Clinics in Surgery

Review Article
Published: 06 Jun, 2017

Evolution of a System to Increase Precision in the Surgical Management of Colorectal Carcinoma

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Abstract

Current surgical procedures for colorectal adenocarcinoma are plagued by a lack of precise information provided by preoperative imaging and the surgeon's exploration of the surgical field using traditional techniques (i.e., inspection and palpation). The staging of colorectal adenocarcinoma begins with preoperative imaging and ends with the pathologist; however, potential sources of error between these two points may result in suboptimal treatment impacting outcome. Using colorectal adenocarcinoma as a model, we developed a System incorporating currently available technologies to increase the precision of tumor imaging before and during surgery as well as intraoperative tumor detection.

The multimodal System focused on the patient evolved over 35 years. The System brings together essential resources (i.e., molecular probes, imaging modalities and detection devices) and expertise of various clinical specialties (i.e., Nuclear Medicine, Oncology, Pathology, Radiology, Radiation Oncology and Surgery) for precision diagnosis and optimal treatment.

Although the diagnosis and treatment of colorectal adenocarcinoma was the focus throughout the System's development, it is applicable to other adenocarcinomas.

Developing the System to increase the precision in the surgical management of colorectal adenocarcinoma began with the selection of the tumor-related antigen, tumor associated glycoprotein-72 (TAG-72). Generations of anti-TAG-72 monoclonal antibodies radiolabeled with ^{125}I were safely used as molecular probes. During surgery, a hand-held gamma probe was used for the detection and excision of TAG-72 positive tissues. Long term follow-up of patients with primary colorectal adenocarcinoma demonstrated a survival advantage in those who TAG-72 "Status @ Closing" was negative. Our proof-of-concept studies demonstrated that this System increases the surgical precision, and thus the quality of care, for individual patients. The proposed use of bioengineered anti-TAG-72 monoclonal antibody fragments radiolabeled with ^{123}I for hand-held gamma probe detection along with pre- and post-resection intraoperative gamma imaging would more precisely answer the question, "Did you get it all?" for those patients with colorectal and other adenocarcinomas.

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Received Date: *03 Nov 2016*
Accepted Date: *22 May 2017*
Published Date: *06 Jun 2017*

Citation:
Hitchcock CL, Magliery TJ, Mojzisik C, Johnson M, Arnold MW, Martin EW. Evolution of a System to Increase Precision in the Surgical Management of Colorectal Carcinoma. Clin Surg. 2017; 2: 1491.

Introduction

The need for precision

Over 1,685,000 new cancers will be diagnosed in the U.S. in 2016, excluding keratinocyte carcinoma. Of these, approximately 85% will be carcinomas with adenocarcinomas making up the majority. Adenocarcinomas of the colon and rectum constitute 134,490 of these. However, the prevalence of adenocarcinomas is four times the incidence rate, which equates to 621,430 patients living with colorectal carcinoma in 2016 [1].

The National Comprehensive Cancer Network (NCCN) developed guidelines and clinical resources to help physicians treat, detect, prevent, reduce risk, provide supportive care, and image a large number of different cancers, including colorectal adenocarcinoma [2]. The TMN staging criteria forms the platform for the guideline for colorectal carcinomas, and its accuracy is critical to

treatment selection and planning. The staging of these tumors begins with preoperative imaging and ends with the pathologist, but there are many potential sources of error between these two points that can impact patient treatment and outcome. In the case of colorectal carcinomas, despite these evidence based guidelines, more than 40% of patients who underwent a "curative resection" of a primary tumor will have recurrent disease, and patients with the same stage of colorectal adenocarcinoma can differ in their clinical course. The reason is a lack of precision.

The National Institutes of Health (NIH) defines the term "Precision Medicine" as "an emerging approach for disease treatment and prevention that takes into account individual variability in genes, environment, and lifestyle for each person [3]." In the case of colorectal adenocarcinoma, the complete removal of all tumor-bearing tissue requires precision in the localization and detection of intraabdominal metastatic disease before and during surgery. There are several factors that impact this precision.

The NCCN guidelines call for using computerized tomography (CT) scans with contrast for preoperative imaging, needed for surgical planning for resection of primary and recurrent disease. This includes respectability of the primary tumor and assessment of the presence of metastatic disease that alters the surgical approach or mandates non-surgical therapies. Despite providing anatomic information, poor spatial resolution has an adverse effect on the specificity and sensitivity of conventional and contrast CT imaging to detect lymph nodes smaller than 5 mm that often contain metastatic disease [4]. The end result is a wide range of reported specificity from only 42% to 70% [5-9].

Patients often ask "Did you get it all?" Current surgical procedures are based on surgical anatomy and traditional planes of resection that are easily violated by cancer cells. Variation in surgeon experience influences the type of tumor resection and surgical precision. Traditional visual and manual evaluation does not necessarily provide surgeons with intra-operative information needed to obtain, not just for a curative resection, but a resection that actually cures. As one of us has previously noted, "[I]f surgeons had real-time information regarding the precise location of all disease and had a real-time assessment of surgical resection margins, they would be able to intervene immediately to accomplish a complete resection without subjecting the patient to subsequent additional surgical procedures [10]."

Advances in Precision Medicine are getting underway. This paper examines how our diverse group of physicians, basic scientists and engineers brought currently available resources and developed new ones that have evolved over 35 years into a multimodal System that provides the surgeon with the approach and tools needed to increase the precision of tumor imaging and detection, before and during surgery, for the individual patient with a solid tumor. Although our focus is on colorectal adenocarcinoma, the proposed system applies to the majority of adenocarcinomas that arise in other organs.

A System to Increase Precision Management of Colorectal Cancer Patients

System components

The components of our multimodal System are seen in Figure 1. With the patient at its center, the System integrates physicians from Nuclear Medicine, Radiology, Surgery, Oncology, Radiation Oncology and Pathology with the tools needed for a more precise

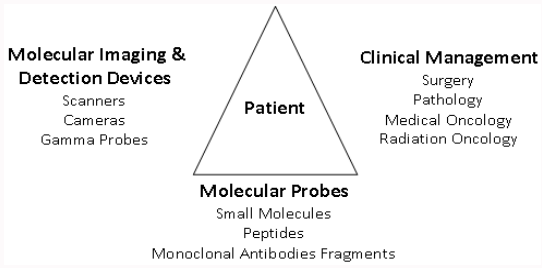

Figure 1: The System.

diagnosis and treatment of the patient's cancer. Molecular probes, specific for the patient's tumor, are the foundation of the System. Based on the results of the initial biopsy and/or laboratory studies, the Pathologist recommends the appropriate molecular probe to be used. Labeling of the molecular probe is dictated by the type of molecular imaging and intraoperative detection devices. The results of molecular imaging determine optimal treatment, such as surgery or undergoing chemotherapy and/or radiation therapy. Precise imaging provides the surgeon with a "mine field map" of where to go with the intraoperative hand-held gamma-detection probe to find and remove the tumor containing tissue. Intraoperative imaging provides real-time verification of complete resection. From a systems standpoint, the complete resection of all tumors is globally cost effective.

The System begins with the patient and their solid tumor. The tumor's pathologic features are used to select the appropriate tumor specific or associated molecular probe and radionuclide or non-radioactive label. The labeled-molecular probe dictates the type of devices that can be used for preoperative and intraoperative imaging and for intraoperative detection. The results of imaging and/or intraoperative detection will aid in treatment decision making before and/or after tissue examination by Pathology.

Molecular probes

In contrast to the anatomic information provided by CT and MRI imaging, molecular imaging is a diagnostic modality that provides functional information about molecular makeup of tissue. Molecular imaging uses a variety of radiolabeled molecular probes for positron emission tomography (PET) and single-photon emission computed tomography (SPECT) alone or in combination with CT or magnetic resonance imaging (MRI). Constantly evolving, molecular imaging provides the necessary versatility needed for the System's multimodality approach to increasing the precision of cancer surgery [11]. There are several categories of tumor-related molecular probes available for molecular imaging [12]. They include small molecules that bind intracellular targets, small peptides that bind to membrane receptors, and monoclonal antibodies (MAbs) and bioengineered MAb fragments that bind to tumor-related antigens. Ongoing studies are directed at the production of molecular probes that rapidly enter the tumor and bind to the specific target in the tumor, lack uptake by non-target tissue, and rapidly clear from the blood and normal tissue. The end result of this optimization is a reduction in unwanted background that will yield the maximum signal-to-noise for the probe [13].

Categorized as a small molecule molecular probe, [^{18}F]-2-fluoro-2-deoxyglucose (^{18}F-FDG) is widely used for preoperative PET or PET/CT imaging of patients with cancer, monitoring patients for recurrent disease, and more recently for assessing response to therapy [14]. However, ^{18}F-FDG is not cancer specific. As a glucose analog,

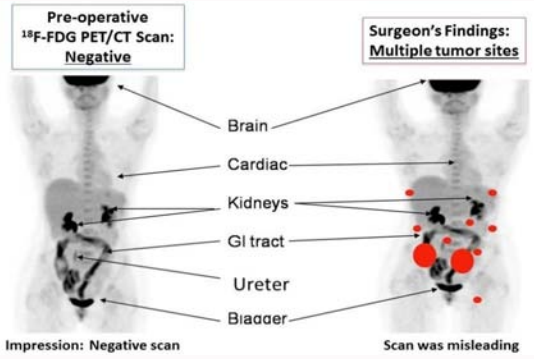

Figure 2: ^{18}F-FDG PET/CT of Patient with Recurrent Colon Cancer.

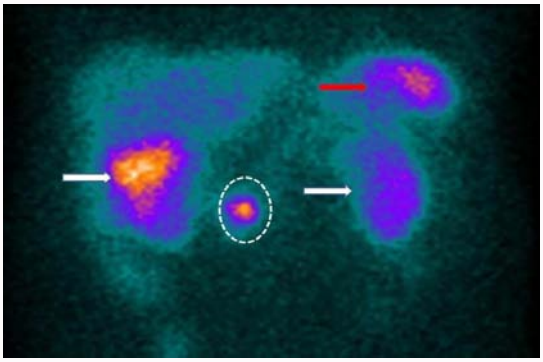

Figure 3: SPECT/CT Scan of ^{111}In-pentetreotide-positive gastrinoma.

FDG is taken up into cells with a high metabolic rate. This includes cells within tumors, normal organs (e.g., brown fat, myocardium, brain, gastrointestinal (GI) tract, thyroid, liver and spleen), inflammatory responses (e.g., infections granulomas and immune hyperplasia), and wound healing. In addition, FDG accumulates in the kidneys and bladder prior to its excretion in the urine [15]. These result in false positive findings. In addition, tumors with a low metabolic rate do not take up FDG. False negative PET and PET/CT scans often occur with invasive bronchioloalveolar carcinoma and carcinoid tumors in the lung, renal cell carcinomas, hepatomas, mucinous tumors of the gastrointestinal tract, and low grade non-Hodgkin lymphomas. The false positive and false negative rates call into question the precision of ^{18}F-FDG PET and ^{18}F-FDG PET/CT for pre- or perioperative staging of tumors [16,17]. The reported sensitivity of ^{18}F-FDG PET/CT for the detection of lymph node metastases is reported as low as 43% for colorectal carcinomas, which is below the needed diagnostic precision for our System (Figure 2) [18].

The left image is the pre-operative PET/CT scan that was interpreted as negative for cancer. Nonspecific uptake of the ^{18}F-FDG was present in the brain and GI tract and compared to the kidneys, ureter and bladder where the molecular probe accumulated. The right image correlates the surgical findings (orange dots) with the same ^{18}F-FDG-PET/CT scan. This false negative finding led to unneeded surgery.

Small peptides of no more than 15 amino acid are used for both SPECT and PET molecular imaging, alone or in combination with CT. These molecular probes act as ligands for various membrane receptors, the most common of which is the multiple types of somatostatin receptors on neuroendocrine tumors (NETs). They have excellent specificity, stability, and low immunogenicity, but are prone to proteolysis [12,19,20]. Radionuclide labeling generally requires an intermediate chelator attached to the peptide. As most gastrinomas and other foregut neuroendocrine tumors (NETS) over express somatostatin receptors, somatostatin receptor imaging using SPECT/CT is the method-of-choice for pre- and/or perioperative staging of gastrinomas (Figure 3) [21,22]. However, the advent of PET/CT probes will replace them in the future, especially for midgut and hind gut NETs [23].

Large field-of-view gamma camera (SPECT) scan of ^{111}In-pentetreotide bound to somatostatin receptors on a gastrinoma cells (dotted circle). There is non-specific uptake in the spleen (red arrow) and accumulation in the gallbladder (right white arrow), and in the kidneys (left kidney- white arrow, right kidney behind the gallbladder). Note the poor spatial resolution.

Monoclonal antibodies (MAb), directed against tumor-related antigens, are being developed as molecular probes for molecular imaging and/or therapy. The efficacy of a given MAb is limited by the type of tumor(s) and the level of expression of the target antigen. Ideally, MAb molecular probes exhibit the properties listed on Table 1. Several different MAbs have been approved by the FDA for molecular imaging [12], the majority of which have been labeled for SPECT imaging. However, numerous other monoclonal antibodies and their bioengineered counterparts are working their way through the clinical trial steps needed for Food and Drug Administration (FDA) approval for molecular imaging both SPECT ^{123}I and PET ^{124}I modalities.

The development of MAbs to meet these desired properties resulted in multiple generations of monoclonal antibodies and their biochemical and genetic engineered protein fragments of antibody molecules (Figure 4). The clinical utility of first generation intact murine IgG molecules was limited by their large size, accumulation

Table 1: Desired Properties of Monoclonal Antibodies for Molecular Imaging.

Features	Desired Properties
Specificity	Specific to tumor factors with no cross reactivity with normal tissue
Affinity	Ability to bind the tumor-related antigen tightly
Avidity	Slow ~~off~~ ***decay?*** rates lead to longer the tumor-related antigen binding times
Uptake	Rapid penetration into the tumor
Clearance Kinetics	Rapid clearance of unbound MAb from the circulation
Low Background	Minimal accumulation in normal tissue
Humanized Protein	No generation of human anti-mouse antibodies (HAMA)
Labeling	Able to be labeled with radionuclides and/or other tracers (e.g., fluorophores) for multimodal detection
Stability	Long shelf life

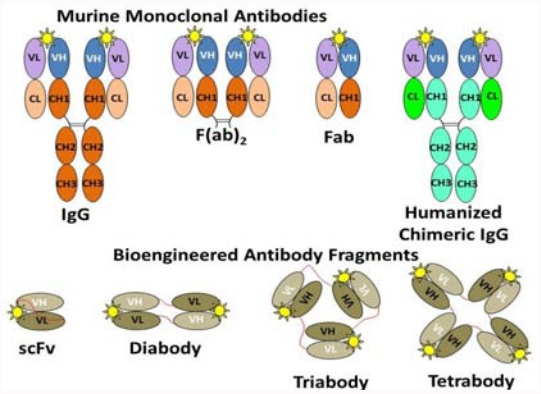

Figure 4: Types of Antibody-Derived Molecules for Molecular Imaging.

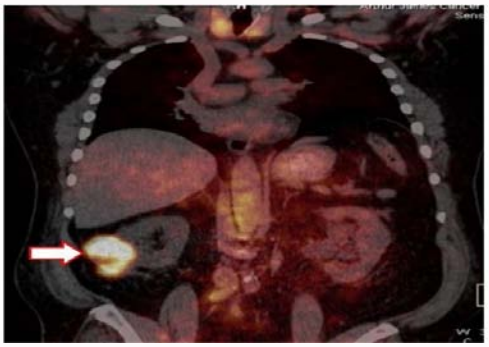

Figure 5: Molecular Imaging of Clear Cell Renal Cell Carcinoma.

in non-target tissue, long serum half-lives, and immunogenicity that induced the formation of human anti-mouse antibodies (HAMA). Their slow clearance and slow uptake in the target tissue necessitated that they be labeled with radionuclides with longer half-lives [^{111}In (2.8 days), ^{89}Zr (3.3 days), ^{124}I (4.2 days), or ^{125}I (60 days)] [24]. Enzymatic digestion of the intact IgG molecules gave rise to smaller F(ab)$_2$ and Fab fragments with better pharmacokinetics; however, these were still immunogenic. Genetic engineering directed at minimizing the immunogenicity resulted in chimeric IgG molecules (Figure 4) that contain amino acids of the murine variable regions attached to the human constant regions. Fully humanized MAbs (not shown) containing only 5% murine-derived amino acids from the antigen binding site [25].

Development of MAb fragments for the desired properties for a given application resulted in small single-chain variable fragments (scFv) and their diabodies. The scFv is monomeric with a 12–15 amino acid linker (Figure 4 - red line) between the V_H and the V_L domains. Linker composition and length can have a significant impact on antigen binding and stability. Diabodies contain two non-covalently associated scFv-like fragments that interact with and bind to their corresponding antigen in a divalent manner. Tribody and tetrabody molecules of these scFv fragments are also possible. The scFv fragments of bispecific diabodies (not shown) have different antigen binding specificities. When compared to intact IgG, F(ab)$_2$ and Fab fragments, scF vs. and diabodies have faster clearance with excellent tumor penetration, and higher tumor-to-blood ratios. The result is low background and a high signal-to-noise ratio resulting in increased precision of molecular imaging to identify malignant tissue [26,27].

Tuning antibody fragments to the exact molecular imaging application remains a significant frontier for engineering and development. The fragment size can be adjusted by genetic engineering, linker manipulation, and chemical modification (for example with PEG, an inert polymer of ethylene glycol), but often these modifications result in poor stability, poor or ablated binding, and aggregation. But adjustments in fragments size translate into adjustments in clearance time suitable for different imaging time lines, modalities and sensitivities.

Yellow star represents the same antigenic epitope attached to the antigen binding site of each of the MAb molecules and their fragments. The murine variable regions are fused to human constant regions to give rise to the humanized and chimeric IgG molecules. The scFv and its corresponding diabody, triabody, and tetrabody variants can contain only a few murine-derived amino acids, which that are essential for antigen binding. The peptide linker between the domains provides many of the desired physical properties of these molecular probes for imaging.

Molecular Imaging & Intraoperative Detection Devices

Detection of tumor-related molecular probes depends on the use of a wide range of radionuclides and non-radioactive labels. The half-life of the radionuclide must be matched to the half-life of the molecular probe to optimize imaging and timing of surgery. As an example, if a particular molecular probe is slow to clear from the blood and normal tissue, then the imaging is delayed for several days or weeks, and the radioisotope with a shorter half-life would not be detected. PET imaging require positron emitting radionuclides, whereas SPECT imaging directly detects photons from gamma emitters.

High energy (511 KeV) radionuclides such as ^{18}F, ^{124}I or ^{68}Ga emit positrons that annihilate electrons, giving rise to two photons that travel in opposite directions and are detected by the PET scanner. PET instruments contain multiple gamma cameras arranged in a circular fashion. More often than not, PET is combined with CT for anatomical information. Lower energy radionuclides such as ^{123}I, ^{99m}Tc, and ^{111}In emit γ-radiation which is detected using planar or tomographical γ-cameras (SPECT). The ability to perform whole body scans and obtain multiple images over time is a major advantage of these types of molecular imaging. The limitless depth of penetration associated with the imaging use of radionuclide-labeled molecular probes induces a loss of spatial resolution due to the inverse square law of intensity as a function of distance. Combining CT or MRI with PET or SPECT along with the ongoing development of new generations of tumor–specific MAbs will only increase the precision of molecular imaging by providing both anatomic and more precise functional localization of primary and metastatic malignancies. As an example, tumor–specific MAbs labeled with high energy molecular probes have been shown to provide high specificity and sensitivity in detecting tumors in patients with clear cell renal cell carcinoma [28] (Figure 5).

PET/CT ^{124}I MAb cG250 (arrow) with clear cell renal cell carcinoma in the lower pole of the right kidney. Focal molecular probe also labels the thyroid glands.

Hand-held gamma probes (HGPs), and to a lesser extent laparoscopic gamma detecting probes, are used for intraoperative detection of radiation that is unbound or bound to a molecular probe

Table 2: Distribution and Survival of Different Stages of Colorectal Adenocarcinomas [35].

Invasive Adenocarcinomas	Stages I-II Localized		Stage III Regional		Stage IV Distant		Unstaged	
	% of cases	5-Year Survival	% of cases	5-Year Survival	% of cases	5-Year Survival	% of cases	5-Year Survival
Colon/Rectum	49%	90.1%	35%	71.2%	21%	13.5%	5%	35.5%

Table 3: Incidence of Adenocarcinomas and TAG-72 Positive Adenocarcinomas.

Organ	**2015 Number of Adenocarcinomas** (1)	**~ % TAG-72 (+) Adenocarcinomas (Number of Cases)** [36,37]
Breast	**210,771**	55% (115,924)
Lung	**91,798**	80% (72,438)
Prostate	**209,760**	80% (167,808)
Colon & Rectum	**135,565**	85% (115,230)
Endometrium	**29,630**	91% (26,963)
Pancreas	**47,021**	90% (42,319)
Stomach	**23,261**	55% (12,794)
Ovary	**18,100**	88% (15,928)
Esophagus	**8,660**	60% (1,443)

[24,29,30]. Widely available, these probes are either like a gamma camera, or they are solid state detectors containing a semiconductor crystal. Our studies have primarily employed a HGP containing a cadmium telluride (CdTe) crystal linked to a control unit that provides both numerical information and an auditory signal when the radioactivity is above three standard deviation above the background radiation [31]. Their precision for routine use in radioguided surgery is operator dependent. The surgeon may not go outside of the planned surgical field or they may not be aware of the instruments restricted field of view or that the sensitivity and specificity increases as the probe moves closer to the source of radiation [24]. The precision of the surgeon, using the HGDP, is enhanced by utilizing intraoperative portable gamma camera that provides real-time intraoperative localization of the low-energy radionuclide labeled molecular probes. The use of these nuclear medicine instruments allows the surgeon in real-time to determine the success of the operation and whether or not he or she *"got it all."*

Commercially available fixed gamma cameras collect the low energy emission to produce a planar image that can be used in surgery to provide real-time images. Small gamma cameras are hand-held and are easily used for intraoperative imaging. However, these instruments take 10-60 seconds to generate an image which may be less than optimal due to an unsteady hand. Larger, portable, gamma cameras require stabilization and can have either a small field of view (5×5 cm^2) or large field of view ($>5 \times 5$ cm^2) such as seen in Figure 3 [32]. We and others have used intraoperative gamma cameras for intraoperative imaging of sentinel lymph nodes, parathyroid adenomas, and a variety of tumors including: gastrinomas, head-and-neck squamous cell carcinomas, breast cancer, and melanoma [28,32-34]. Gamma cameras have a larger field of view than the HGDP, and thus provide the surgeon with a unique visual assessment of the extent of disease and its complete resection.

A System Engineered to Increase Surgical Precision for Colorectal Carcinoma

Based on initial conventional imaging studies, up to 80% of patients with colorectal adenocarcinoma lack clinical stage IV disease and undergo curative surgery with or without adjuvant therapy. (Table 2) However, more than 40% of these patients will have recurrent disease, which primarily occurs in the lymph nodes, liver and/or lungs. The best survival potential for patients undergoing curative surgery for colorectal adenocarcinoma is the complete removal of all tissue containing tumor. One has to remember the adage that *it's not what the surgeon removes during surgery that kills the patient, but it is what is left behind* (residual cancer).

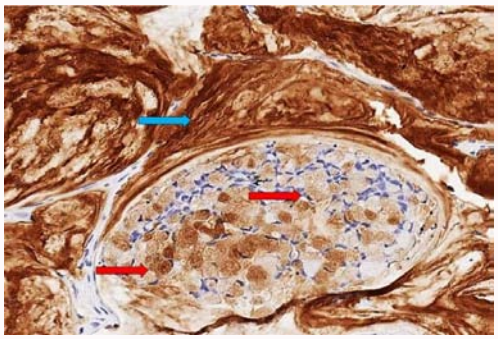

Figure 6: Adenocarcinoma of the Colon - Immunohistochemical Staining TAG-72 Antigen.

The proposed "System" brings together the surgeon, radiologist, nuclear medicine physician, and pathologist in order to increase the precision of "getting it all." Using molecular imaging, they identify where the malignant tumor sites are and intraoperatively refine the "map" to ensure that the surgeon does a more complete resection. Increasing the precision of intraoperative detection of tumor will increase the pathologist's ability to "physiologically," as well as anatomically, stage the tumor. In the last 35 years, our group generated several lines of evidence supporting this clinical claim, especially for colorectal adenocarcinomas.

The "System" begins with the selection of the most appropriate tumor-related antigen. For colorectal carcinoma we selected Tumor Associated Glycoprotein-72 (TAG-72). TAG-72 is an oncofetal antigen that is expressed by the majority of human adenocarcinomas (Table 3). TAG-72 is a large mucin-like molecule consisting of 80% carbohydrate moieties [27].

Immunohistochemical staining for TAG-72 (Figure 6) demonstrates these molecules in cytoplasmic vacuoles of the tumor

Table 4: Evolution of Anti-TAG-72 Monoclonal Antibodies.

Anti-TAG-72 Antibody	Generation (Year)	Type	Size	Radionuclide (Half-life)	MAb Biologic Half-life
B72.3	1st (1981)	Murine IgG	150kD	^{125}I (60 days)	2-3 days
CC49	2nd (1988)	Murine IgG	150kD	^{125}I (60 days)	2-3 days
CC83	2nd (1988)	Murine IgG	150kD	^{125}I (60 days)	2-3 days
HuΔCH2CC49	3rd (1997)	CDR-Humanized CH2 Domain Deleted	125kD	^{125}I (60 days)	18 hrs
3E8	4th (2006)	SDR-Humanized CC49 IgG	~150kD	^{125}I (60 days)	2-3 days
3E8 Proteins	5th (2015)	SDR-Humanized scFv, diabody tetrabody	~75kD	^{124}I (4 days) ^{123}I (13 hrs)	2-3 hrs

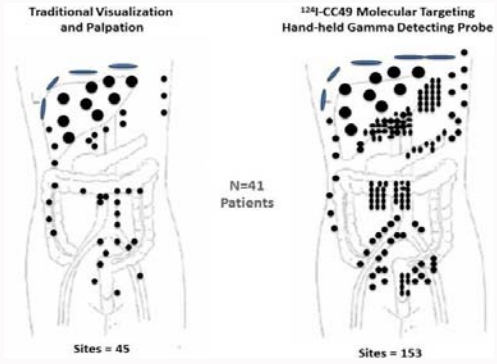

Figure 7: Intraoperative Tumor Location of Metastatic Disease: Traditional Visualization and Palpation vs. Hand-Held Gamma Detection Probe (HGDP) of Occult Tumor Binding ^{125}I-CC49 in 41 cases of primary colorectal adenocarcinoma [45].

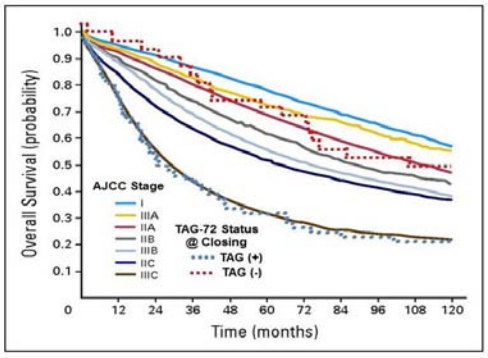

Figure 8: Ten Year Patient Survival: Traditional Surgery vs. HGDP Intraoperative Detection.

cells that release the molecule into the lumen of tumor acini and into the extracellular matrix where it accumulates. The extracellular accumulation of TAG-72 facilitates its targeting by radiolabeled-antibodies and subsequent localization by molecular imaging. These features results in the ideal target molecule for molecular imaging and intraoperative detection.

The TAG-72 molecule is a complex array of different antigenic epitopes, to which multiple MAbs have been developed [38]. Of these, we selected B72.3 murine MAb and its subsequent generations. The evolution of antibodies to TAG-72 followed the prescribed path previously noted for MAbs as molecular probes (Table 4). The initial four generations of antibodies to TAG-72 were generated in the same laboratory at the NIH [39-41], and were used by us to increase the precision of radioimmunoguided surgery (RIGS) in an attempt to detect all tumor in real-time and to remove it from patients with either primary or recurrent colorectal adenocarcinoma [29].

Clinical studies using the first three generations of the murine anti-TAG-72 MAbs were complicated by several factors. The immunogenicity of murine IgG molecules resulted development HAMA, whose only clinical significance was interference with several clinical laboratory tests [42]. That fact that these were whole IgG molecules with a long half-life required labelling with ^{125}I with half-life of 60 days. These resulted there being a delay of up to four weeks before surgery was performed and a tumor-background (signal-noise) ratio of 2:1. The somewhat smaller size of the 3rd generation MAb to TAG-72 doubled the tumor-background ratio and halved its clearance time to allow for an improved time to surgery, and did not induce significant HAMA [43,44].

Numerous clinical studies have used one of these first three generations of ^{125}I-labelled MAbs to TAG-72 to study over 1,000 patients with either primary or recurrent adenocarcinomas, with a focus on colorectal carcinomas. (Reviewed in 29, 31) Figure 7 demonstrates the increased precision by which the surgeon can detect remove TAG-72 containing metastatic disease using a HGDP as compared to that obtained by traditional visual inspection and palpation [45]. These findings, found in numerous other studies [46-50], had significant impact in altering clinical decision making in up to 50% of cases. These decisions included abandoning surgery due to extensive disease (e.g., carcinomatosis), increasing the area of resection, and up-staging leading to adjuvant chemotherapy [45-54].

The increased precision that intraoperative detection and removal of occult metastatic disease provides a significant survival advantage to patients with primary colorectal adenocarcinoma. (Figure 8) A longitudinal follow-up of 97 patients with primary colorectal adenocarcinoma demonstrated that patient survival at 5, 10, and 15 years [31,55,56] was significantly improved when all of the TAG-72 positive tissue was surgically removed. The TAG-72 "Status @ Closing" is a bimodal, real-time, intraoperative assessment of the patient's survival potential at the time of closing that is independent of the TNM stage. The survival of those patients in the TAG (+) category mimics that of patients with Stage IIIC disease. In contrast, those patients where all TAG-72 containing tissue was removed, classified as TAG (-), regardless of the TNM stage, the survival was consistent with disease confined to the bowel wall with or without minimal nodal involvement.

Based on current AJCC TNM staging criteria, the solid lines represent the 10-year survival for 128,853 primary colon carcinoma patients in the SEER Database [57]. Using the presence [TAG (+ - blue dotted line)] or absence [(TAG (-) – red dotted line] of radioactivity at the time of closing (TAG-72 Status @ Closing) the dotted lines represent survival data from 97 patients that were given ^{125}I-CC49 and subsequently underwent HGDP directed intraoperative detection

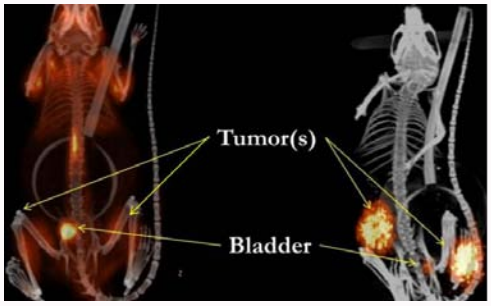

Figure 9: ^{18}F-FDG vs. ^{124}I 3E8 fragment PET Scan Images of Human Colon Cancer Xenografts in Mice.

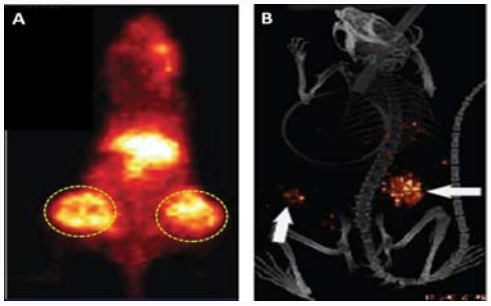

Figure 10: ^{124}I-IgG 3E8 PET scan vs. ^{124}I-diabody 3E8 PET/CT Scan of Human Colon Cancer Xenografts in Mice.

with possible resection of radioactive tissue [56].

TAG-72 positive tissues that lack evidence of tumor on routine H&E staining is considered to be a false-positive finding [50,58]. However, several lines of evidence indicate that this is a misconception. Clinically, the data in Figure 8 indicates that all TAG-72 positive tissue, regardless of H&E staining status, has clinical significance if left behind. Secondly, the non-regional periportal lymph nodes often contain TAG-72 activity with the HGDP. Subsequent recurrent disease was found in these nodes if they had been previously respected [59]. Just as important, routine pathologic examination of these "false positive" lymph nodes lacks precision. Additional sections submitted for H&E staining and/or immunohistochemical staining demonstrated metastatic disease; however, the detection sensitivity of the light microscope appears to have its limits as well [60-62]. More sensitive molecular studies detected metastatic cells where the microscope could not [63,64].

Many of these previous studies were complicated by the use of the first three generations of murine MAbs to TAG-72. They were potentially immunogenic and their large molecular size resulted in poor pharmacokinetics and the need for ^{125}I labelling with its less than optimal long half-life that delayed surgery up to four weeks after injection [27,63]. Despite these obvious disadvantages, the precision in the surgical management of colorectal adenocarcinoma, as well as other tumors, can be further increased by using the previously mentioned (above) multimodal approach, where the tumor-related antigen TAG-72 is targeted using 5th generation scFv or other fragment MAbs, labelled with radionuclides ^{123}I or ^{124}I with their short half-lives. The excellent pharmacokinetics of these molecules provide little background to impair preoperative and/or perioperative, molecular imaging while facilitating next-day-surgery using a HGDP and intraoperative and post-operative molecular imaging.

This can be accomplished by targeting TAG-72 using humanized single chain Fv fragments (scFv) and its bi- tri- and tetravalent forms (Figure 4). These smaller molecules retain the specificity and affinity of the previous generation murine CC49 (unpublished data). Their small size optimizes their pharmacokinetics, yielding molecular imaging with a much higher signal-to-noise (i.e., tumor-to-background) ratio (unpublished data) as well as providing for same day surgery and intraoperative detection. Studies with xenografts human adenocarcinoma cells clearly demonstrate ^{18}F-FDG and the humanized 4th generation MAb to TAG-72 (3E8), lack the precision obtained using humanized 3E8 fragment, a 5th generation MAb to TAG-72 (Figure 9 and 10).

The clinical significance of this proposed approach has been addressed in recent proof-of-concept (POC) studies that combined pre- and perioperative molecular imaging with intraoperative imaging and the use of a HGDP to ensure that the surgeon "*got it all.*" Gastrinomas often characterized by their over expression of somatostatin receptors on their membrane which bind the peptide ligand ^{111}In-labeled octreotide as a molecular probe for imaging. A proof-of-concept study clearly demonstrated that this probe can be used for preoperative SPECT/CT followed by planar imaging with a portable large field-of-view gamma camera (LFOVGC) before incision, and at the completion of surgery, intraoperatively. The precision of the surgery was furthered by the intraoperative use of a HGDP for locating primary and metastatic tumor [28,34]. A second POC study used the same approach for the molecular imaging ^{99m}Tc-Sestamibi (MIMI) binding to parathyroid adenomas in 20 patients [33]. Although a benign disease, primary hyperparathyroidism requires the resection of the related parathyroid adenomas to prevent development of debilitating sequelae. Resection of involved gland is often complicated by its anomalous location in the neck and mediastinum. The portable LFOVGC was again used to ensure complete resection prior to closure. The resulting increase in precision significantly decreased time in the operating room by reducing the need to confirm complete resection by delaying Parathyroid hormone (PTH) studies until the patient was in recovery [65].

Conclusion

The current guidelines for colorectal cancer surgery do not take into account the limited precision of visual inspection and palpation, and even CT scans, to accurately detect nodal metastases outside of the traditional planes of dissection, which clearly have a significant influence on patient survival. Although used for molecular imaging and for HGDP intraoperative localization and resection of malignant tissue, the use of ^{18}F-FDG-PET/CT for molecular imaging lacks the necessary precision needed to identify these same lymph nodes. The identification and excision of these malignant lymph nodes requires a multimodal System. As proposed here, this System brings together the necessary resources and the expertise of various clinical specialties needed to present the surgeon with real-time, intraoperative, information needed to locate, identify and resects all malignant tissue expressing the radiolabeled molecular probe. That is, the System provides a map of the "tumor's mine field" and the position of the "malignant mines" within it that will allow for their safe removal. Two small proof-of-concept studies used this approach with great success; however, these studies require expansion. The model system for these expanded studies should be one where the number of potential patients is large and the clinical impact can be determined with statistical confidence. We propose that such a

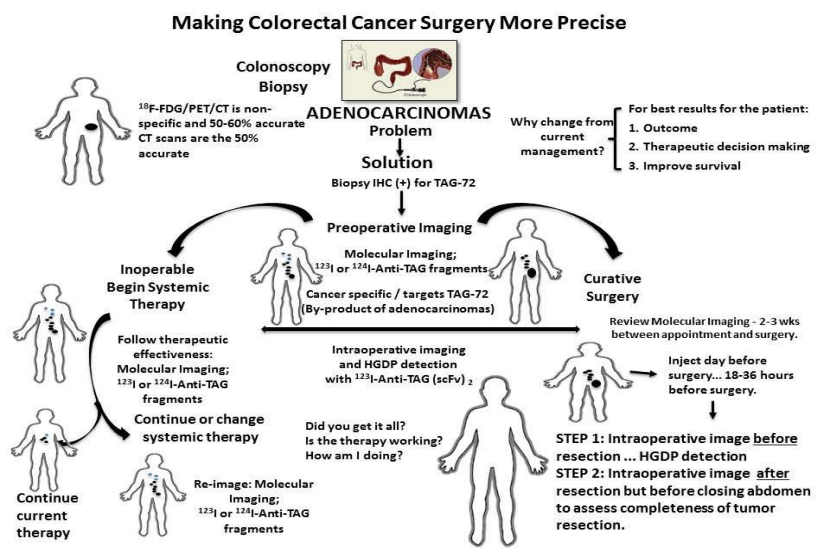

Figure 11: Increasing the Precision to Colorectal Cancer Surgery.

study be undertaken with primary colorectal adenocarcinomas that examine the role of the proposed System on making colorectal cancer surgery more precise (Figure 11).

Patient presenting with sign and symptoms of colorectal cancer undergo laboratory studies, including CEA serum levels, and colonoscopy with a biopsy of all relevant lesions. If an invasive adenocarcinoma is noted, the pathologist with perform IHC staining to determine the presence or absence of TAG-72 expression. The fact that TAG-72 is expressed in 85% of colorectal adenocarcinomas makes anti-TAG-72 the ideal foundational molecular probe for the System in these patients. If the initial biopsy is shown to express TAG-72, the patient is injected with a ^{124}I- or ^{123}I-anti-TAG-72 antibody fragment and imaged using PET/CT or PET/MRI, or SPECT/CT, respectively. The results of this molecular imaging determine if the patient can undergo surgery for cure or undergo chemotherapy and/or radiation therapy.

If clinically resectable, the day before surgery the patient is given a ^{123}I-anti-TAG fragment cocktail to facilitate localization of TAG-72 antigen-expressing malignant tissue. Intraoperative use of a HGDP in conjunction with a portable LFOVGC allows the surgeon to precisely identify all TAG-72 positive tissue, including surgical margins for excision, and to ensure that it is excised. Prior to closing, a planar image will tell the surgeon the patient's TAG-72 status at closing. This real-time intraoperative information about each tissue specimen will be available to the pathologist to aid in clearly identifying where to sample the respected specimens for subsequent processing and microscopic examination. In addition, this information will be available for more precise post-operative treatment planning before the patient leaves the recovery room. Where molecular imaging demonstrated inoperable disease, the patient is referred to an oncologist for treatment planning that may include chemotherapy and/or radiation therapy. Here again the molecular imaging using either ^{124}I or ^{123}I labeled anti-TAG-72 fragments will be used to follow therapeutic effectiveness. In the end, increased precision leads to increased quality of patient care.

References

1. Rasooly RS, Gossett DR, Henderson MK, Hubel A, Thibodeau SN. High-Throughput Processing to Preserve Viable Cells: A Precision Medicine Initiative Cohort Program Workshop. Biopreserv Biobank. 2017.
2. Herrera-Ornelas L, Justiniano J, Castillo N, Petrelli NJ, Stulc JP, Mittelman A. Metastases in small lymph nodes from colon cancer. Arch Surg. 1987;122(11):1253-6.
3. Thoeni RF. Colorectal cancer. Radiologic staging. Radiol Clin North Am. 1997;35(2):457-85.
4. Jeune F, Brouquet A, Caramella C, Gayet M, Abdalla S, Verin AL, et al. Cardiophrenic angle lymph node is an indicator of metastatic spread but not specifically peritoneal carcinomatosis in colorectal cancer patients: Results of a prospective validation study in 91 patients. Eur J Surg Oncol. 2016;42(6):861-8.
5. Jeune F, Brouquet A, Caramella C, Gayet M, Abdalla S, Verin AL, et al. Cardiophrenic angle lymph node is an indicator of metastatic spread but not specifically peritoneal carcinomatosis in colorectal cancer patients: Results of a prospective validation study in 91 patients. Eur J Surg Oncol. 2016;42(6):861-8.
6. Jeune F, Brouquet A, Caramella C, Gayet M, Abdalla S, Verin AL, et al. Cardiophrenic angle lymph node is an indicator of metastatic spread but not specifically peritoneal carcinomatosis in colorectal cancer patients: Results of a prospective validation study in 91 patients. Eur J Surg Oncol. 2016;42(6):861-8.
7. Dighe S, Purkayastha S, Swift I, Tekkis PP, Darzi A, A'Hern R, et al. Diagnostic precision of CT in local staging of colon cancers: a meta-analysis. Clin Radiol. 2010;65(9):708-19.
8. Wiegering A, Kunz M, Hussein M, Klein I, Wiegering V, Uthe FW, et al. Diagnostic value of preoperative CT scan to stratify colon cancer for neoadjuvant therapy. Int J Colorectal Dis. 2015;30(8):1067-73.
9. de Vries FE, da Costa DW, van der Mooren K, van Dorp TA, Vrouenraets BC. The value of pre-operative computed tomography scanning for the assessment of lymph node status in patients with colon cancer. Eur J Surg Oncol. 2014;40(12):1777-81.
10. Edward W. Martin Jr. In: Herrmann K, Nieweg OE, Povoski SP, editors. Radioguided Surgery: Current Applications and Innovative Directions in Clinical Practice (pages vii-ix). Cham, Heidelberg, New York, Dordrecht, and London: Springer. 2016.
11. Weber J, Haberkorn U, Mier W. Cancer stratification by molecular imaging. Int J Mol Sci. 2015;16(3):4918-46.
12. James ML, Gambhir SS. A molecular imaging primer: modalities, imaging

agents, and applications. Physiol Rev. 2012; 92:897-965.

13. Frangioni JV. The problem is background, not signal. Mol Imaging. 2009;8(6):303-4.

14. Brush J, Boyd K, Chappell F, Crawford F, Dozier M, Fenwick E, et al. The value of FDG positron emission tomography/computerised tomography (PET/CT) in pre-operative staging of colorectal cancer: a systematic review and economic evaluation. Health Technol Assess. 2011;15(35):1-192.

15. Long NM, Smith CS. Causes and imaging features of false positives and false negatives on 18F-PET/CT in oncologic imaging. Insights Imaging. 2011;2(6):679-98.

16. Lu YY, Chen JH, Ding HJ, Chien CR, Lin WY, Kao CH. A systematic review and meta-analysis of pretherapeutic lymph node staging of colorectal cancer by 18F-FDG PET or PET/CT. Nucl Med Commun. 2012;33(11):1127-33.

17. Park K, Jang G, Baek S, Song H. Usefulness of combined PET/CT to assess regional lymph node involvement in gastric cancer. Tumori. 2014;100(2):201-6.

18. Gade M, Kubik M, Fisker RV, Thorlacius-Ussing O, Petersen LJ. Diagnostic value of (18) F-FDG PET/CT as first choice in the detection of recurrent colorectal cancer due to rising CEA. Cancer Imaging. 2015;15(1):11.

19. Andreas K, Ulrich K. Use of radioactive substances in diagnosis and treatment of neuroendocrine tumors. Scand J Gastroenterol. 2015;50(6):740-7.

20. Johnbeck CB, Knigge U, Kjaer A. PET tracers for somatostatin receptor imaging of neuroendocrine tumors: current status and review of the literature. Future Oncol. 2014;10:2259-77.

21. Béhé M, Gotthardt M, Behr TM. Imaging of gastrinomas by nuclear medicine methods. Wien Klin Wochenschr. 2007;119(19-20):593-6.

22. Termanini B, Gibril F, Reynolds JC, Doppman JL, Chen CC, Stewart CA, et al. Value of somatostatin receptor scintigraphy: a prospective study in gastrinoma of its effect on clinical management. Gastroenterology. 1997;112(2):335-47.

23. Jimenez Londoño GA, García Vicente AM, Soriano Castrejon AM, Gómez López OV, Palomar Muñoz A, Vega Caicedo CH, et al. Role of 99mTc-HYNIC-Tyr3-octreotide scintigraphy in neuroendocrine tumors based on localization of the primary tumor. Minerva Endocrinol. 2016;41(1):10-18.

24. Povoski SP, Neff RL, Mojzisik CM, O'Malley DM, Hinkle GH, Hall NC, et al. A comprehensive overview of radioguided surgery using gamma detection probe technology. World J Surg Oncol. 2009:7:11.

25. Kaur S, Venktaraman G, Jain M, Senapati S, Garg PK, Batra SK. Recent trends in antibody-based oncologic imaging. Cancer Lett. 2012;315(2): 97-111.

26. Wu AM. Engineered antibodies for molecular imaging of cancer. Methods. 2014;65(1):139-47.

27. Povoski SP, Mojzisik CM, Sullivan BJ. Radioimmunoguided Surgery: Intraoperative Radioimmunodetection for the Radioguided Localization and Resection of Tumors. In: Herrmann K, Nieweg OE, Povoski SP, editors. Radioguided Surgery: Current Applications and Innovative Directions in Clinical Practice. Cham, Heidelberg, New York, Dordrecht, and London: Springer. 2016: 371-417.

28. Povoski SP, Hall NC, Murrey DA Jr, Sharp DS, Hitchcock CL, Mojzisik CM, et al. Multimodal imaging and detection strategy with 124 I-labeled chimeric monoclonal antibody cg250 for accurate localization and confirmation of extent of disease during laparoscopic and open surgical resection of clear cell renal cell carcinoma. Surg Innov. 2013;20(1):59-69.

29. Povoski SP. The History of Radioguided Surgery: Early Historical Milestones and the Development of Later Innovative Clinical Applications. In: Herrmann K, Nieweg OE, Povoski SP, editors. Radioguided Surgery: Current Applications and Innovative Directions in Clinical Practice. Cham, Heidelberg, New York, Dordrecht, and London: Springer. 2016: 3-12.

30. Barrio AV, Cody HS III. Radioguided Sentinel Lymph Node Mapping and Biopsy in Breast Cancer. In: Herrmann K, Nieweg OE, Povoski SP, editors. Radioguided Surgery: Current Applications and Innovative Directions in Clinical Practice. Cham, Heidelberg, New York, Dordrecht, and London: Springer. 2016: (115-123).

31. Sun D, Bloomston M, Hinkle G, Al-Saif OH, Hall NC, Povoski SP, et al. Radioimmunoguided surgery (RIGS), PET/CT image-guided surgery, and fluorescence image-guided surgery: past, present, and future. J Surg Oncol. 2007;96(4):297-308.

32. Hellingman D, Vidal-Sicart S. The Use of Intraoperative Small and Large Field of View Gamma Cameras for Radioguided Surgery. In: Herrmann K, Nieweg OE, Povoski SP, editors. Radioguided Surgery: Current Applications and Innovative Directions in Clinical Practice. Cham, Heidelberg, New York, Dordrecht, and London: Springer. 2016: 35-56.

33. Hall NC, Plews RL, Agrawal A, Povoski SP, Wright CL, Zhang J, et al. Intraoperative Scintigraphy Using a Large Field-of-View Portable Gamma Camera for Primary Hyperparathyroidism: Initial Experience. BioMed Res Int. 2015;2015:930575.

34. Hall NC, Nichols SD, Povoski SP, James IA, Wright CL, Harris R, et al. Intraoperative Use of a Portable Large Field of View Gamma Camera and Handheld Gamma Detection Probe for Radioguided Localization and Prediction of Complete Surgical Resection of Gastrinoma: Proof of Concept. J Am Coll Surg. 2015; 221(2):300-8.

35. Siegel R, Ma J, Zou Z, Jemal A. Cancer Statistics, 2014. CA Cancer J Clin. 2014;64(1):9-29.

36. Julien S, Videira PA, Delannoy P. Sialyl-Tn in cancer: How did we miss the target? Biolmolecules. 2012;2:435-66.

37. Molinolo A, Simpson JF, Thor An, Schlom J. Enhanced tumor binding using immunohistochemical analyses by second generation anti-Tumor-associated Glycoprotein 72 monoclonal antibodies versus monoclonal antibody B72.3 in human tissue. Cancer Res, 1990;50:1291-8.

38. Kuroki M, Fernsten PD, Wunderlich D, Colcher D, Simpson JF, Poole DJ, et al. Serological mapping of the TAG-72 tumor-associated antigen using 19 distinct monoclonal antibodies. Cancer Res. 1990;50(16):4872-9.

39. Colcher D, Horan Hand P, Nuti M, Schlom J. A spectrum of monoclonal antibodies reactive with human mammary tumor cells. Proc Nati Acad Sci. 1981;78(5):3199-203.

40. Sheer DG, Schlom J, Cooper HL. Purification and Composition of the Human Tumor-associated Glycoprotein (TAG-72) Defined by Monoclonal Antibodies CC49 and B72.3. Cancer Res. 1998;48(23):6811-18.

41. Muraro R, Kuroki M, Wunderlich D, Poole DJ, Colcher D, Thor A, et al. Generation and characterization of B72.3 second generation monoclonal antibodies reactive with tumor-associated glycoprotein 72 antigen. Cancer Res. 1988;48(16):4588-96.

42. Sosolik RC, Hitchcock CL, Becker WJ. Heterophilic antibodies produce spuriously elevated CK-MB concentrations in a selected patient population. Am J Clin Pathol. 1997;107(5):506-10.

43. Agnese DM, Abdessalam SF, Burak WE Jr, Arnold MW, Soble D, Hinkle GH, et al. Pilot study using a humanized CC49 monoclonal antibody (HuCC49DeltaCH2) to localize recurrent colorectal carcinoma. Ann Surg Oncol. 2004;11(2):197-202.

44. Fang L, Holford NH, Hinkle G, Cao X, Xiao JJ, Bloomston M, et al. Population pharmacokinetics of humanized monoclonal antibody HuCC49?CH2 and murine antibody CC49 in colorectal cancer patients. J Clin Pharmacol. 2007;47:227-37.

45. Arnold MW, Hitchcock CL, Young DC, Burak WE Jr, Bertsch DJ, Martin EW Jr. Intra-abdominal patterns of disease dissemination in colorectal cancer identified using radioimmunoguided surgery. Dis Colon Rectum.

1996;39(5):509-13.

46. Burak WE Jr, Schneebaum S, Kim JA, Arnold MW, Hinkle G, Berens A, et al. Pilot study evaluating the intraoperative localization of radiolabeled monoclonal antibody CC83 in patients with metastatic colorectal carcinoma. Surgery. 1995;118(1):103-8.

47. Arnold MW, Schneebaum S, Berens A, Petty L, Mojzisik C, Hinkle G, et al. Intraoperative detection of colorectal cancer with radioimmunoguided surgery and CC49, a second-generation monoclonal antibody. Ann Surg. 1992;216(6):627-32.

48. Arnold MW, Schneebaum S, Berens A, Mojzisik C, Hinkle G, Martin EW Jr. Radioimmunoguided surgery challenges traditional decision making in patients with primary colorectal cancer. Surgery. 1992;112(4):624-30.

49. Haddad R, Avital S, Troitsa A, Chen J, Baratz M, Brazovsky E, et al. Benefits of radioimmunoguided surgery for pelvic recurrence. Eur J Surg Oncol. 2001;27:298-301.

50. Schneebaum S, Troitsa A, Haddad R, Avital S, Kashtan H, Baratz M, et al. Immunoguided lymph node dissection in colorectal cancer: a new challenge? World J Surg. 2001;25(12):1495-8.

51. Avital S, Haddad R, Troitsa A, Kashtan H, Brazovsky E, Gitstein G, et al. Radioimmunoguided surgery for recurrent colorectal cancer manifested by isolated CEA elevation. Cancer. 2000;89(8):1692-8.

52. Nieroda CA, Mojzisik C, Sardi A, Ferrara PJ, Hinkle G, Thurston MO, Martin EW Jr. Radioimmunoguided surgery in primary colon cancer. Cancer Detect Prev. 1990;14(6):651-6.

53. Percivale P, Bertoglio S, Meszaros P, Schenone F, Gipponi M, Moresco L, et al. Radioimmunoguided surgery with different iodine-125 radiolabeled monoclonal antibodies in recurrent colorectal cancer. Semin Surg Oncol. 1998;15(4):231-4.

54. Sickle-Santanello BJ, O'Dwyer PJ, Mojzisk C, Tuttle SE, Hinkle GH, Rousseau M, et al. Radioimmunoguided Surgery Using the Monoclonal antibody B72.3 in colorectal tumors. Dis Colon Rectum. 1987;30(10):761-4.

55. Bertsch DJ, Burak WE Jr, Young DC, Arnold MW, Martin EW Jr. Radioimmunoguided surgery for colorectal cancer. Ann Surg Oncol. 1996;3(3):310-6.

56. Povoski SP, Hatzaras IS, Mojzisik CM, Arnold MW, Hinkle GH, Hitchcock CL, et al. Antigen-directed cancer surgery for primary colorectal cancer: 15-year survival analysis. Ann Surg Oncol. 2012;19(1):131-8.

57. SEER Cancer Statistics Factsheets: Colon and Rectum Cancer. National Cancer Institute. Bethesda.

58. Cornelius EA, West AB. False tumor-positive lymph nodes in radioimmunodiagnosis and radioimmunoguided surgery: etiologic mechanisms. J Surg Oncol. 1996;63(1):23-35.

59. Schneebaum S, Arnold MW, Houchens DP, Greenson JK, Cote RJ, Hitchcock CL, et al. The significance of intraoperative periportal lymph node metastasis identification in patients with colorectal carcinoma. Cancer. 1995;75(12):2809-17.

60. Greenson JK, Isenhart CE, Rice R, Mojzisik C, Houchens D, Martin EW Jr. Identification of occult micrometastases in pericolic lymph nodes of Duke's B colorectal cancer patients using monoclonal antibodies against cytokeratin and CC49. Correlation with long-term survival. Cancer. 1994;73(3):563-9.

61. Cote RJ, Houchens DP, Hitchcock CL, Saad AD, Nines RG, Greenson JK, et al. Intraoperative detection of occult colon cancer micrometastases using 125 I-radiolabled monoclonal antibody CC49. Cancer. 1996;77(4):613-20.

62. Hitchcock CL, Sampsel J, Young DC, Martin E Jr, Arnold MW. Limitations with light microscopy in the detection of colorectal cancer cells. Dis Colon Rectum. 1999;42(8):1046-52.

63. Martinez DA, Barbera-Guillem E, LaValle GJ, Martin EW Jr. Radioimmunoguided surgery for gastrointestinal malignancies: an analysis of 14 years of clinical experience. Cancer Control. 1997;4(6):505-16.

64. Hitchcock CL, Arnold MW, Young DC, Schneebaum S, Martin EW Jr. TAG-72 expression in lymph nodes and RIGS. Dis Colon Rectum. 1996;39(4):473-5.

65. Povoski SP, Murrey DA Jr, Hall NC. 18F-FDG-Directed Surgery and 18F-FDG-Directed Interventional. In: Herrmann K, Nieweg OE, Povoski SP, editors. Radioguided Surgery: Current Applications and Innovative Directions in Clinical Practice. Cham, Heidelberg, New York, Dordrecht, and London: Springer. 2016: 421-445.