# Radioimmunoguided surgery for colorectal cancer – An overview

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**ABSTRACT:** Many different monoclonal antibodies used experimentally and clinically are highly tumour-specific. Radiolabelling of these antibodies has been successfully accomplished. Immunoscintigraphy of primary and metastatic cancers has a reported sensitivity of 59 to 70%. However, in many studies, operative and histologic confirmation is lacking. Radioimmunoguided surgery is a promising new adjunctive technique for the surgical treatment of colorectal cancer. Its reported sensitivity ranges between 70 and 100% and specificity between 66 and 100%. In approximately one-third of patients with colorectal cancer, additional intraoperative information concerning the presence of subclinical tumours was gained using radioimmunoguided surgery. This system has the potential to assist the surgeon in performing complete resection of cancer and decrease the local recurrence rate. This could be of particular clinical importance for rectosigmoid tumours where the reported local recurrence rate is as high as 30%. Despite the advances made, many problems still need to be resolved. The important ones include: finding an antibody with high tumour specificity and at the same time rapid clearance from the blood pool and normal tissue – this would avoid the delay between monoclonal antibody injection and surgery and would make this approach more easily accepted by the patient; and use of alternative isotopes for radiolabelling. Radioimmunoguided surgery has the potential to change the way surgery for colorectal cancer is being performed. It offers the possibility of improvement in patient survival. **Can J Gastroenterol 1990;4(5):215-218**

**Key Words:** *Colorectal cancer, Monoclonal antibodies, Radioimmunoguided surgery*

## La chirurgie radioimmunoguidée et le cancer recto-colique: Un aperçu

**RESUME:** Bon nombre d'anticorps monoclonaux divers utilisés expérimentalementn et cliniquement sont hautement spécifiques de tumeurs. Le radiomarquage de ces anticorps a été réalisé avec succès. L'immunoscintigraphie des cancers primaires et métastatiques a une sensibilité rapportée de 59 à 70%. Dans de nombreuses études, une confirmation opératoire et histologique fait

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THE DEVELOPMENT OF MONOCLONAL antibodies against a variety of antigens, including human cancer-specific antigens, holds promise of new treatment modalities for malignant disease the ability to label these antibodies with radioactive isotopes allowed the development of external scintigraphy for primary and metastatic tumours. The invention of portable, sterilizable, gamma-detecting probes for intraoperative use has made clinical application of the concept of radioimmunoguided surgery possible. The purpose of this article is to review the scientific background of radioimmunoguided surgery and to project future uses of this technique in colorectal surgery.

## MONOCLONAL ANTIBODIES

To date, many different monoclonal antibodies have been developed. Most of them are murine antibodies. Examples of antigens used for murine monoclonal antibody production include: a membrane-enriched fraction of a human carcinoma metastasis (1), carcinoembryonic antigen (2) and a beta-anomer of the Thomsen-Friedenreich antigen (3). An interesting concept involves the derivation of monoclonal antibodies of predetermined specificity using a synthetic antigen. This has been successfully accomplished at the

toutefois défaut. La chirurgie radioimmunoguidée est une nouvelle technique d'appoint prometteuse dans le traitement chirurgical du cancer recto-colique. La sensibilité rapportée oscille entre 70 et 100% et la spécificité entre 66 et 100%. Chez environ un tiers des patients atteints de cancer recto-colique, la chirurgie radioimmunoguidée permet d'obtenir des données periopératoires supplémentaires sur la présence de tumeurs subcliniques. Cette modalité offre la possibilité d'aider le chirurgien à effectuer la résection complète du cancer et peut diminuer le taux de récurrence locale. Ceci pourrait s'avérer d'une importance clinique particulière pour les tumeurs rectosigmoïdiennes, la récurrence locale pouvant en effet atteindre 30%. En dépit des progrès réalisés, il reste encore à résoudre de nombreux problèmes; parmi les plus importants figure notamment celui de trouver un anticorps doté à la fois d'une spécificité élevée et d'un taux de clairance rapide du sang et des tissus sains. Ces caractéristiques permettraient d'éviter le délai entre l'injection d'anticorps monoclonaux et l'opération, et rendrait l'approche plus aisément acceptable par le patient. Le recours aux isotopes pour le radiomarquage pourrait également être considéré. La chirurgie radioimmunoguidée pourrait éventuellement changer la chirurgie du cancer recto-colique. Elle offre la possibilité d'améliorer les chances de survie du patient.

University of Alberta against a synthetic beta-anomeric analogue of the Thomsen-Friedenreich antigen – the terminal disaccharide of asialo–GM1 described as beta-D-Gal-(1-3)-beta-Gal-NAc-R (4,5).

Monoclonal antibodies can be used for specific staining of malignant tissue on frozen section and paraffin section slides. Histologic methods are among the most important in screening newly developed monoclonal actibodies and testing their tumour specificity before clinical use (2).

## RADIOIMMUNO-SCINTIGRAPHY

Immunoscintigraphy of various human primary and metastatic cancer sites using radiolabelled monoclonal antibodies has been widely evaluated. It clearly has the potential to overcome the limitations of both ultrasound (problems with detection of small, solid masses) and computed tomography scanning (differentiation of invasion by tumour from benign scarring).

Monoclonal antibodies labelled with different isotopes have been used for imaging. An anti-carcinoembryonic antigen antibody labelled with $^{111}$In was reported to image 69% of primary colorectal cancers, but none of six extrahepatic recurrences from this disease (6). In the same study the authors reported difficulty imaging hepatic metastases due to high activity in the normal liver when $^{111}$In-labelled antibody was used. In a different study using antibody against tumour-specific antigen (designated as 17-1A) and labeled with $^{131}$I, a sensitivity of 59% (27 of 46) was achieved. Another antibody designated 19-9 (against a monosialoganglioside) labelled with $^{131}$I was 66% (19 of 29) sensitive (7) Similar results were obtained when F(ab')$_2$ fragments rather than whole antibodies were injected.

Significant differences were observed in some cases in antibody pick-up between the primary tumour and metastases in the same patient, showing heterogenicity of antigen expression. An 'antibody cocktail' rather than a single antibody might therefore prove useful to increase the chances of tumour detection. Positive images were sharpest seven to eight days after injection for the intact antibody and four to five days for the F(ab')$_2$ fragments. Epenetos et al (8) have used three different types of antibody labelled with $^{131}$I against tumour-associated antigens. They observed that the 'tumour to normal' tissue ratio of radioactivity rose from the time of injection to day 12 and was the highest between days 5 and 12. Overall, they found that an average of only 0.015% of the injected dose of radioactivity could be found in the tumour, per gram of tissue, one day after injection.

The usefulness of monoclonal antibodies derived against a synthetic anomeric analogue of the Thomsen-Friedenreich antigen for immunoscintigraphy of human adenocarcinomas has been evaluated at the University of Alberta (9,10). Authors showed that two antibodies of different specificities (determined by in vitro studies) could be used for scintigraphic detection of the various primary and metastatic adenocarcinomas. Initially $^{131}$I was used as a radiolabel for the antibodies. Recently $^{111}$In has been used with success. In that study an interesting observation was made. The quality of the image as well as the number of metastatic sites identified appeared to depend on the dose of radiolabelled antibody used.

## RADIOIMMUNO-GUIDED SURGERY

An area of special appeal to the surgeon is the prospect of using tumour-specific, radiolabelled monoclonal antibodies for intraoperative localization of primary and metastatic cancers. Local spread of tumour, adequacy of resection margins and completeness of lymph node resection could also be assessed immediately. The rationale of this method is based on faster clearance of antibody-isotope complex from the blood pool and normal tissue than from the tumour, as discussed previously (7,8). With a difference in radioactivity between normal tissue and tumour, the surgeon can then 'home in' on the malignant tissue using a hand-held gamma-detecting probe. This technique requires counting of gamma radiation over various areas of the operative field per unit time, comparison with known 'background noise.' If the ratio of counts is 1.5:1, this area is likely to bear malignant tissue and resection should be carried out if possible. All investigators agree that the higher the ratio, the more sensitive this method becomes (9,11).

Gamma counting depends on the inverse square law, which states that the number of detected radioactivity counts is inversely proportional to the square of the distance separating a radioactive source from the detecting device. Radioimmunoguided surgery

has an advantage over immunoscintigraphy in that the probe can be placed directly over the radioactive tumour, and therefore the effect of the inverse square law can be almost eliminated. As a result, the dose of radioactive isotope necessary for radioimmunoguided surgery can be much lower than that used for external scintigraphy. Also, the use of a low energy isotope becomes feasible.

Only a few hand-held gamma-detecting probes for intraoperative use are available on the market today. One of them has been manufactured by Radiation Monitoring Devices Inc (Watertown, Massachusetts). It uses a cadmium telluride crystal to convert gamma radiation into a light impulse that can in turn be counted and converted into an electrical impulse. No results from experimental or clinical use of this instrument have been reported. Another probe has been manufactured by Stratec Inc (West Germany) using the same principle. It has been evaluated in several centres in Europe and seems to be gaining popularity (personal communication). The system for intraoperative gamma detection and counting has also been constructed at Ohio State University (12). It has been thoroughly evaluated and engineered to include a 'squelch' mechanism for the elimination of background noise and for activation of a siren tone only when the number of counts detected is two standard deviations greater than background (personal communication).

Early in the development of radioimmunoguided surgery, animals were implanted with human carcinoembryonic antigen-producing tumours. They were injected with an anti-carcinoembryonic antigen antibody labelled with $^{131}I$ (13,14). These studies stressed the ability of the gamma probe to differentiate between the carcinoembryonic antigen-producing tumour and surrounding normal tissue. The authors also confirmed the presence of a time-dependent ratio between malignant and nonmalignant tissue by calculating the number of counts per gram of biopsied material.

Soon after the first clinical trials were undertaken (15), Martin et al (16) studied intraoperative probe counts in patients with colorectal, gastric and ovarian adenocarcinomas. They demonstrated the probe's ability to identify tumours in 83% of cases with primary colon cancer (five of six), 79% with recurrent colon cancer (31 of 39), 80% with gastric cancer (four of five) and 50% with ovarian adenocarcinoma (four of eight). Of particular importance was the fact that the authors were able to localize metastatic deposits in the liver. They demonstrated an excellent correlation between positive probe counts and positive tumour to normal tissue radioactivity ratios, determined by a gamma counter (16). They also showed the probe's ability to localize $^{125}I$ in tissue in which the antigen could be demonstrated – the antigen from which the antibody was derived – using the immunoperoxidase technique. Sardi et al (17) analyzed a series of thirty-two patients with recurrent colorectal cancer. A gamma-detecting probe was used during laparotomy in these patients. The authors found that in 18% of cases (six of 32), tumour not detected clinically at exploration was identified by the probe.

## POSTOPERATIVE STAGING

Another area of benefit to the patient would be in assisting a pathologist examining operative specimens of colorectal cancer in identifying metastatic lymph nodes which appear clinically normal. It would be of particular importance to stage fully patients with Duke's C lesions, since an effective adjuvant chemoimmunotherapy has been found for this group, producing an improved survival rate (18-20).

## REFERENCES


1. Schlom J, Colchier D, Roselli M, et al. Tumor targeting with monoclonal antibody B72.3. Nucl Med Biol 1989;16:137-42.
2. Primus FJ, MacDonald R, Goldenberg DM, Hansen HJ. Localization of GW-39 human tumours in hamsters by affinity purified antibody to carcinoembryonic antigen. Cancer Res 1977;37:1544-7.
3. Longnecker BM, Willans DJ, MacLean GD, Selvaraj S, Suresh MR, Noujaim AA. Monoclonal antibodies and synthetic tumor associated glycoconjugates in the expression of Thomsen-Friedenreich- like and Tn-like antigens on human cancers. J Nucl Med 1987;78:489-96.
4. MacLean GD, Noujaim AA, McEwan AJB, et al. A novel strategy to derive monoclonal antibodies for successful imaging of cancer in humans. In: Kaplan JG, Green DR, Bleackley RC, eds. Cellular Basis of Immune Modulation. New York: Alan R Liss Inc, 1989:587-99.
5. MacLean GD, McEwan AJ, Noujaim AA, Hooper HR, Sykes TR, Longenecker BM. The clinical relevance of the expression of Asialo-G.M1 for gynecologic cancer imaging. Eur J Nucl Med. (In press)
6. Beatty JD, Duda RB, Williams LE, et al. Preoperative imaging of colo rectal carcinoma with $^{111}In$-labelled anticarcinoembryonic antigen monoclonal antibody. Cancer Res 1986;46:6494-502.
7. Chatal JF, Saccavini JC, Fumoleau P, et al. Immunoscintigraphy of colon carcinoma. J Nucl Med 1984;25:307-14.
8. Epenetos AA, Snook D, Durbin H, Johnson PM, Taylor-Papadimitriou J. Limitations of radiolabelled monoclonal antibodies for localization of human neoplasms. Cancer Res 1986;46:3183-91.
9. MacLean GD, McEwan AJ, Noujaim AA, et al. A novel strategy for cancer immunoscintigraphy. Antibody Immunoconjugates and Radiopharmaceuticals 1989;2:15-27.
10. MacLean G, McEwan A, Mackie E, et al. The potential of synthetic tumor-associated glycoconjugates (S-tags) for generating monoclonal antibodies for breast cancer imaging and for specific immunotherapy. In: Seriani RL, ed. Breast Cancer Immunodiagnosis Immunotherapy. New York: Plenum Press. (In press)
11. Sickle-Santanello BJ, O'Dwyer PJ, Mojzisik C, et al. Radioimmunoguided surgery using the monoclonal antibody B72.3 in colorectal tumors. Dis Colon Rectum 1987;30:761-4.
12. Martin ET, Aitken D, Thurston M, et al. Successful experimental use of a self contained gamma detecting device. Curr Surg 1984;41:193-4.
13. Aitken DR, Thurston MO, Hinkle GH Jr, et al. Portable gamma probe for radioimmune localization of experimental colon tumor xenografts.

J Surg Res 1984;36:480-9.
14. Aitken DR, Hinkle GH, Thurston MO, et al. A gamma-detecting probe for radioimmune detection of CEA-producing tumors. Dis Colon Rectum 1984;27:279-82.
15. Tuttle SE, Jewell SD, Mojzisik CM, et al. Intraoperative radioimmunolocalization of colorectal carcinoma with a hand-held gamma probe and MAb B72.3: Comparison of in vivo gamma probe counts with in vitro MAb radiolocalization. Int J Cancer 1988;42:352-8.
16. Martin EW Jr, Mojzisik CM, Hinkle GH Jr, et al. Schlom-radioimmunoguided surgery using monoclonal antibody. Am J Surg 1988;156:386-92.
17. Sardi A, Workman M, Mojzisik C, Hinkle G, Nieroda C, Martin EW Jr. Intraabdominal recurrence of colorectal cancer detected by radioimmunoguided surgery (RIGS System). Arch Surg 1989;124:55-9.
18. National Institute of Health: The efficacy and the group C status of levamisole and 5-fluorouracil for patients with Duke's C colon cancer. National Cancer Institute Update, Bethesda, Maryland, November 1989.
19. Laurie JA, Moertel CG, FlemingTR. Surgical adjuvant therapy of large bowel carcinoma: An evaluation of levamisole and the combination of levamisole and fluorouracil. J Clin Oncol 1989;7:1447-56.
20. Moertel CG, Fleming TR, MacDonald JS, et al. Levamisole and fluorouracil for adjuvant therapy of resected colon carcinoma. N Engl J Med 1990;322:352-8.