

Adjuvant Radioimmunotherapy Trial with Iodine-131-Labeled Anti-Carcinoembryonic Antigen Monoclonal Antibody F6 F(ab')₂ after Resection of Liver Metastases from Colorectal Cancer.

Marc Ychou, David Azria, Cathy Menkarios, Patrick Faurous, François Quenet, Bernard Saint-Aubert, Philippe Rouanet, Monique Pèlerin, Caroline Bascoul-Mollevi, Dominique Guerreau, et al.

► **To cite this version:**

Marc Ychou, David Azria, Cathy Menkarios, Patrick Faurous, François Quenet, et al.. Adjuvant Radioimmunotherapy Trial with Iodine-131-Labeled Anti-Carcinoembryonic Antigen Monoclonal Antibody F6 F(ab')₂ after Resection of Liver Metastases from Colorectal Cancer.. *Clinical Cancer Research*, American Association for Cancer Research, 2008, 14 (11), pp.3487-93. 10.1158/1078-0432.CCR-07-4698 . inserm-00292535

HAL Id: inserm-00292535

<https://www.hal.inserm.fr/inserm-00292535>

Submitted on 2 Jun 2009

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Adjuvant radioimmunotherapy trial with iodine-131-labeled anti-carcinoembryonic antigen monoclonal antibody F6 F(ab)₂ after resection of liver metastases from colorectal cancer

Ychou Marc^{1,2}, Azria David^{1,2}, Menkarios Cathy^{2,3}, Faurous Patrick², Quenet François², Saint-Aubert Bernard², Rouanet Philippe², Pèlerin Monique^{1,2}, Bascoul-Mollevi Caroline², Guerreau Dominique⁴, Saccavini Jean-Claude⁴, Mach Jean-Pierre⁵, Artus Jean-Claude², Pèlerin André^{1*}

¹ Immunociblage et radiobiologie en oncologie INSERM : U860, Université Montpellier I, CRLC val d'aurelle - paul lamarque parc euromedecine centre de recherche en cancerologie 34298 MONTPELLIER CEDEX 5,FR

² CRLC Val d'Aurelle - Paul Lamarque CRLCC Val d'Aurelle - Paul Lamarque, Montpellier,FR

³ Department of Radiation Oncology Université de Montréal, Pavillon Maisonneuve 5415, boul. de l'Assomption Montréal (Québec) HIT 2M4,CA

⁴ Schering-CIS Bio International B.P 32 F-91192 Gif sur Yvette,FR

⁵ Département de Biochimie Université de Lausanne, CH-1066 Epalinges,CH

* Correspondence should be adressed to: André Pèlerin <andre.pelerin@valdorel.fnclcc.fr>
Marc Ychou and David Azria contributed equally to this work

Abstract

Purpose

To evaluate the feasibility of radioimmunotherapy (RIT) with radiolabeled anti-CEA antibodies after complete resection of liver metastases (LM) from colorectal cancer.

Patients and Methods

Twenty-two patients planned for surgery of one to four LM received a pre-operative diagnostic dose of a ¹³¹I-F(ab')₂ labelled anti-CEA monoclonal antibody F6 (8–10 mCi/5 mg). ¹³¹I-F(ab')₂ uptake was analyzed using direct radioactivity counting and tumor-to-liver (TTL) ratios were recorded. Ten patients with TTL ratios > five and three others were treated with a therapeutic injection (180–200 mCi ¹³¹I/50 mg F(ab')₂) 30 to 64 days after surgery.

Results

Median ¹³¹I-F(ab')₂ immunoreactivity in patient serum remained at 91% of initial values for up to 96h after injection. The main and dose-limiting-toxicity was hematological, with 92% and 85% grade 3–4 neutropenia and thrombocytopenia, respectively. Complete spontaneous recovery occurred in all patients. No human anti-mouse antibody (HAMA) response was observed after the diagnosis dose, however 10 of the 13 treated patients developed HAMA approximately three months later. Two treated patients presented extra-hepatic metastases at the time of RIT (one bone and one abdominal node) and two relapsed within three months of RIT (one in the lung and the other in the liver). Two patients are still alive, and one of these is disease-free at 93 months after resection. At a median follow-up of 127 months, the median disease-free survival is 12 months and the median overall survival is 50 months.

Conclusion

RIT is feasible in an adjuvant setting after complete resection of LM from colorectal cancer and should be considered for future trials, possibly in combination with chemotherapy, because of the generally poor prognosis of these patients.

MESH Keywords Adenocarcinoma ; mortality ; radiotherapy ; secondary ; Adult ; Antibodies, Monoclonal ; therapeutic use ; Carcinoembryonic Antigen ; immunology ; Chemotherapy, Adjuvant ; Colorectal Neoplasms ; mortality ; pathology ; therapy ; Female ; Hepatectomy ; Humans ; Immunoglobulin Fab Fragments ; therapeutic use ; Iodine Radioisotopes ; pharmacokinetics ; therapeutic use ; Liver Neoplasms ; Male ; Middle Aged ; Radioimmunotherapy ; methods ; Radiotherapy, Adjuvant

Author Keywords Radioimmunotherapy ; radiolabeled monoclonal antibodies ; colorectal cancer ; hepatic metastases

INTRODUCTION

Surgical resection is the most effective therapy for isolated liver metastases (LM) from colorectal cancer (CRC) (1–4) and offers the only possibility of cure in these patients. When complete resection is achieved, 5-year survival rates ranging from 25–41% have been reported (2, 3, 5–7). However, approximately two-thirds of patients relapse, often in the first two-years, demonstrating the need for efficient post-operative therapies capable of sterilizing microscopic disease. Even though chemotherapy in this setting has enhanced disease-free survival (DFS), it has not clearly demonstrated a survival advantage. Efforts have thus been focused on immunotherapy and

radioimmunotherapy with native or radiolabeled monoclonal antibodies (MAb) based on impressive results in the treatment of non-hodgkin lymphoma (8–10). Studies of radiolabeled MAb in the adjuvant setting of CRC or with small volume-disease have been reported (11, 12), although success with bulky, metastatic tumors from CRC has been limited (13–15). At our institution, an initial phase I study involving patients with non-resectable and chemorefractory LM from colorectal cancer (CRC) showed that the maximal tolerated dose (MTD) of ^{131}I -anti-CEA $\text{F}(\text{ab}')_2$ was 200 mCi. No patient at this dose level had greater than grade 2 hematologic toxicity (WHO classification), thus alleviating the need for autologous bone-marrow rescue that was observed with a dose of 300 mCi (16). Based on the results of this study and on the favorable biodistribution showing high tumor uptake of $\text{F6 F}(\text{ab}')_2$ in the majority of the patients with metastatic CRC, we conducted a phase II study of post-operative radioimmunotherapy (RIT) after complete resection of LM using ^{131}I $\text{F6 F}(\text{ab}')_2$.

PATIENTS AND METHODS

MAb production and purification

MAb F6 is a murine IgG_{1a} directed against the Gold-1 CEA-specific epitope (17). This antibody has undergone extensive preclinical analyses, localization (18) and therapeutic studies in patients (16).

Clinical grade $\text{F6 F}(\text{ab}')_2$ was obtained from Schering-CIS Biointernational (Gif-sur-Yvette, France). It was purified from culture supernatant by protein A chromatography. $\text{F}(\text{ab}')_2$ was obtained by pepsin digestion and purified by ion-exchange chromatography. The cell banks and the purified MAb and $\text{F}(\text{ab}')_2$ tested negative for viruses and mycoplasma contamination.

Radioiodination of $\text{F6 F}(\text{ab}')_2$ fragments

Radiolabeling was performed by Schering-CIS Biointernational. The diagnostic dose consisted of 5 mg $\text{F6 F}(\text{ab}')_2$ labeled with 8–10 mCi of ^{131}I (1 mCi = 37 MBq) in sterile phosphate-buffered saline containing 1.6% human serum albumin. The therapeutic dose consisted of 50 mg $\text{F6 F}(\text{ab}')_2$ labeled with 200 mCi of ^{131}I in the same buffer. Each therapeutic dose was stored in two vials of nine ml to reduce radiolysis of the antibody. The radiochemical purity, as determined by radio-thin layer chromatography, was greater than 90%. The endotoxin (limulus assay) was lower than 175 endotoxin units/dose. The immunoreactivity of ^{131}I $\text{F}(\text{ab}')_2$ was determined as described by Buchegger et al. (19) using a direct binding assay to CEA coupled to CNBr-Sepharose (Pharmacia, Uppsala, Sweden) and ranged from 44 % to 85% (median 71%).

Patient selection

Patients were eligible if complete resection (R0) of LM from colorectal cancer was deemed possible after assessment by an experienced committee of surgeons, medical and radiation oncologists, and nuclear physicians. Other eligibility criteria included absence of chemotherapy in the six weeks prior to the injection, a serum creatinine concentration less than 120 $\mu\text{mol/ml}$, bilirubinemia less than 30 $\mu\text{mol/ml}$, prothrombine time greater than 60%, a platelet count greater than 100.000/ mm^3 and a granulocyte count greater than 2000/ mm^3 . Patients with severe cardiac, pulmonary or infectious disease, clinical evidence of central nervous system tumor involvement, history of prior administration of mouse-derived antibodies, protein constructs or human antimouse antibody serum reactivity were excluded as were those with a performance status greater than 2 according to the CTC v. 2.0 classification (20). Patients with prior pelvic radiation therapy could be included. Post-operative chemotherapy was not permitted in the absence of recurrence. The clinical protocol and consent form were approved by our ethical committee and written informed consent was obtained from all patients before study entry.

Dose and administration of ^{131}I $\text{F6 F}(\text{ab}')_2$

Three days prior to antibody administration and continuing for two weeks, patients received a daily iodine solution (150 mg/day) to prevent uptake of the ^{131}I by the thyroid gland. A first diagnostic dose (8–10 mCi $^{131}\text{I}/5$ mg) was administered as an i.v. bolus 5–7 days before the surgery. Patients were eligible to receive a second, therapeutic injection (200 mCi $^{131}\text{I}/50$ mg) 4–8 weeks after surgery if the tumor-to-normal liver (TTL) uptake ratio of ^{131}I $\text{F6F}(\text{ab}')_2$ was superior to 5, based on analysis of tissues obtained during surgery. This ratio corresponds to the lower limit proposed by Welt et al. for the screening of patients potentially eligible for RIT (21). The therapeutic dose was administered in the form of an i.v. 250 ml isotonic sodium chloride solution in a 60-minute perfusion. The radionuclide doses were not adjusted for body weight or surface area because we believe that individualized calculation of doses should be based on antibody biodistribution and pharmacokinetics rather than body weight or surface area. In the future, we will adjust doses based on data from this trial. Patients were hospitalized in a protected nuclear medicine ward allowing for safe radioactive urine collection and adequate radiation-isolation. Strict radioprotection regulations were reviewed with nursing and auxiliary staff. Visits were forbidden during the entire week. Patients were discharged five to seven days after treatment, depending on the results of dose-rate measurements. They were given recommendations on safety precautions to follow for the next week with the aim of reducing radiation exposure to family members.

Scintigraphic and metabolic monitoring

The day prior to surgery (four to six days after the ^{131}I F6 F(ab')₂ diagnostic dose), 0.08 mCi/kg $^{99\text{m}}\text{Tc}$ -sodium phytate (Phytacis[®], CisBio International, Gif-sur-Yvette, France) was administered intravenously to image normal liver and a whole body scan and single photon emission computed tomography (SPECT) were carried out with a Sopha Medical NXT gamma camera and a high-energy, parallel-hole, general purpose collimator. Scintigraphic data was collected through a double isotope acquisition, each with a 20% symmetrical window around 140 keV and 364 keV respectively. SPECT was implemented with a 360° elliptical rotation, 64 projections, and 30s/projection, while slice reconstruction was completed by a Weiner-filtered backprojection technique.

Following the therapeutic dose, complete blood cell counts were performed at least twice weekly until week eight or until the neutrophil and platelet counts returned to normal. Blood chemistry tests and a coagulation profile were done before discharge from the hospital and within four to eight weeks after the therapeutic dose.

Blood samples were collected at the end of the therapeutic injection (t_0), at 1, 2, 4, 8 hours post-injection and then once daily until day five. These samples were used to determine the circulating half-lives (α and β) using the biexponential model, and the immunoreactivity of the injected F(ab')₂ fragments as described by Buchegger (19).

Blood samples were also collected from weeks 3 to 15 to detect the formation of human anti-mouse antibodies (HAMA), using an ELISA assay which provided a quantitative result concerning the anti-isotype human anti-mouse IgG response (HAMA-ELISA, Medac Diagnostika, Hamburg, Germany).

Carcinoembryonic antigen (CEA) values were determined before surgery, before the RIT treatment, every month for the first three months and then every 3 months.

Surgical procedures

Of the 13 patients receiving a therapeutic dose of RIT, 7 underwent a major hepatectomy, 3 underwent a minor hepatectomy, and the remaining 3 patients underwent wedge resections. Major hepatectomy was defined as resection of more than three segments according to the Couinaud classification. Minor hepatectomy was defined as resection of 1 to 3 liver segments. Intraoperative ultrasound was systematically used for complete hepatic evaluation. Postoperative parameters of hepatocyte damage and recovery, including serum transaminase, bilirubin levels and prothrombin time (expressed as a percentage of controls), were measured on postoperative days 1, 2, 5, and 7. Histologic margin to the nearest metastasis was characterized as negative (≥ 1 cm), close (< 1 cm), or positive.

Patient follow-up

All patients underwent a CT of the chest, abdomen, and pelvis before therapy. Follow-up consisted of clinical evaluation with routine blood chemistry, tumor marker dosage (CEA), HAMA production, CT or X-rays of the chest, and CT or ultrasound of the abdomen every three months during the first two years, and then every 6 months for the following 3 years. Clinical and radiologic response were assessed until relapse or death.

RESULTS

Pre-operative imaging and surgery

Twenty-two patients planned for surgery for 1 to 4 LM from colorectal cancer received a diagnostic dose of ^{131}I -labeled F(ab')₂ fragments (^{131}I -F(ab')₂) from anti-CEA monoclonal antibody F6. Preoperative SPECT imaging, performed 4 to 6 days after ^{131}I F6 F(ab')₂ injection (corresponding to 24h before surgery), detected at least one LM in 19 of 22 patients (negative for #2, 12 and one untreated patient).

All surgical resections of LM were performed with curative intent at a single institution. Complete resection (R0) was obtained for all 22 patients. Intraoperative ultrasound did not detect LM other than those found by the preoperative staging procedures described above. No postoperative bleeding or hepatocellular failure was observed.

For one patient (#6), the suspected LM on pre-operative imaging in segment IV was not palpable intra-operatively after neo-adjuvant chemotherapy. However, a hyperdense image on intraoperative ultrasound was noted and an immunoscintigraphy of segment IV allowed the visualization of a metastatic nodule (0.7 cm diameter). The patient underwent a metastatectomy of segment VII and a resection of segment IV. This nodule demonstrated an uptake of 34.5×10^{-3} %ID/g and a metastasis-to-normal liver uptake ratio of 68 (Figure 1).

Anti-CEA F(ab')₂ uptake in LM and patient selection for therapeutic dose

Metastases and normal liver biopsies obtained during surgery were analyzed for ^{131}I -F(ab')₂ uptake by direct radioactivity counting. All TTL uptake ratios ranged from 1.7 to 32.4, except for one value of 68 as previously discussed (Table 1). Only patients with ratios greater than five were selected for the therapeutic injection. Three other patients (#7, 12 and 13) that did not meet this criteria also received

the therapeutic dose. Patient 12 (TTL ratio of 2) was considered to be at high risk of recurrence and no other adjuvant therapy could be offered at that time. Patients 7 and 13 did not receive the pre-operative diagnostic injection but were included because their hepatic recurrence occurred within 1 year of the adjuvant chemotherapy for their primary cancer, and they both refused additional chemotherapy. It was felt that a post-operative treatment should be offered in the presence of short disease-free intervals, most likely representing aggressive disease. Three other patients with TTL ratios > 5 did not receive the therapeutic dose for various reasons: one had suspected pulmonary metastases and chemotherapy was offered, one died of post-hepatectomy complications and the third had negative SPECT imaging with a borderline ratio. The treating physician judged that this patient might benefit more from chemotherapy as he was chemotherapy naïve. Clinical characteristics and outcomes of the 13 RIT treated patients are given in Table 2.

Therapeutic injection

The therapeutic injections were performed between day 30 and day 64 after surgery (median = 37), depending on each patient's post-operative recovery. All patients presented a postoperative circulating CEA value below 10 ng/ml (which is the upper limit of normal for our CEA assay) except for patient number 2 who had a value of 12 ng/ml. The administered doses ranged from 180 to 200 mCi. Post-treatment SPECT imaging was negative in all patients.

Pharmacokinetic analysis

Extensive analysis of the radiolabeled F(ab')₂ serum clearance rates was performed for 12 of the 13 treated patients. This was done using radioactivity counting of serum samples collected until day 5 as previously described. The calculated circulating α and β half-lives were found to range from 30 min to 6 h 37 min and from 23 h 09 min to 40 h 53 min, respectively. The median mean residence time (MRT) was 51 h 42 min, with extreme values of 37 h 45 min for patient 2 and 65 h 09 min for patient 1. Table 3 shows the kinetic parameters and HAMA responses for each of the 13 treated patients.

The immunoreactivity of ¹³¹I-F(ab')₂ determined from all serum samples was stable for up to 96 h after the injection. It ranged from 70 % to 100% (median 91%) of the injected product in the serum of the 11 patients sampled at 72 hours post-injection, and remained in the same range in the 6 patients sampled at 96 hours.

Human anti-mouse antibody (HAMA) response

All 22 patients tested negative for HAMA before the study and they all remained negative after the diagnostic injection. Of the 13 RIT treated patients, 12 were analyzed further for HAMA response. Ten developed HAMA and two patients remained negative until testing was stopped at 15 weeks after RIT (Table 3).

Toxicity

One patient experienced an allergic reaction to the diagnostic dose. Symptomatic clinical toxicity was mild to moderate (grade 1 or 2) and consisted mostly of fatigue and anorexia, occurring in 61.5% and 23%, respectively. Unexplained fever was observed in one patient without concurrent neutropenia. Hematologic toxicity was significant (Table 4), but there were no toxicity-related deaths. Cytopenic nadirs usually occurred between weeks 4 and 6, with recovery by week 6 to 9. No febrile neutropenia was observed and no patient required granulocyte colony-stimulating factor (G-CSF). The grade 4 thrombocytopenias lasted from 9 to 33 days. No patient experienced symptomatic bleeding and only one patient (#6) required platelet transfusions.

Patient 2 presented no toxicity at all which may be related to the short mean residual time observed (37 h 45 min). Two other patients (#3 and 9) experienced no leukocyte toxicity despite grade 4 thrombopenia. There was no correlation to the MRT of the therapeutic injection (52 h 54 min and 43 h 34 min, respectively).

Patient follow-up

At the time of writing, with 127 months of median follow-up, 2 of the 13 treated patients are still alive. Median DFS was 12 months and median OS was 50 months.

Patient #13 has no evidence of disease at 93 months and his CEA levels remain within normal limits. Patient #8 presented liver recurrences that were resected in 1999 and in 2001 (one of which was treated with neo-adjuvant chemotherapy). At 127 months of follow-up, he has non-resectable LM but is responding well to chemotherapy. Six other patients developed further LM and were not candidates for curative treatment. No patient underwent retreatment with RIT.

DISCUSSION

Over the past two decades, RIT has made important progress in cancer treatment, particularly in radiosensitive lymphomas where two biologically different radiopharmaceutical agents targeting CD20 surface antigen have demonstrated clinical safety and efficacy (8, 9).

Both ^{90}Y -ibritumomab tiuxetan and ^{131}I -tositumomab have shown impressive overall response rates and complete response rates in patients with relapsed, refractory or transformed CD20+ indolent B-cell lymphoma.

The natural radiosensitivity of lymphomas make them ideal tumors for this treatment when given as a single agent and may explain in part why similar success has not been obtained with solid, bulky malignancies, notably in CRC (13–15). Other explanations may include the more heterogeneous antigen expression and density of solid tumors or factors that limit agent delivery such as: location in solid organs or on peritoneal surfaces, heterogeneous tumor vascularity or slow diffusion of Abs to tumoral tissues (22, 23). Furthermore, it has been shown in solid tumors that MAb uptake and the resulting radiation doses are inversely correlated with tumor size (13, 18). Uptake is also higher in the periphery of the tumor than at its core (18). Hence, microscopic disease is probably more suited for RIT and clinical benefit might be observed only in the presence of smaller tumor burden. Behr et al. tested this hypothesis in 19 metastatic CRC patients with small-volume disease. All lesions were smaller than three cm and patients received 60 mCi/m² of ^{131}I -hMN14, a humanized anti-CEA antibody (11). Partial response rate of 16% were reported and the overall response rate was 58%. In the same study, 9 other patients were treated post-operatively after surgical resection of LM and 7 of these remained disease-free for up to 36 months. Transient myelosuppression was the only toxicity observed.

The present study is a phase II trial of radioimmunotherapy in which the feasibility and toxicity of high-dose ^{131}I F6 F(ab')₂ after resection of LM were determined. One of the major limitations in RIT of clinical tumors is the small percentage of injected MAb that is selectively delivered to the tumor (19). In nude mice xenografted with human colon cancer, we demonstrated that ^{131}I -labeled F(ab')₂ fragments are more efficient and less toxic than their intact anti-CEA counterpart (18). These results can be explained by the fact that F(ab')₂ fragments give higher tumor-to-normal tissue ratios and have a shorter half-life than intact MAbs. In the present study, as in our initial phase I study (18), most TTL uptake ratios ranged from 2 to 30. Treatment was well tolerated, and similar to others studies of RIT, the main toxicity was significant but transient myelosuppression. Ten of 13 patients experienced grade 3 or 4 neutropenia and 11 of 13 patients had grade 3 or 4 thrombopenia. However, no patient experienced spontaneous bleeding, none required G-CSF and only one patient required platelet transfusions.

We cannot draw conclusions as to the efficacy of this treatment since this was not taken into account during the design of our phase II study. However, median OS (50 months) and DFS (12 months) are provided for information and seem to be comparable to historical controls despite the small number of patients, which included 2 patients with metastases at the time of treatment.

Although much effort has been put into investigating peri-operative treatment to improve the outcome of these patients, there is no standard approach in this setting. Studies have included hepatic arterial infusion (HAI) with or without systemic chemotherapy (24–29) and systemic 5-FU/LV-based chemotherapy alone (6). Some of these treatments have demonstrated enhancement of DFS but an overall survival benefit over surgery alone remains unclear. To date, none of these regimens has established itself as standard and toxicities from these treatments are nonnegligible, especially with regards to hepatic catheter complications with HAI. Moreover, there are no published randomized studies using active chemotherapy agents such as oxaliplatin, irinotecan or bevacizumab that have shown superiority in stage III and stage IV patients with measurable disease, although a phase-II trial of post-operative irinotecan has been shown to be tolerable after major liver resection (30). Preliminary results of the EORTC 40983 trial which randomizes patients with potentially resectable liver-only metastases to surgery alone or 3 months of pre-operative and 3 months of post-operative FOLFOX4 chemotherapy were presented at the American Society of Clinical Oncology (ASCO) 2007 annual meeting. They found an 8.1% increase in DFS at 3 years, which was the primary end-point (HR 0.77, $p = 0.041$). The ongoing NSABP C-09 trial is evaluating the role of systemic capecitabine and oxaliplatin alone or alternating with hepatic arterial infusion (HAI) chemotherapy in these patients.

According to clinical dosimetric data obtained in a previous study, the maximum delivered dose to LM can be estimated as being 10 Gy per 100 mCi of injected ^{131}I -F6 F(ab')₂ (18). This is insufficient to eradicate macroscopic metastases (which require at least 50 to 70 Gy), or even to eradicate microscopic disease. One strategy to improve the results of RIT in CRC would be repeated administration of anti-tumor radiolabeled Mab in order to increase the cumulative dose. This could be done using humanized Ab to avoid HAMA formation in response to repeated injection of murine Ab. A limitation of this approach is the need to wait at least 2 or 3 months to allow full bone marrow recovery before delivering a second therapeutic dose. However, this opens the possibility of tumor cell repopulation during this relatively long waiting period. Another option would be to combine RIT with chemotherapy which could also act as a radiosensitizer, but cumulative hematologic toxicity is of concern. Wong et al. conducted a phase I dose-escalation study of ^{90}Y -anti-CEA chimeric T84.66 RIT in combination with continuous infusion 5-FU in heavily pretreated patients with chemotherapy-refractory metastatic CRC (31). Thirteen patients received one cycle and eight patients received 2 cycles. The MTD of both agents in combination were comparable with MTD levels of each agent alone. Hematologic toxicity was dose-limiting and reversible with 52% grade 3–4 platelet toxicity and 29% grade 3 WBC toxicity. RIT did not seem to enhance the non-hematological toxicities usually associated with 5-FU. They concluded that RIT in combination with chemotherapy is feasible. At this time, it is not possible to compare adjuvant RIT with adjuvant chemotherapy in this particular setting but a further trial incorporating RIT combined with chemotherapy seems indicated.

There is only one other published trial delivering RIT to metastatic CRC patients in the post-operative setting (12). In this phase II trial, Liersch et al. treated 23 patients who underwent hepatectomies for LM of CRC with 40–60mCi/m² of ¹³¹I-labetuzumab, a humanized monoclonal antibody against CEA. Recently updated data with 91 months median follow-up show a median overall survival (OS) of 58 months, a 5-year survival rate of 42% and a median DFS of 18 months (32). Although retrospective comparisons must always be interpreted with caution, this seems encouraging compared to the median survival of 28 to 40 months of historical controls (3) and of 31 months for a group of 19 contemporaneous patients with similar prognostic scores at their center. Of note, 13 of these 19 patients used for comparison had received adjuvant chemotherapy whereas this was not permitted in the RIT treated patients. Similar to our study, the only significant toxicity was transient myelosuppression with a 52% rate of grade 3–4 cytopenias. These effects seemed to be independent of prior chemotherapy.

In our study, a « single-shot » treatment protocol was utilized. Behr et al. tested retreatment with the same dose (60 mCi/m²) in five patients with metastatic lesions having previously responded to RIT with ¹³¹I-hMN14, after a delay of 8 to 16 months. Two patients experienced partial responses and another experienced disease stabilization. Of importance, there was no evidence of increased toxicity, with no hematological toxicity greater than grade 3 (11). Liersch et al. also retreated 2 patients for recurrent disease 5 and 31 months after the first treatment and reported no cumulative toxicity (12). Retreatment with RIT is feasible and deserves further study.

Regarding HAMA response, 10 of the 12 patients with longer follow-up eventually tested positive after the therapeutic injection. Future research should be geared towards humanized anti-CEA antibodies such as ¹³¹I-labetuzumab and ¹³¹I-hMN14. A fully human anti-CEA IgG2κ has been developed at our center and is a promising candidate for RIT in intact form, as a F(ab')₂, or as a bispecific antibody (33). Other promising avenues include use of affinity enhancement systems with pre-targeting bispecific MAb (34).

In conclusion, this phase II study demonstrates that RIT after complete resection of LM in CRC is both feasible and well tolerated. These findings support those of others and suggest that RIT, either alone with surgery or in a multimodality setting including chemotherapy, should be evaluated in a randomized trial.

Acknowledgements:

We are grateful to the Ligue Nationale Contre Le Cancer, the Fondation Gustave Prévot and the Fondation de France for financial support, and to Mrs. S. Bousquié for excellent technical assistance.

References:

1. Scheele J, Stang R, Altendorf-Hofmann A, Paul M Resection of colorectal liver metastases. *World J Surg.* 1995; 19: 59- 71
2. Fong Y, Cohen AM, Fortner JG Liver resection for colorectal metastases. *J Clin Oncol.* 1997; 15: 938- 46
3. Fong Y Surgical therapy of hepatic colorectal metastasis. *CA Cancer J Clin.* 1999; 49: 231- 55
4. Lehnert T, Otto G, Herfarth C Therapeutic modalities and prognostic factors for primary and secondary liver tumors. *World J Surg.* 1995; 19: 252- 63
5. Jaeck D, Bachellier P, Guiguet M Long-term survival following resection of colorectal hepatic metastases. *Association Francaise de Chirurgie Br J Surg.* 1997; 84: 977- 80
6. Portier G, Elias D, Bouche O Multicenter randomized trial of adjuvant fluorouracil and folinic acid compared with surgery alone after resection of colorectal liver metastases: FFCD ACHBTH AURC 9002 trial. *J Clin Oncol.* 2006; 24: 4976- 82
7. Gayowski TJ, Iwatsuki S, Madariaga JR Experience in hepatic resection for metastatic colorectal cancer: analysis of clinical and pathologic risk factors. *Surgery.* 1994; 116: 703- 10
8. Witzig TE, Gordon LI, Cabanillas F Randomized controlled trial of yttrium-90-labeled ibritumomab tiuxetan radioimmunotherapy versus rituximab immunotherapy for patients with relapsed or refractory low-grade, follicular, or transformed B-cell non-Hodgkin's lymphoma. *J Clin Oncol.* 2002; 20: 2453- 63
9. Kaminski MS, Zelenetz AD, Press OW Pivotal study of iodine I 131 tositumomab for chemotherapy-refractory low-grade or transformed low-grade B-cell non-Hodgkin's lymphomas. *J Clin Oncol.* 2001; 19: 3918- 28
10. Coiffier B, Lepage E, Briere J CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. *N Engl J Med.* 2002; 346: 235- 42
11. Behr TM, Liersch T, Greiner-Bechert L Radioimmunotherapy of small-volume disease of metastatic colorectal cancer. *Cancer.* 2002; 94: 1373- 81
12. Liersch T, Meller J, Kulle B Phase II trial of carcinoembryonic antigen radioimmunotherapy with ¹³¹I-labetuzumab after salvage resection of colorectal metastases in the liver: five-year safety and efficacy results. *J Clin Oncol.* 2005; 23: 6763- 70
13. Behr TM, Sharkey RM, Juweid ME Variables influencing tumor dosimetry in radioimmunotherapy of CEA-expressing cancers with anti-CEA and antimucin monoclonal antibodies. *J Nucl Med.* 1997; 38: 409- 18
14. Welt S, Divgi CR, Kemeny N Phase I/II study of iodine 131-labeled monoclonal antibody A33 in patients with advanced colon cancer. *J Clin Oncol.* 1994; 12: 1561- 71
15. Knox SJ, Goris ML, Tempero M Phase II trial of yttrium-90-DOTA-biotin pretargeted by NR-LU-10 antibody/streptavidin in patients with metastatic colon cancer. *Clin Cancer Res.* 2000; 6: 406- 14
16. Ychou M, Pelegrin A, Faurous P Phase-I/II radio-immunotherapy study with Iodine-131-labeled anti-CEA monoclonal antibody F6 F(ab')₂ in patients with non-resectable liver metastases from colorectal cancer. *Int J Cancer.* 1998; 75: 615- 9
17. Hammarstrom S, Shively JE, Paxton RJ Antigenic sites in carcinoembryonic antigen. *Cancer Res.* 1989; 49: 4852- 8
18. Ychou M, Ricard M, Lumbroso J Potential contribution of ¹³¹I-labelled monoclonal anti-CEA antibodies in the treatment of liver metastases from colorectal carcinomas: pretherapeutic study with dose recovery in resected tissues. *Eur J Cancer.* 1993; 29: 1105- 11
19. Buchegger F, Mach JP, Pelegrin A Radiolabeled chimeric anti-CEA monoclonal antibody compared with the original mouse monoclonal antibody for surgically treated colorectal carcinoma. *J Nucl Med.* 1995; 36: 420- 9
20. Trotti A, Byhardt R, Stetz J Common toxicity criteria: version 2.0. an improved reference for grading the acute effects of cancer treatment: impact on radiotherapy. *Int J Radiat Oncol Biol Phys.* 2000; 47: 13- 47
21. Welt S, Divgi CR, Real FX Quantitative analysis of antibody localization in human metastatic colon cancer: a phase I study of monoclonal antibody A33. *J Clin Oncol.* 1990; 8: 1894- 906
22. Bertagnolli MM Radioimmunotherapy for colorectal cancer. *Clin Cancer Res.* 2005; 11: 4637- 8

- 23. Sgouros G Radioimmunotherapy of micrometastases: sidestepping the solid-tumor hurdle. *J Nucl Med.* 1995; 36: 1910- 2
- 24. Lygidakis NJ , Sgourakis G , Vlachos L Metastatic liver disease of colorectal origin: the value of locoregional immunochemotherapy combined with systemic chemotherapy following liver resection. Results of a prospective randomized study. *Hepato-gastroenterology.* 2001; 48: 1685- 91
- 25. Asahara T , Kikkawa M , Okajima M Studies of postoperative transarterial infusion chemotherapy for liver metastasis of colorectal carcinoma after hepatectomy. *Hepato-gastroenterology.* 1998; 45: 805- 11
- 26. Rudroff C , Altendorf-Hoffmann A , Stangl R , Scheele J Prospective randomised trial on adjuvant hepatic-artery infusion chemotherapy after R0 resection of colorectal liver metastases. *Langenbeck's archives of surgery/Deutsche Gesellschaft fur Chirurgie.* 1999; 384: 243- 9
- 27. Lorenz M , Muller HH , Schramm H Randomized trial of surgery versus surgery followed by adjuvant hepatic arterial infusion with 5-fluorouracil and folinic acid for liver metastases of colorectal cancer. German Cooperative on Liver Metastases (Arbeitsgruppe Lebermetastasen). *Ann Surg.* 1998; 228: 756- 62
- 28. Kemeny MM , Adak S , Gray B Combined-modality treatment for resectable metastatic colorectal carcinoma to the liver: surgical resection of hepatic metastases in combination with continuous infusion of chemotherapy—an intergroup study. *J Clin Oncol.* 2002; 20: 1499- 505
- 29. Kemeny NE , Gonen M Hepatic arterial infusion after liver resection. *N Engl J Med.* 2005; 352: 734- 5
- 30. Mackay HJ , Billingsley K , Gallinger S A multicenter phase II study of “adjuvant” irinotecan following resection of colorectal hepatic metastases. *Am J Clin Oncol.* 2005; 28: 547- 54
- 31. Wong JY , Shibata S , Williams LE A Phase I trial of 90Y-anti-carcinoembryonic antigen chimeric T84.66 radioimmunotherapy with 5-fluorouracil in patients with metastatic colorectal cancer. *Clin Cancer Res.* 2003; 9: 5842- 52
- 32. Liersch T , Meller J , Bittrich M , Kulle B , Becker H , Goldenberg DM Update of carcinoembryonic antigen radioimmunotherapy with (131i)-labetuzumab after salvage resection of colorectal liver metastases: comparison of outcome to a contemporaneous control group. *Ann Surg Oncol.* 2007; 14: 2577- 90
- 33. Garambois V , Glausel F , Foulquier E Fully human IgG and IgM antibodies directed against the carcinoembryonic antigen (CEA) Gold 4 epitope and designed for radioimmunotherapy (RIT) of colorectal cancers. *BMC Cancer.* 2004; 4: 75-
- 34. Goldenberg DM , Sharkey RM , Paganelli G , Barbet J , Chatal JF Antibody pretargeting advances cancer radioimmunodetection and radioimmunotherapy. *J Clin Oncol.* 2006; 24: 823- 34

Figure 1

An immunoscintigraphy of liver segment IV was performed and allowed the visualization of an occult metastasis (0.7 cm diameter) with a TTL ratio of $^{131}\text{I-F(ab')}_2$ uptake of 68 (Patient #6). A, scanning of the (Sn)Phytate in the $^{99\text{m}}\text{Tc}$ window; B, scanning of the anti-CEA F(ab')_2 in the ^{131}I window.

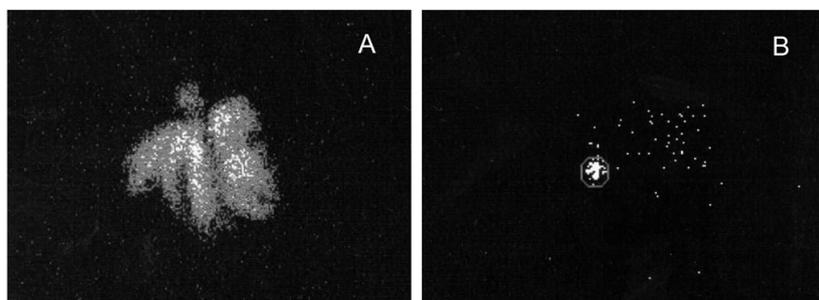


Table 1Diagnostic injection: Anti-CEA F(ab')₂ uptake in liver metastases and normal liver

Patient No	%ID/kg of liver metastases			Tumor to normal liver ratios (TTL)		
	Median	Minimum	Maximum	Median	Minimum	Maximum
1	2.1	2.1	2.1	-	-	-
2	1.7	1.6	1.8	6.6	6.3	7.0
3	3.6	2.7	6.3	8.9	6.6	15.7
4	8.3	8.0	9.8	9.8	9.4	11.5
5	6.6	4.3	9.3	8.3	5.4	11.8
6	4.9	4.9	34.5	9.7	9.6	68.0
7	*	-	-	-	-	-
8	3.2	0.5	8.2	12.6	1.9	32.4
9	0.8	0.6	1.4	6.5	4.8	11.5
10	2.9	2.3	4.9	12.0	9.3	20.0
11	1.3	1.2	1.8	5.3	4.8	7.3
12	0.3	0.2	0.4	2.0	1.7	2.8
13	*	-	-	-	-	-

* Patients did not receive the diagnostic dose.

Table 2

Therapeutic injection: Patient summary

Patient No	Age/Sex	Prior Treatment	Pre-operative SPECT	Pre-operative CEA (ng/ml)	Type of Surgery	Number resected metastasis	Site of first recurrence	DFS (months)	OS (months)
1	61/M	CT(FU-LV)+ RT	Positive	1	1 Met.	1	lung	3	26
2	53/M	CT(FU)+ RT	Negative	7	Seg. IV	2	bone	0	34
f3	54/M	CT (FU-LV)	Positive	5	Left Hep.	4 + 1epipl.	Liver and nodes	3	5
4	65/F	CT (FU-LV) + RT	Positive	2	1 Met.	1	liver	12	35
5	59/M	-	Positive	25	Biseg. + 1Met.	3	Pre-sacral (local)	12	84
6	51/M	CT (FU-HAI MMC)	Positive	4	Seg. IV and met. VIII	2	liver	50	110
7	56/M	CT (FU-LV-oxa)	Not Done	218	R. Hep. + 1 Met.	2	liver	13	14
8	64/M	CT (FU-LV)	Positive	4	L. Lob. + 2 Met.	3	liver	36	>127
9	42/M	CT (FU-LV)	Positive	17	Seg. IV + 1 Met.	2	pleura and cutaneous scar	4	50
10	50/M	-	Positive	1	L. Lob. + 1 Met.	3	liver	16	60
11	54/M	CT (FU-LV) + RT	Positive	3	Seg. VII +1 Met.	2	liver	16	54
12	50/F	CT (FU-LV)	Negative	36	Right Hep.	1	lung	5	21
13	47/M	RT + FU-LV	Not Done	2	Right Hep.	1	NED	>93	>93

Abbreviations: SPECT, single photon emission computed tomography; CEA, carcinoembryonic antigen; RIT, radioimmunotherapy; CT, chemotherapy; FU-LV, 5-fluorouracil-leucovorin; MMC, mitomycin-C; HAI, hepatic arterial infusion; oxa, oxaliplatin; RT, radiotherapy; DFS, disease-free survival; Met, metastatectomy; Lob, lobectomy; Seg, segmentectomy; Hep, hepatectomy; NED, no evidence of disease.

Table 3

Therapeutic injection: kinetic parameters and HAMA responses

Patient No	Pre-RIT CEA (ng/ml)	Surgery-RIT Delay (days)	Kinetic parameters			
			T _{1/2} α (h:mn)	T _{1/2} β (h:min)	MRT (h:min)	HAMA (ng/ml/delay)
1	2	30	4:34	40:35	65:09	64/D99*
2	12	34	3:00	23:09	37:45	41/D76
3	2	36	2:44	33:55	52:54	129/D74
4	2	36	1:07	32:01	47:50	negative
5	3	41	6:37	37:39	54:20	184/D126
6	5	64	00:52	36:19	53:39	392/D182
7	3	44	ND†	ND†	ND†	219/D99
8	3	37	2:00	37:57	57:39	112/D94
9	ND	45	1:59	28:12	43:34	873/D121
10	1	36	1:03	34:59	52:00	negative
11	1	35	0:30	37:11	54:22	ND
12	1	52	2:24	40:53	62:27	871/D141
13	1	37	2:31	35:40	55:05	99/D51

Abbreviations: CEA, carcinoembryonic antigen; RIT, radioimmunotherapy; MRT, Mean Residual Time; HAMA, human anti-mouse antibody; ND, not done.

* RIT-HAMA assay delay (days);

† Samples did not permit calculation

Table 4Hematologic Toxicity of Therapeutic ¹³¹I-anti-CEA F(ab')₂

Patient No	Neutrophils (WHO Grade/Nadir)	Recovery from neutropenia(>1000/mm ³)	Platelets (WHO Grade/Nadir)	Recovery from thrombopenia(>75 000/mm ³)
1	3/D44	D54	4/D29	D43
2	1	-	0	-
3	3/D42	D51	4/D31	D41
4	3/D39	D50	2/D32	D42
5	4/D43	D60	3/D30	D43
6	3/D37	D61	4/D28	D61
7	4/D39	D53	4/D32	D46
8	4/D43	D54	4/D32	D41
9	3/D41	D68	3/D32	D49
10	4/D38	D52	4/D31	D45
11	4/D41	D56	4/D39	D54
12	4/D38	D46	4/D27	D38
13	4/D36	D45	4/D34	D45