advantage of repurposing therapeutic antibodies for imaging is that the pharmacokinetic profile, biodistribution, side effects and potential toxicity of these FDA-approved antibodies are generally well known. Moreover, because the dosing of the antibodies as imaging agents often requires less than therapeutic levels, the toxicity profile is usually limited to non-dose dependent events such as immunological reactions. This makes the antibody-based approach ideal for safely pioneering the use of targeted imaging agents in the clinic. Compared to most imaging agents, the plasma half-life is long (days to weeks) due to the increased size of the protein (~150kD) and retained clearance profile. This requires that imaging occur days

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after systemic administration due to a high blood background, which persists for at least 24 hours after administration. However, this is advantageous considering the long half-life may lead to a higher cancer specific uptake compared to smaller particles like nanobodies or peptide fragments, whose relatively fast clearance hinders bioavailability of the targeting agent.

The ideal imaging vector would permit selective and sensitive tumor imaging immediately after systemic administration of the diagnostic agent. The pharmacokinetics of antibodies is non-linear, such that repeated dosing lengthens the half-life. When administered as a single dose at non-therapeutic levels, however, the half-life is shorter. Although abbreviated antibody derivatives have very short half-lives, conventional IgG1 or IgG2 antibodies have half-lives around 24 hours which will likely require administration several days before imaging to allow for accumulation within the tumor and clearance of blood borne background. However, there may be some advantages to a longer circulating half-life. It can be imagined that if there is lower expression of a certain target, it is advantageous to have a targeting moiety with a longer half-life in order to achieve a sufficient tumor-to-background level. For example, in this personalized approach to imaging, a full-core preoperative biopsy and immunohistochemistry is used to determine the ideal targeting agent for the individual patient (Figure 1). Currently, no human data exist on which moiety will emerge as the superior scheme. Realistically, the ideal agent will depend on factors specific to each cancer. Currently, there are fifteen antibodies approved for cancer therapy by the FDA [1]. Moieties include those that target the receptor of interest, such as epidermal growth factor (EGFR) or HER2/neu, and also antibodies that are covalently modified with cytotoxic microtubule antagonists (antibody-drug conjugates, ADCs) thereby providing targeted chemotherapy [2]. A similar approach is used in the field of targeted molecular imaging.

Almost any kind of imaging probe can be linked to antibodies. Figure 2 provides an overview of imaging probes currently available for targeted imaging purposes in oncology. The imaging probe is covalently linked to the antibody at very low molar ratios to prevent interference with the antigen-binding site and to prevent a high rate of hepatic clearance [3]. Furthermore, the imaging efficiency of the diagnostic probe provides a strong signal even at these relatively low molar ratios. Several strategies for antibody-tracer conjugates (ATCs) are currently in clinical practice. For nuclear imaging purposes, a modality-specific radionuclide is conjugated to the antibody and combined with either SPECT or PET imaging (Table 1). Paramagnetic or superparamagnetic particles for antibody labelling are used in combination with magnetic resonance imaging [4]. Optical dyes are dependent on the properties of light and as a result, have limited depth of tissue penetration, but high resolution when imaged at the surface. These agents are considered optimal for the surgical setting where the tissue planes are exposed and wide-field, high-resolution imaging can be applied in real-time. Furthermore, optical probes can be designed to emit fluorescence and toxic reactive oxygen species after light based activation. This imaging strategy is referred to as photoimmunotherapy (PIT) and has concurrent diagnostic and therapeutic application.

Antibody-based optical imaging

Fluorescently labeled antibodies are emerging as a powerful tool for cancer localization in various clinical applications. Fluorescent probes are largely non-toxic and have been widely used in the clinical setting (indocyanine green, ICG) with very limited toxicity in humans. However, limitations in ICG use, such as low quantum yield and absence of a bioactive group for conjugation, have led to the exploration of alternate dyes to ensure consistent drug manufacturing and superior performance (Table 2). Antibody-conjugated fluorophores can be visualized either in the visible spectrum, such as fluoresceine isothiocyanate (FITC) [5], or in the near-infrared spectral range, as in IRDye800CW [6,7]. Optical agents have limited application in whole body imaging considering the depth of penetration is reduced to less than a centimeter. However, they are well suited for endoscopic or surgical procedures since they can be used for repeated and real-time imaging.

The use of cancer-targeted fluorescence imaging has progressed from predominantly preclinical animal studies [8] towards human clinical applications [5,9]. Various strategies are employed with respect to the development of imaging systems and companion oncologic tracers [10-18]. These tracers include blood pool agents (ICG) [19,20,9,21,22], folate-based probes [5], RGD-based probes [23,24], smart activatable probes such as matrix metalloproteinase or pH activatable probes [12,25,26], and those based on tumor-specific receptor targeting (i.e. immuno-imaging) using (therapeutic) antibodies [27] or smaller fragments like affibodies or nanobodies [28].

The use of real-time imaging inside the operating room has been investigated for some time. Intraoperative ultrasound, portable CT scanners, and image-guided surgical navigation systems have been developed in response to this need for greater resolution of surgical anatomy. The introduction of optical imaging into the operating room, however, is in its early development. To date, 5-aminolevulinic acid (5-ALA) for use in malignant glioma surgery is the only agent that has been shown to have clinical benefit and is approved for fluorescence-guided cancer resection in Europe. 5-ALA leverages the differential metabolic activity of brain tumors and is visible in the red region of the visible spectrum [29]. While the application of 5-ALA has been shown effective, limitations such as natural autofluorescence of brain tissue in the deep red region have made adequate contrast a challenge. Furthermore, because 5-ALA metabolism is a hallmark of a limited set of cancer types, it is difficult to extend 5-ALA beyond malignant gliomas. ICG has shown promise predominantly in sentinel lymph node mapping and is currently being studied in liver cancer [30] and breast cancer [31,32]. The greatest barrier for broader application of ICG is the lack of specificity for cancer, since the primary method of preferential accumulation within the tumor is limited to the enhanced permeability and retention (EPR) effect.

Antibody-based optical imaging during surgery confers the advantage of targeting tumor-specific markers for fluorescence. One such approach utilizes antibodies to ligands that are over-expressed by tumors such as vascular endothelial growth factor (VEGF). Rapid growth of tumor tissue beyond 1-2cm in diameter requires signalling for angiogenesis by way of increased VEGF production. In preclinical studies, bevacuzimab-IRDye800 administered in

a murine subcutaneous breast cancer model demonstrated modest fluorescence within flank tumors [33,34].

Another strategy is to employ antibodies to molecular targets that are resident within the tumor such as epidermal growth factor receptor (EGFR), human epidermal growth factor receptor-2 (HER-2), and vascular endothelial growth factor receptor (VEGFR). Conjugating a fluorescent dye to a tumor-targeting antibody provides a rational design for visualizing cancer in real-time. Numerous preclinical studies have demonstrated tumor selectivity using antibody-based dyes. Preferential tumor fluorescence has been demonstrated using cetuximab-IRDye800 in head and neck cancer[35], panitumumab-IRDye800 in head and neck cancer [36], and trastuzumab-IRDye800 in breast cancer [34]. A phase I trial using cetuximab-IRDye800 optical imaging during head and neck cancer surgery is currently underway (clinicaltrials.gov: NCT01987375).

With all these strategies being introduced, the concept of immuno-imaging using clinical grade therapeutic antibodies and the more recent development of affibody and nanobody imaging are of the highest interest for clinical translation [27]. The use and efficacy of antibodies as therapeutic agents in cancer was recently described in a review by Sliwkowski and Mellman [1]. Similarly, the application of antibody engineering for molecular imaging was also recently detailed by Wu [37]. Interestingly, current antibody-based therapeutic agents are also useful as imaging agents and the strategy of repurposing therapeutics agents for imaging agents was described and outlined in perspective of regulatory issues and (dis)advantages [38,39]. The first clinical studies are currently underway to assess feasibility in patients with breast cancer (Clinicaltrials.gov: NCT01508572), colorectal cancer (Clinicaltrials.gov: NCT01972373), and head and neck cancer (Clinicaltrials.gov: NCT01987375). The clinical approaches for these agents have utilized microdosing methodology [38,40,41,39,42] and classical dose-escalation design based on pre-clinical data.

Antibody-based ultrasound imaging

Ultrasound provides real-time imaging with nonionizing radiation for detection and monitoring of various disease states. The addition of ultrasound contrast agents, or microbubbles, has greatly improved the sensitivity of ultrasound imaging, providing an enhanced method for measuring blood flow, vasculature structure, lesion characterization, and assessment of therapeutic response [43]. Microbubbles are polymer, protein or phospholipid shelled, compressible gas-filled bubbles ranging in size from 1-5 μm . These intravascular contrast agents non-linearly oscillate in response to high frequency ultrasound waves, allowing enhancement of signal in the blood to tissue ratio during ultrasound imaging. In the last two decades, the addition of targeted antibodies to the outer surface of these microbubbles [44] has propelled ultrasound into the field of molecular imaging, providing novel approaches for imaging inflammation [45-48], cancer vascularity and angiogenesis [49-51] and atherosclerosis [52,53].

Functionalizing microbubbles to various endothelial markers improves local accumulation of contrast enhancement, therefore further heightening specificity of contrast-enhanced

ultrasound imaging and visualization of vascularity [43,54,55]. Utilizing either covalent or noncovalent (i.e. avidin-biotin) bonds, antibodies are attached to the outer surface of the bubbles, while retaining the same general properties as untargeted microbubbles. Targeted microbubbles are systemically injected, providing vascular and organ delineation before binding to their respective receptors, as shown in Figure 1. Specifically, increasing visualization of tumor angiogenesis can improve detection and monitoring of cancer. Targeted ultrasound imaging provides molecular information regarding vascular receptors, such as VEGFR2 and P-selectin. Molecular ultrasound imaging has been applied to visualize changes in tumor vasculature and response to drug treatment [56-58]. VEGFR2 targeted agents revealed improved vasculature signal enhancement compared to unspecific contrast-enhanced ultrasound in pancreatic cancer, and detected correlative changes in response to anti-VEGF treatment as detected by histological analysis [59]. VEGFR2-targeted microbubbles have also been utilized to improve visualization in various cancer types and quantify response from targeted agents [60], chemotherapy, and radiation treatment [61]. Biodistribution of VEGFR2 targeted microbubbles [62] and P-selectin targeted microbubbles [63] were recently reported that revealed both increased localization in the tumor as well as improved clearance from filtering organs, such as kidney and spleen. More recent advancements have investigated dual- and triple-targeted microbubbles, revealing that multi-targeted microbubbles demonstrate an overall synergistic effect by increasing tumor vessel visualization compared to single-targeted constituents [49,64,65]. Multi-targeted microbubbles have since been used to determine early response to anticancer treatment in preclinical studies prior to changes in tumor size [58]. The synergistic benefit from simultaneously targeting multiple receptors enhances the ability of the MBs to attach within the desired region-of-interest, and provides increased potential to monitor treatment response, as well as improved cancer staging and prognosis.

Antibody targeted microbubbles and traditional non-targeted microbubbles have also been explored as drug delivery vehicles by pre-loading or coating MBs with drugs or molecules [66,67] for localized or stimulated delivery. Furthermore, microbubbles have been utilized as mechanisms for an ultrasound induced drug or gene delivery approach through improving extravasation and gateways for current systemic therapies [68,69]. The high sensitivity and specificity when imaged *in vivo*, and ability of antibody-targeted microbubbles to function as drug delivery and imaging agents [70-72] permits them to be a dual-purpose vehicle. This theranostic approach of utilizing targeted microbubbles in combination with packaging a gene (or drug) has opened up new interest in the application of microbubbles for imaging and therapy. Additionally, the ability to also use targeted microbubbles for ultrasound therapy or sonoporation demonstrates potential to create a dual localized and targeted approach, further increases the potential for delivery of anticancer agents to the desired region-of-interest [73,74,54]. Theranostic targeted microbubbles utilized for both specific noninvasive imaging and localized delivery reveal an exciting new area of research in antibody-based cancer imaging.

Antibody-based MR imaging

Magnetic resonance imaging (MRI) generates image contrast based on the difference in intrinsic features of tissues such as T1 and T2 values or proton density. MRI contrast agents

alter these properties of a target tissue (or background tissue) to further enhance the contrast. Paramagnetic or superparamagnetic metal ions chelated to various kinds of antibodies have been employed as MRI contrast agents altering T1 and T2 values.

Gadolinium is a commonly used paramagnetic substance for MRI contrast agents. Most of clinically available gadolinium-based contrast media are systemically delivered into tissues by vascular perfusion, improving contrast between hypervascular and hypovascular tissues. Gadolinium can be readily conjugated with an antibody using DTPA (diethylenetriaminepentaacetic acid) as a chelating material [75]. Gd-DTPA has been conjugated to monoclonal antibodies targeting sialylated lewis (a) antigen in colon adenocarcinoma demonstrating high tumor uptake of the bioconjugates using a murine model [76]. Similarly, Gd-DTPA has been conjugated to antibodies against NG2, CD33, and MUC1 targeting melanoma, leukemia, and breast adenocarcinoma, respectively, and revealed that the tumor uptake of the novel contrast agents were significantly higher than those of non-targeted gadolinium compounds in animal models [77]. However, it has been reported that the conjugation between antibody and DTPA is not stable in human serum at body temperature (37°C) [78]. More recently, another bi-functional chelate, isothiocyanobenzyl-EDTA, was validated for conjugating gadolinium with antibodies. Kuriu *et al* investigated bound Gd-EDTA with A7, a monoclonal antibody targeting colon adenocarcinoma, and reported that the %ID/g of ¹²⁵I labeled Gd-EDTA-A7 in colorectal tumor xenograft model was significantly higher than that of ¹²⁵I labeled Gd-EDTA-control antibody for 96 hours after dosing [79]. However, a high dose of antibody (10 mg) had to be administered to achieve adequate image contrast [79].

Iron oxide is the most common superparamagnetic substance for MRI. However the bare iron oxide nanoparticles (IONPs) cannot be utilized for clinical use, due to their inherent tendency to suffer rapid clearance by macrophages and agglomerate by plasma protein interaction [80]. Thus, the surface of the IONPs is typically coated with polymer like dextran, polyethylene glycol (PEG), and polyethylene oxide (PEO) [81-83]. The polymer layer can serve as the foundation to attach antibodies targeting biomarkers. This strategy has been used, for example, with the anti-HER2 antibody (herceptin) and *in vivo* experiments confirmed binding of anti-HER2 antibody-IONPs to HER2 positive human breast cancer cell lines. Similar techniques have been used for coating IONPs with anti-EGFR antibodies. It was demonstrated that this novel agent could induce therapeutic effect in an orthotopic glioma murine model, in addition to sufficient contrast enhancement [84]. One major concern of IONP based MR contrast agents is that these particles are easily trapped in the spleen and liver when administered systemically.

Freon-like substances like PFOB (perfluoro-octyl bromide), fatty emulsions, and barium sulfate have been used to decrease the proton density of a target lesion, and thus to make the lesion darker than background tissue in MR images. Wei *et al* have recently achieved success in conjugating PFOB with monoclonal antibodies targeting intercellular adhesion molecule-1 (ICAM-1), and verified the specific binding of the compound to ICAM-1 overexpressing cardiomyocytes in an *in vitro* model [85].

Antibody-based PET imaging

As biomarkers for cancer dissemination are continually being identified, imaging techniques are evolving to leverage these new targets for cancer localization. Radiolabeling of monoclonal antibodies has been a widely used application for disease localization. These methods uniquely permit membrane-specific biomarkers to be noninvasively profiled in situ. Although at the cost of spatial resolution, the unlimited tissue penetration of radionuclides allows measurement of whole-body antibody biodistribution and localization.

During the early 1990's, single-photon radionuclides (^{111}In and $^{99\text{m}}\text{Tc}$) were used in combination with planar and single-photon emission computed tomography (SPECT) for tumor detection. While SPECT could successfully image radiolabeled antibodies, deficiencies in sensitivity and spatial resolution caused by single photon physics and computed tomography hindered the clinical utility. The real potential of antibody-based nuclear imaging was not realized until positron emission tomography (PET) was introduced by radiolabeling antibodies with positron emitters to combine the power and resolution of PET imaging with the specificity of antibody targeting.

Immuno-PET is the *in vivo* imaging and quantification of antibodies radiolabeled with positron-emitting radionuclides. These application-matched radionuclides are conjugated to chimeric, humanized, or fully human antibodies to provide real-time, target-specific information with high sensitivity. There are fifteen antibodies (with many more under investigation) that have been approved by the FDA for the treatment of solid and hematological cancers [1]. For patient application, matching the appropriate positron-emitting radionuclide for antibody labeling depends on several factors. Firstly, the decay characteristics must match the antibody pharmacokinetics for optimal resolution and quantitative precision. Secondly, the radionuclide must be readily manufactured and labeled in a cost-efficient manner using current Good Manufacturing Practices (GMP). Thirdly, the radiolabeling of the antibody must not affect the pharmacokinetics and biodistribution of the targeting agent. While copper-64 (^{64}Cu , $t_{1/2}=12.7\text{hr}$) and yttrium-86 (^{86}Y , $t_{1/2}=14.7\text{hr}$) are suitable immuno-PET radionuclides, iodine-124 (^{124}I , $t_{1/2}=100.3\text{hr}$) and zirconium-89 (^{89}Zr , $t_{1/2}=78.4\text{hr}$) more appropriately matches the time needed (2-4 days for intact antibodies) to achieve optimal tumor-to-background ratios [86,87]. Shorter-lived positron emitters, such as gallium-68 (^{68}Ga , $t_{1/2}=1.13\text{hr}$) and fluorine-18 (^{18}F , $t_{1/2}=1.83\text{hr}$), are typically used with antibody fragments that have much shorter pharmacological half-lives. Currently, ^{89}Zr is considered the optimal positron emitter due to its compatible half-life, ideal physicochemical characteristics for protein conjugation, and availability [27]. For these reasons, we will focus on ^{89}Zr for the remainder of this section.

Early steps in the clinical translation of immuno-PET were hindered by arduous radiolabeling techniques. However, recent advances in conjugation chemistry have improved the efficiency, reducing the synthesis steps, and identified new chelators. The labeling of radionuclides is performed directly or indirectly using a bifunctional linker such as desferrioxamine (DFO), a popular metal chelator [88]. Recently, Lewis *et al* introduced the novel concept of pretargeting the antibody using biorthogonal click chemistry [89].

Using this strategy, the antibody is modified with a cycloalkane to produce a highly immunoreactive complex that is systemically administered without radioactivity. Twenty-four hours post injection, a NOTA-modified tetrazine, which binds with high affinity to the cycloalkane, is conjugated to a positron emitter and the radioactive tag is administered. The radionuclide then rapidly binds to the prelocalized antibody and the radiolabeling occurs in situ, allowing sufficient time for antibody clearance and optimal target-site binding. While advancements in radiolabeling for immuno-PET are ongoing, characterizing GMP standards for radionuclide and chelator production, radiochemical purity, and stability data, in addition to retention of pharmacokinetic properties remain paramount for successful patient translation.

Currently, there are over 30 ongoing clinical trials evaluating the utility of immuno-PET in multiple cancer types using FDA-approved and experimental antibodies. Clinical trials using ^{89}Zr as a positron-emitter (Table 2) include studies utilizing immuno-PET to monitor treatment response, detect recurrent cancers, and patient screening for tailored therapies. Beyond simple detection, some trials are focused on using immuno-PET for molecular characterization of the tumor. For example, “*Treatment Optimization of Cetuximab in Patients With Metastatic Colorectal Cancer Based on Tumor Uptake of ^{89}Zr -labeled Cetuximab Assessed by PET*” (Clinicaltrials.gov: NCT01691391) is using ^{89}Zr -cetuximab to assess K-Ras mutation and explore the relationship between cetuximab uptake and treatment response in colorectal cancer. In this study they are using immuno-PET to ascertain whether differences in treatment response are due to antibody pharmacokinetics or molecular mutations within the tumor. Further highlighting the widespread utility of immuno-PET, the phase 2 HER2 imaging trial, “*HER2 Imaging Study to Identify HER2 Positive Metastatic Breast Cancer Patient Unlikely to Benefit From T-DMI (ZEPHIR)*” (Clinicaltrials.gov: NCT01565200) is using ^{89}Zr -trastuzumab to screen potential candidates for successful T-DMI cytotoxic agent therapy based on HER2 expression, as determined by ^{89}Zr -trastuzumab immuno-PET imaging.

Results from completed clinical trials have demonstrated high sensitivity and specificity for immuno-PET probes to detect cancer. The first-in-human trial was conducted between 2003 and 2005 at the VU Medical Center in Amsterdam using ^{89}Zr -cmAb U36 [90,91]. In 20 head and neck squamous cell carcinoma (HNSCC) patients, immuno-PET was shown to provide greater sensitivity and specificity than both CT and MRI in positively identifying primary tumors and 18/25 metastatic lymph nodes. In another trial evaluating HER2 expression using ^{89}Zr -N-SucDf—labeled trastuzumab to detect metastatic breast cancer, immuno-PET detected all known lesions in six of 12 patients while identifying unknown lesions that were later determined positive for metastasis [92]. In yet another immuno-PET trial using the ^{124}I positron emitter, ^{124}I -labeled anti-CA9 chimeric cG250 was used to positively identify clear cell carcinomas in 15 out of 16 patients while correctly diagnosing nine patients with nonclear cell renal masses (sensitivity of 94%, specificity of 100%)[93].

Radiolabeled antibodies provide a gateway for personalized imaging to preselect patients that would benefit from antibody use. In addition, immuno-PET may be used as a companion diagnostic to preselect patients for conventional antibody based therapy. Furthermore, as the technology becomes more established, immuno-PET may select patients

for photoimmunotherapy or fluorescence-guided surgery using patient-specific antibodies conjugated to near infrared dyes. While these advantages are apparent, logistical hurdles exist that must be overcome. Firstly, the cost and availability of positron emitting radionuclides and commercially available chelators has hindered widespread clinical development. However an increasing number of institutions are now installing on-site cGMP facilities to produce in-house radiolabeled compounds, which provides a significant opportunity for development. Another challenge facing the widespread use of immuno-PET is reducing the radiation dose. Patient exposures of up to 40mSv are common for most ^{89}Zr doses, which are relatively high and eliminate the opportunity for repeated administrations [94]. To solve this problem, ongoing work is being performed to introduce more sensitive PET scanners that would require less radiation while providing the same resolution and sensitivity as current scanners.

Antibody-based phototherapy

Photodynamic therapy (PDT) has been a treatment modality for over several decades that employs a systemically injected nontoxic light-sensitive compound (photosensitizer), which can serve as both a diagnostic and therapeutic agent. Photosensitizers absorb light at a certain wavelength and generate measurable fluorescence as well as highly cytotoxic singlet oxygen molecules [95]. Photosensitizers accumulate within the tumor due to their higher metabolic activity compared to the surrounding normal tissue, which allows some selectivity between normal tissues and cancer. Light of the appropriate wavelength will excite the photosensitizer for fluorescence imaging, but will also generate cytotoxic singlet oxygen molecules which directly kills tumor cells [96]. Furthermore, indirect PDT-mediated effects induce damage to the vascular system resulting in hypoxia and deprivation of nutrients [97-99]. Collapse of tumor circulation is followed by a strong inflammatory reaction [100], which includes the phagocytosis of PDT-damaged tumor cells by macrophages. These macrophages are capable of antigen presentation, which promotes tumor specific immunity.

Use of PDT has been limited to treatment of tumors that are anatomically accessible for repeated light-based treatments including head and neck cancer [101-107], locoregional breast recurrences [108,109], and skin cancers (squamous cell carcinoma and basal cell carcinoma) [110-112]. However, the off-target uptake of the photosensitizer in normal surrounding tissue results in skin photosensitivity and normal tissue damage. As a result, PDT is currently only applied for palliative treatment of cancers that are not amenable to curative therapy. This is in part because of the damage to adjacent normal tissues, but also because this technique limits the histological information available – it is not possible to measure the tumor depth or margin assessment after treatment.

Development of highly selective photosensitizers would have the advantage of tumor specific killing without off-target effects. Antibody-based photodynamic therapy, which is called photoimmunotherapy (PIT), is a highly innovative novel approach designed to improve tumor selectivity. Every tumor has a unique biological profile, which includes the expression of different cell surface antigens [113]. Targeting tumor-specific antigens by using monoclonal antibodies (mAbs) as vectors for selective delivery of photosensitizers

would thereby overcome the severe off-target toxic side effects of conventional PDT, increase therapeutic efficacy, and decrease morbidity.

Conjugation of conventional photosensitizers to antibodies has been reported in the literature using preclinical models but has met with a range of success. Poor results in these studies are attributed to the molar absorption coefficient, i.e. molar extinction coefficient, which is a measurement of how strong a chemical compound absorbs light at a given wavelength. Conventional photosensitizers have low molar absorption coefficients (i.e. absorb photons with low efficiency), meaning that successful therapy requires delivery of a large number of photosensitizers to the tumor. Since there is only a low number of photons per antibody a high dose of the antibody-photosensitizer bioconjugate needs to be delivered or conjugation of a large number of photosensitizers to a single antibody. The first would be associated with antibody-related toxicity and increased costs, whereas the latter would alter the antibody pharmacokinetics, biodistribution [114-118], and the binding affinity [119,120]. Another barrier to success of PIT is the hydrophobicity of conventional photosensitizers, which compromises the physico-chemical properties of the mAb during the conjugation process [121,122]. Furthermore, conventional photosensitizers absorb light in the visible range (400-750nm). Because molecules like hemoglobin and lipid that are physiologically abundant in tissue largely resorb in the visible spectral range, excitation of the mAb-photosensitizer conjugate is reduced.

Recent developments of the near-infrared (NIR) phthalocyanine dye, IRDye700DX (IR700) as a suitable photosensitizer in PIT has provided significant promise for targeted photodynamic therapy due to its highly desirable chemical properties [123]. IR700 bears a more than fivefold higher molar absorption coefficient (at the absorption maximum of 689nm) than conventional photosensitizers [124]. Moreover, IR700 is a relatively hydrophilic dye that can be easily conjugated to antibodies without compromising immunoreactivity and *in vivo* target accumulation. Another highly desirable feature of PIT using fluorescent mAb-IR700 conjugate is that the NIR light excitation wavelength (peaking at 689 nm) penetrates tissue significantly better, has limited absorption by biological tissues and is also a conventional optical dye which can be used to visualize the tumor to be treated with PIT [125]. As newer generations of photosensitizing dyes become available, the potential of this technology for treatment and imaging needs to be re-evaluated.

In cancer treatment, the extent of cytoreduction greatly influences patient prognosis with clear surgical margins defining outcomes in most solid tumor types. Use of adjuvant post-ablation PIT after surgical excision may have significant patient benefits. Conventional surgical resection is often incomplete due to microscopic residual disease or in transit metastasis, which are often managed with post-operative radiation or chemotherapy. To improve patient outcomes, a more detailed detection and elimination of residual tumor tissue is crucial. It is possible that PIT allows for visualization of microscopic residual disease through optical imaging (Figure 4a) and direct subsequent light based phototherapy to eliminate residual microscopic disease (Figure 4b-c). A major advantage over current treatment modalities is that the therapeutic effects of PIT can provide real-time information at the conclusion of the oncologic surgery, thereby aiding decision making whether additional doses of NIR light exposure are necessary or not during the same surgical

procedure [126]. Potential for a ‘personalized surgical intervention’ may decrease the extent of resection and the need for adjuvant treatment.

The advantages of tumor targeting may be obvious, however successful clinical implementation may depend on finding the appropriate target for high tumor specificity and limiting accumulation in normal tissues. For PIT to be effective it is required that the antibody conjugate is distributed homogeneously throughout the tumor or among residual disease. The expression of tumor-associated antigens varies enormously between and within tumors. Consequently, thorough research is required to identify the best tumor-specific target for each tumor type. Fortunately, to date, over 25 tumor associated antigen targeting antibodies have been approved by the US Food and Drug Administration [127,128], several of which are in clinical trials for optical imaging (clinical trials.gov, cetuximab and bevacizumab). Moreover it may be possible that a cocktail of antibodies targeting a range of antigens may yield better PIT results and thereby overcome the problem of the high degree of diversity and heterogeneity in tumor antigen expression [129].

Conclusion

The main advantages to antibody-based imaging in the field of oncology are low toxicity and high specificity. A plethora of imaging fields, as previously described, have the capability to incorporate antibody-based techniques into their unique field creating a universal avenue to improve overall cancer imaging. It has been well established that in preclinical cancer investigations, these techniques improve overall detection, monitoring and therapy. The future of antibody-based techniques in clinical cancer imaging will significantly advance cancer identification and monitoring.

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Figure 1.
Concept of personalized approach to cancer-specific imaging using antibodies strategically targeted to ideal antigen.

Figure 2.
Modality-specific probes available for antibody labeling for the purpose of cancer imaging and localization.

Figure 3.

Functionalizing contrast agents through attachment of antibodies on the surface of the microbubbles creates a molecular ultrasound imaging strategy to target specific receptors on the endothelium, thereby improving localization and further increasing vasculature visualization.

Figure 4.

Prior to surgery, the mAb-photosensitizer construct is administered systemically. Targeting a tumor-specific antigen with the fluorescent photosensitizer by means of a tumor-targeted monoclonal antibody will allow for real-time fluorescent guided surgery (A), but will also will generate highly reactive singlet oxygen molecules which directly kills unresectable microscopic residual disease (B-C).

Table 1

Radionuclides available for antibody-based nuclear imaging

PET				SPECT		
Radionuclide	Half-life	Residualizing	Radionuclide	Half-life	Residualizing	
⁸⁹ Zr	78.4 h	+	¹¹¹ In	67.3 h	+	
¹²⁴ I	100.3 h	–	¹³¹ I	192.5 h	–	
⁶⁴ Cu	12.7 h	+	¹²³ I	13.2 h	–	
⁸⁶ Y	14.7 h	+	^{99m} Tc	6.0 h	–	

Table 2
Optical probes available for diagnostic purposes

	Cy5/7 dyes	ICG	Fluorescein (FITC)	IRDye700/ 800
Manufacturer:	GE Healthcare	Various	Various	LICOR
Human use:	No	Yes	Yes	Yes
Functional group:	Yes	No	Yes	Yes
Available cGMP:	No	Yes	Yes	Yes
Near-infrared emission:	Yes	Yes	No	Yes

Table 3
Ongoing clinical trials using ^{89}Zr labeled antibodies for Immuno-PET imaging of cancer

Biological agent	Trial phase	Condition	Clinicaltrials.gov Identifier	Sponsor
RO5429083	I	Malignant solid tumors	NCT01358903	Hoffmann-La Roche
Hu1591	I, II	Prostate cancer	NCT01543659	Memorial Sloan-Kettering Cancer Center
Df-IAB2M	I, II	Metastatic prostate cancer	NCT01923727	Memorial Sloan-Kettering Cancer Center
MSTP2109A	I, II	Prostate cancer	NCT01774071	Memorial Sloan-Kettering Cancer Center
MMOT0530A	I	Ovarian, adnexal, pancreatic, digestive system neoplasms	NCT01832116	University Medical Centre Groningen
Cetuximab	Pilot	Colorectal cancer	NCT01691391	VU University Medical Center
Bevacizumab	0	Inflammatory breast carcinoma	NCT01894451	Dana-Farber Cancer Institute
Trastuzumab	Pilot	Esophagogastric cancer	NCT02023996	Memorial Sloan-Kettering Cancer Center
Trastuzumab	II	Metastatic breast cancer	NCT01832051	University Medical Centre Groningen
Trastuzumab	I, II	Metastatic breast cancer	NCT01957332	University Medical Centre Groningen
Bevacizumab	Pilot	Metastatic renal cell carcinoma	NCT01028638	University Medical Centre Groningen
Trastuzumab	II	Breast cancer	NCT01565200	Jules Bordet Institute
Trastuzumab	I	Breast cancer	NCT01420146	Jules Bordet Institute
Cetuximab	I	Stage IV cancer	NCT00691548	Maastricht Radiation Oncology
RO5323441	II	Glioblastoma	NCT01622764	University Medical Centre Groningen
Bevacizumab	Pilot	Multiple myeloma	NCT01859234	University Medical Centre Groningen