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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 due to lack of sensitivity and specificity of preoperative imaging and surgical limitations in the Operating Room using traditional techniques (i.e., inspection and palpation). The staging of colorectal adenocarcinoma begins with preoperative imaging and ends with the pathologist. There are many potential sources of error between these two points that may result in suboptimal treatment. Using colorectal adenocarcinoma as a model, we developed a System incorporating currently available technologies to increase the precision of Preoperative and Intraoperative imaging as well as intraoperative tumor detection.

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 cases. 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 practice guidelines and clinical resources to help physicians treat, diagnose, prevent, reduce risk, provide supportive care, and image a large number of different cancers, including colorectal adenocarcinoma [2]. The TNM 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 have recurrent disease, and patients with the same stage of colorectal adenocarcinoma can differ in their clinical course. These statistics occur due to 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 recommend using Computerized Tomography (CT) scans with contrast for preoperative imaging. This assessment of extent of disease is needed for surgical planning for resection of primary and recurrent disease. This includes resectability 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, the specificity and sensitivity of CT

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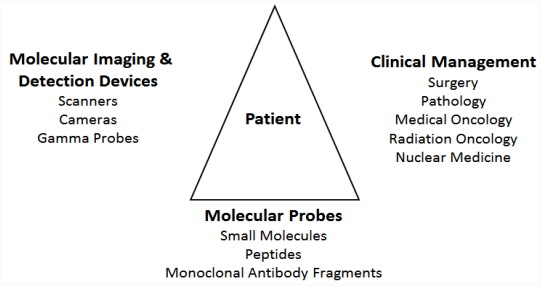

Figure 1: The System. The System begins with the patient and the solid tumor. The tumor's pathologic features are used to select the appropriate tumor specific or associated molecular probe and radionuclide or non-radioactive label. The labeled-molecular probe dictates the devices that can be used for preoperative and intraoperative imaging and intraoperative detection. The results will aid in treatment decision making before and/or after tissue examination by Pathology.

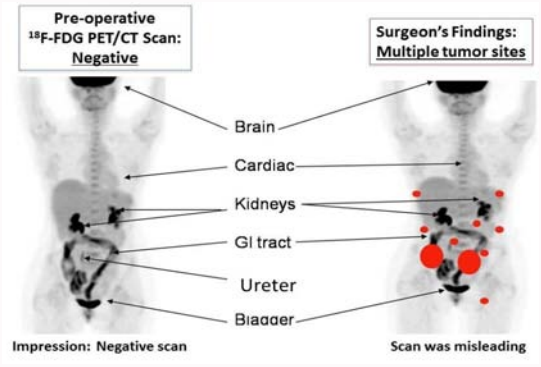

Figure 2: ^{18}F-FDG PET/CT of Patient with Recurrent Colon Cancer. The left image is the pre-operative PET/CT scan was interpreted as negative for cancer. Nonspecific uptake of the ^{18}F-FDG was present in the brain, GI tract, kidneys, ureter and bladder. The right image correlates the surgical findings of cancer (orange dots) with the same ^{18}F-FDG-PET/CT scan.

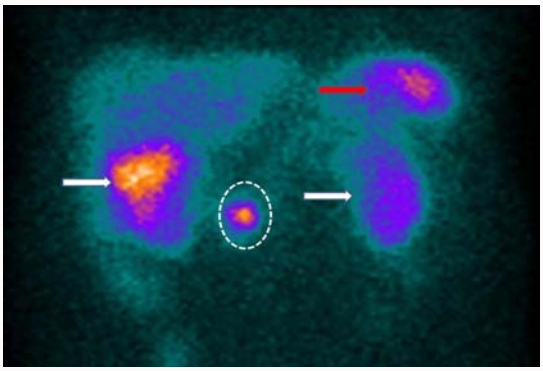

Figure 3: SPECT/CT Scan of ^{111}In-pentetreotide-positive gastrinoma. Large field-of-view gamma camera (SPECT) scan of ^{111}In-pentetreotide bound to somatostatin receptors on a gastrinoma cells (dotted circle). There is non-specific uptake in the spleen (red arrow) and accumulation in the gallbladder (right white arrow), and in the kidneys (left kidney- white arrow, right kidney behind the gallbladder). Note the poor spatial resolution.

imaging to detect lymph node metastases is limited by its inability to: 1) identify lymph nodes smaller than 5 mm that often contain metastatic disease, 2) distinguish non-enlarged lymph nodes under a centimeter containing tumor from normal physiologic non-enlarged lymph nodes, and 3) distinguish enlarged lymph nodes containing tumor from lymph nodes that are enlarged due to reactive/inflammatory changes. [4] The end result is a wide range of reported specificity from only 42% to 70% [5-9].

Patients and their families 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 surgical techniques (i.e. visual inspection and palpation) do not necessarily provide surgeons with accurate information regarding location and extent of disease needed to obtain a "curative" resection. As one of us has previously noted, "surgeons had real-time information regarding the precise location of all disease and had a real-time assessment of surgical resection margins, they may be able to intervene immediately and accomplish a complete resection without subjecting the patient to subsequent surgical procedures [10].

Advances in precision medicine are underway. This paper examines how a diverse group of physicians, basic scientists and engineers brought together currently available resources and new developments 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 patient's solid tumors. Although the focus is colorectal adenocarcinoma, the proposed System applies to the majority of adenocarcinomas that arise in other organs.

Methods

A System to increase precision management of colorectal cancer patients

System components: The components of the multimodal System are seen in Figure 1. With the patient at its center, the System integrate s physicians from Nuclear Medicine, Radiology, Surgery, Oncology, Radiation Oncology and Pathology with the tools needed for a more precise 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" for intraoperative detection using a hand-held gamma detection probe to find and excise/resect the tumor containing tissue. Intraoperative imaging provides real-time verification of complete resection. From a systems standpoint, the complete resection of all tumor is globally cost effective.

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 increase 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, 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 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, FDG is taken up into cells with a high metabolic rate. This includes cells within malignant and some benign tumors, normal organs (e.g., brown fat, myocardium and other muscle, 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 due to its excretion in the urine [15]. Uptake of FDG as described results 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 well differentiated adenocarcinomas of the lung such as invasive bronchioloalveolar carcinomas, carcinoid tumors in the lung, renal cell carcinomas, hepatocellular carcinomas in the liver, mucinous tumors of the gastrointestinal tract, and low grade non-Hodgkin lymphomas. The false positive and false negative rates limit 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 the System (Figure 2) [18].

Small peptides of ≤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 are 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 overexpress 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 may replace them in the future, especially for midgut and hind gut NETs [23].

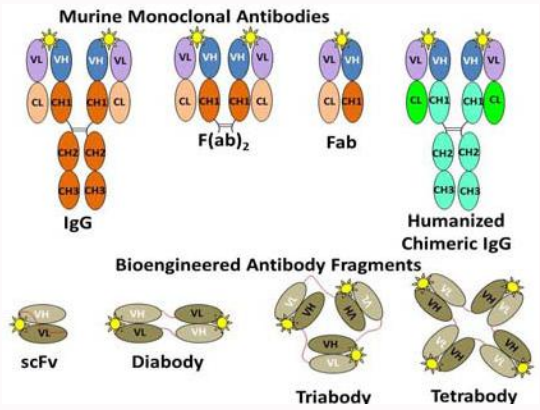

Figure 4: Types of Antibody-Derived Molecules for Molecular Imaging and Intraoperative Detection.

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 are essential for antigen binding. The peptide linker between the domains provides many of the desired physical properties of these molecular probes for imaging.

Monoclonal Antibodies (MAb), directed against tumor-related antigens, are being developed as molecular probes for molecular imaging, intraoperative detection, 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 in 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 in non-target tissue, long serum half-lives, and immunogenicity that induced the formation of Human Anti-Mouse Antibodies (HAMA). Slow clearance and uptake in the target tissue necessitated radiolabeling with radionuclides with longer half-life isotope [^{111}In (2.8 days), ^{89}Zr (3.3 days), ^{124}I (4.2 days), or ^{125}I (60 days)] [24].

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 rates lead to longer 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

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 of 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, scFvs and diabodies have faster clearance with excellent tumor penetration and higher tumor-to-blood ratios. The low background and high signal-to-noise ratio increases the 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 with an inert Polymer of Ethylene Glycol (PEG), but often these modifications result in poor stability, poor or ablated binding, and aggregation. However, adjustments in fragments size translate into adjustments in clearance time suitable for different imaging time lines, modalities and sensitivities.

Molecular imaging and 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 requires 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. PET is now typically 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. For 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).

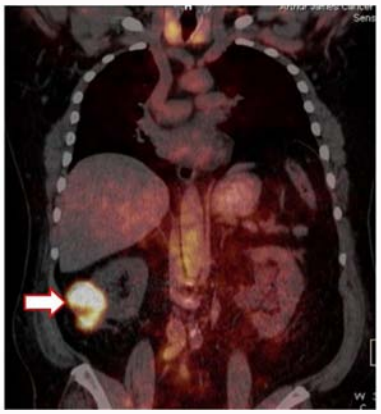

Figure 5: Molecular Imaging of Clear Cell Renal Cell Carcinoma.
^{124}I MAb cG250 PET/CT with clear cell renal cell carcinoma in the lower pole of the right kidney (arrow). Focal molecular probe also labels the thyroid glands.

Hand-Held Gamma Detection Probes (HGDPs), 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 [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 HGDP containing a cadmium telluride (CdTe) crystal linked to a control unit that provides both numerical information and an auditory signal when the radioactivity is higher than three standard deviations 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, may not be aware of the instrument's restricted field of view, or understand 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 an intraoperative portable gamma camera that provides real-time intraoperative localization of 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 portable 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 ($5cm^2$ x $5cm^2$) or large field of view (>5 cm^2 x$5cm^2$) 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.

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	40%	90.1%	35%	71.2%	20%	13.5%	5%	35.5%

Table 3: Incidence of Adenocarcinomas and TAG-72 Positive Adenocarcinomas in the United States.

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)

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.

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 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 cells that release the molecule into the lumen of tumor acini and extracellular matrix where it accumulates. The extracellular accumulation of TAG-72 facilitates its targeting by radiolabeled antibodies and subsequent localization by molecular imaging and hand-held probes. These features result 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

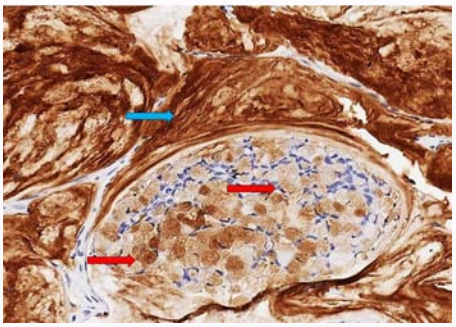

Figure 6: Adenocarcinoma of the Colon - Immunohistochemical Staining of the TAG-72 Antigen.
Anti-TAG-72 scFv with ABC immunohistochemical staining of a mucinous adenocarcinoma. Extensive accumulation of TAG-72 in the extracellular matrix is seen as dark brown staining (blue arrow). Intracellular TAG-72 containing vesicles exhibit a lesser staining intensity (red arrows). (20x Virtual Slide).

four generations of antibodies to TAG-72 were generated in the same laboratory at the National Cancer Institute (NCI) [39-41], and were used by us to increase the precision of radioimmunoguided surgery (RIGS) in an attempt to detect all Tag-72 bearing tissue 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 in development of HAMA, whose only clinical significance was interference with several clinical laboratory tests [42]. The fact that these were whole IgG molecules with a long half-life required labelling with ^{125}I with half-life of 60 days resulted in a delay of surgery up to four weeks and a tumor-background (signal-noise) ratio of 2:1. The smaller size of the 3rd generation MAb 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]. 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

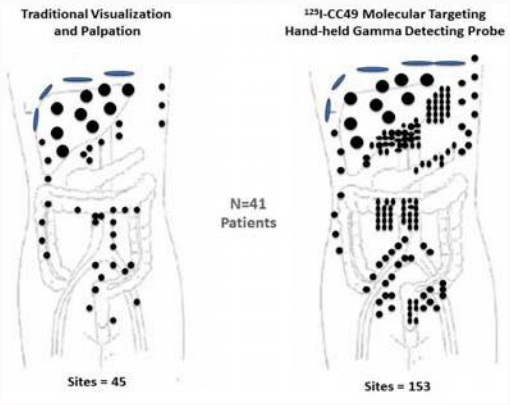

Figure 7: Intraoperative Tumor Location of Metastatic Disease.
Traditional Visualization and Palpation *vs.* Hand-Held Gamma Detection Probe (HGDP) of Occult Tumor Binding ^{125}I-CC49 in 41 Cases of Primary Colorectal Adenocarcinoma [45]. The left drawing represents the 45 individual sites (black dots) of occult metastatic disease detected at traditional visual inspection exploration of the abdomen and pelvis. The right drawing demonstrates the 153 sites (black dots) of occult metastases found in the same 41 patients with primary colorectal adenocarcinoma using a HGDP.

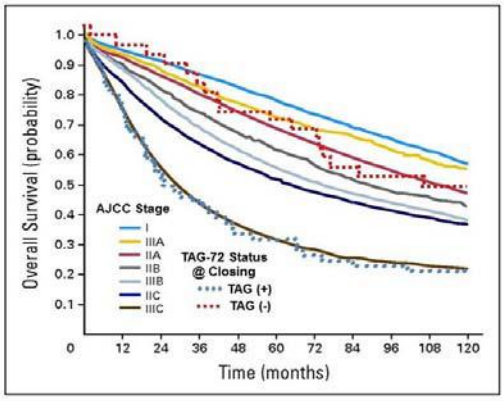

Figure 8: Ten Year Patient Survival: Traditional Surgery vs HGDP Intraoperative Detection. Based on current AJCC TNM staging criteria, the solid lines represent the 10-year survival for 128,853 primary colon carcinoma patients in the SEER Database. (57) Using the presence [TAG (+ - blue dotted line)] or absence [(TAG (-) – red dotted line] of radioactivity at the time of closing (TAG-72 Status at Closing) the dotted lines represent survival data from 97 patients that were given ^{125}I-CC49 and subsequently underwent HGDP directed intraoperative detection with possible resection of radioactive tissue [56].

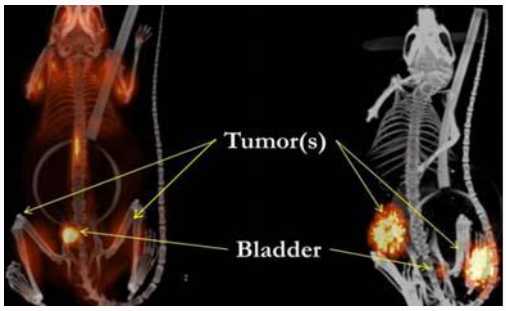

Figure 9: ^{18}F-FDG vs. ^{124}I 3E8 fragment PET Scan Images of Human Colon Cancer Xenografts in Mice.
The ^{18}F-FDG-PET/CT (left image) was obtained 2 hours after injection. The ^{124}I 3E8 fragment PET/CT (right image) was obtained 24 hours after injection. The tumor xenografts are clearly demonstrated in the ^{124}I 3E8 fragment injected mouse image, whereas the ^{18}F-FDG-PET image only demonstrates physiologic excreted urinary activity in the bladder, nonspecific activity in spine, and a diffuse background of nonspecific/physiologic activity predominantly in muscle.

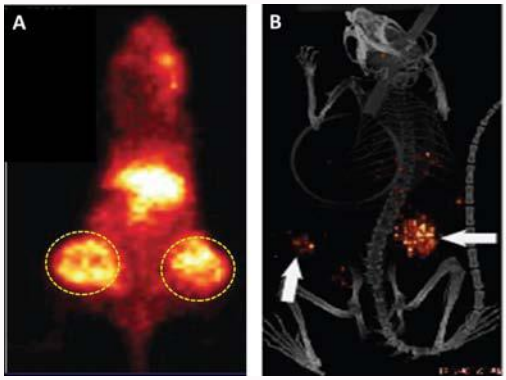

Figure 10: ^{124}I-IgG 3E8 PET Scan *vs.* ^{124}I-diabody 3E8 PET/CT Scan of Human Colon Cancer Xenografts in Mice.
The ^{124}I-IgG 3E8 PET scan image A demonstrates a high background signal, i.e., noise, with accumulation in the liver and head as compared to the significantly lower background seen in the ^{124}I-3E8 fragment PET/CT scan image at 24 hours.

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 of 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 at the end of surgery 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, for those patients in whom 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.

TAG-72 positive tissue that lacks 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 indicate 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 not been previously resected [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 did demonstrate metastatic disease, though 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

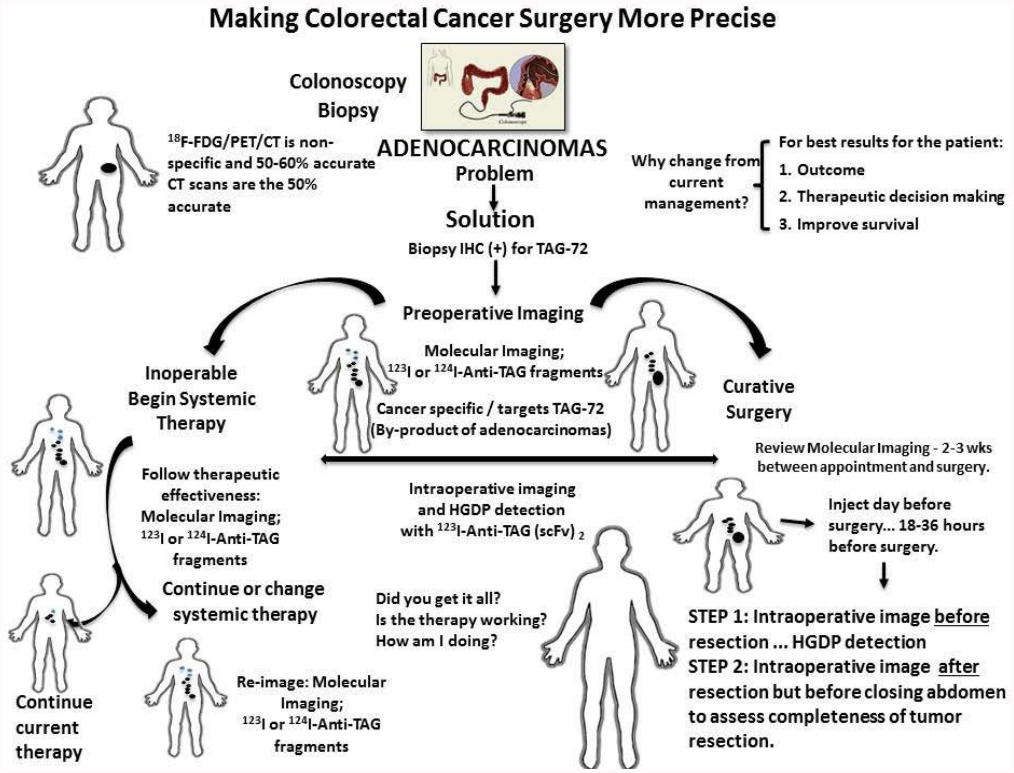

Figure 11: Increasing the Precision of Colorectal Cancer Surgery.

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 of human adenocarcinoma cells clearly demonstrate ^{18}F-FDG and the humanized 4th generation MAb to TAG-72 (3E8), yet lack the precision obtained using humanized 3E8 fragment, a 5th generation MAb to TAG-72 (Figures 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 are often characterized by over expression of somatostatin receptors on their membrane which bind the peptide ligand ^{111}In-labeled octreotide as a molecular probe for imaging. A POC study clearly demonstrated that the 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 tumors [28,34]. A second POC study used the same approach for the molecular imaging ^{99m}Tc-Sestamibi (MIBI) 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 the involved gland is often complicated by its variable 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.

Conclusion

The current guidelines for colorectal cancer surgery do not take into account the limited precision of Preoperative CT scans and intraoperative visual inspection and palpation to accurately detect nodal metastases outside of the traditional planes of dissection. This lack of accurate information, has a significant impact on long term survival. Despite its use as a molecular imaging agent, ^{18}F-FDG lacks accuracy in the identification of metastatic lymph nodes. The identification and excision of 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

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

information needed to locate, identify and resect all malignant tissue expressing the radiolabeled molecular probe. 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 study be undertaken with primary colorectal adenocarcinomas that examines the role of the proposed System on making colorectal cancer surgery more precise (Figure 11).

The initial workup for a patient presenting with colorectal cancer is laboratory studies, including CEA serum levels, and colonoscopy with biopsy. If an invasive adenocarcinoma is noted, the pathologist will 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 ^{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 ^{123}I-anti-TAG-72 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 resected 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. If molecular imaging demonstrates inoperable disease, the patient is referred to a medical 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. This targeted approach will result in increased identification and treatment of cancer and improve long term survival for patients with a variety of types of cancer.

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