

PDF hosted at the Radboud Repository of the Radboud University Nijmegen

The following full text is a publisher's version.

For additional information about this publication click this link.

<http://hdl.handle.net/2066/50387>

Please be advised that this information was generated on 2021-03-07 and may be subject to change.

Targeting of Biliary Cancer with Radiolabeled Chimeric Monoclonal Antibody CG250

Baudewijn W. Hendrickx,¹ Cees J.A. Punt,² Otto C. Boerman,¹ Ernst J. Postema,¹
Egbert Oosterwijk,³ Agapi Mavridu,⁴ Frans H.M. Corstens,¹ and Wim J.G. Oyen¹

¹Departments of Nuclear Medicine, ²Medical Oncology, and ³Urology, Radboud University Nijmegen Medical Center, Nijmegen, the Netherlands

⁴Wilex AG, Munich, Germany

ABSTRACT

Objective: Carbonic anhydrase 9 recognized by chimeric monoclonal antibody cG250 is overexpressed on biliary cancers. The aim of this study was to determine the targeting of radiolabeled cG250 in patients with biliary cancer to explore a potential role of radioimmunotherapy.

Methods: Three (3) patients received a diagnostic dose ¹¹¹In-cG250, and images were acquired 2 hours and 5 days after injection. Immediately after the last imaging session, ¹³¹I-cG250 was administered and images were acquired after 2 hours and 5 days. Visual and quantitative analyses was performed and tumor-to-background, tumor-to-normal liver-uptake ratios, and tumor uptake were calculated.

Results: Administration of ¹¹¹In-cG250 in patients with biliary cancer did not reveal enhanced uptake in the cancer lesions on whole-body scans. The scans obtained after the ¹³¹I-cG250 administration showed slightly enhanced tumor uptake in 1 patient with cholangiocarcinoma stage II. In 2 patients with gallbladder carcinoma stage IV, neither ¹¹¹In-cG250 nor ¹³¹I-cG250 showed targeting of known tumor lesions. Immunohistochemical analysis demonstrated CAIX expression in all 3 cases. There were no adverse events related to radiolabeled cG250 administration.

Conclusions: ¹¹¹In- or ¹³¹I-labeled cG250 is not suitable for biliary cancer targeting. Therefore, there is no basis to develop radioimmunotherapy based on radiolabeled cG250 in biliary cancer.

Key words: cG250, radioimmunoscintigraphy, radioiodinated antibody, biliary cancer

INTRODUCTION

Cancer of the biliary tract (cholangiocarcinoma and gallbladder cancer) is a malignancy arising from the epithelial cells of the intrahepatic and

extrahepatic bile ducts. Approximately 7480 cases of extrahepatic biliary tract cancer are diagnosed annually in the United States,¹ two thirds of which comprise gallbladder cancer. Biliary cancer accounts for approximately 3% of all gastrointestinal malignancies, with a prevalence in autopsy studies of 0.01–0.46 percent.² Although biliary cancer is a relatively rare malignancy, it is associated with a high mortality rate, and as such, represents an unmet clinical need. The poor prognosis is most likely related to the advanced stage at diagnosis, which is owing to the lack of specific clinical signs and symptoms and to the

Address reprint requests to: Baudewijn W. Hendrickx; Department of Nuclear Medicine (565), Radboud University Nijmegen Medical Center; Postbox 9101, 6500 HB Nijmegen, The Netherlands; Tel.: +31-24-3614048; Fax: +31-24-3618942

E-mail: b.hendrickx@nucmed.umcn.nl

This study was supported by a grant from Wilex AG (Munich, Germany).

anatomic position of the gallbladder. In the majority of patients, tumors are found incidentally when undergoing surgical exploration for cholelithiasis. Biliary cancer is diagnosed in 1%–2% of such cases.³

Monoclonal antibody (MoAb) G250 has been the subject of investigation in renal-cell cancer (RCC) for many years.^{4,5} This MoAb recognizes carbonic anhydrase isotype IX (CAIX) expressed on the majority of clear-cell-type RCCs.^{6,7} Histological studies have demonstrated expression of the CAIX antigen on the epithelium of the bile ducts.⁸ *In vivo* expression of the CAIX antigen in this compartment is exemplified by the saturable uptake of G250 antibody in the liver: When a low-protein dose (< 2 mg) of G250 antibody is administered, high liver uptake is observed (mean, 3.4 percent injected dose [%ID]). When higher protein doses (\geq 5 mg) are administered, uptake in the liver is reduced (mean, 2.1 %ID).⁹ Additionally, biliary tumors express CAIX, and CAIX expression has been suggested to be a potential biomarker for biliary cancer.⁹ The expression of CAIX in this notoriously difficult tumor suggested that MoAb G250 might be useful in the treatment of biliary cancer.

The aim of this study was to determine the tumor-targeting potential of radiolabeled chimeric monoclonal antibody cG250 in patients with biliary cancer, preceding the development of a radioimmunotherapy (RIT) protocol. Furthermore, iodine or metallic radiolabeled cG250 was studied to determine which radiolabel might be better suited for biliary cancer targeting.

MATERIALS, PATIENTS, AND METHODS

Monoclonal Antibody cG250

The isolation and the immunohistochemical reactivity of MoAb G250 have been described previously.^{10,11} To reduce the immunogenicity of the antibody, a chimeric version has been developed.¹² The reactivity of MoAb cG250 to normal human tissues is restricted to the (upper) gastrointestinal mucosa (stomach, ileum, proximal, and middle colon) and gastrointestinal-related structures (intra- and extrahepatic biliary system, pancreas).^{13,14} MoAb cG250 is reactive with the CAIX antigen, which is expressed on the cell surface of the majority of the clear-cell-type RCCs^{15,16} and in various other carcinomas (e.g., colon and squamous cell carcinomas).

Radiolabeling and Quality Control

The DTPA-cG250 conjugate was prepared, as described previously.¹⁷ The cG250-DTPA conjugate (5 mg, 1.0 mg/mL acetate buffer, pH 5.5) was radiolabeled with ¹¹¹InCl₃ (Tyco Healthcare; Petten, the Netherlands). The specific activity of the final preparation was adjusted to 44.4 MBq ¹¹¹In/mg MoAb cG250 by adding unlabeled MoAb cG250. MoAb cG250 was radioiodinated with ¹³¹I (MDS Nordion; Fleurus, Belgium), according to the IodoGen method, using a remote system, as described previously.^{18,19} Radioiodinated cG250 was purified by AG1-XR resin filtration (Bio-Rad Laboratories; Hercules, CA) in phosphate buffered saline (PBS). Again, the specific activity of the final preparation was adjusted to 44.4 MBq ¹³¹I/mg MoAb cG250 by adding unlabeled antibody. The radiochemical purity of each of the radiolabeled cG250 preparations was determined by instant thin-layer chromatography (ITLC) using ITLC silica gel strips (Gelman Sciences, Inc.; Ann Arbor, MI), using 0.15 M citrate buffer (pH 5.0) as the mobile phase, and always exceeded 95%. The immunoreactive fraction at infinitive antigen excess of both radiolabeled cG250 preparations was determined on freshly trypsinized SK-RC-52 RCC cells, essentially as described by Lindmo and Bunn with minor modifications.^{20,21} The immunoreactive fraction of all preparations, used in these studies, exceeded 80%.

Patients

Patients with histological and/or cytological proven advanced biliary cancer were eligible for the study. Inclusion criteria included: interval between any anticancer therapy and cG250 administration of at least 4 weeks. Patients needed to be at least 18 years of age, in female patients of childbearing age, a pregnancy test had to be negative, Karnofsky score needed to be higher or equal to 70%, hematological parameters in the peripheral blood within normal limits, and liver enzymes not exceeding five times of the upper limit of normal, if liver metastases were present. Patients with cardiac disease (New York Heart Association Classification of 3 or 4), with unrelated serious illness (e.g., active infection), or a life expectancy shorter than 4 months were excluded from this study.

This study was approved by the Institutional Review Board of the Radboud University Nijmegen Medical Centre (Nijmegen, the Netherlands). Prior to study entry, written, informed

consent was obtained from all patients before study entry.

Study Protocol

A CT of the chest and abdomen was obtained less than 4 weeks prior to cG250 administration. Patients were injected intravenously with 222 MBq (6 mCi) ^{111}In -cG250 in 0.9% NaCl (5 mg protein; total volume, 10 mL) over a 5-minute period. Vital signs were monitored up to 2 hours after injection. After 2 hours and 5 days after injection, anterior and posterior whole-body planar images were recorded using a double-headed gamma camera (E-cam, Siemens Inc.; Hoffman Estates, IL) equipped with parallel-hole medium energy collimators (scan speed 8 cm/minute and 4 cm/minute, respectively). An aliquot of the injected dose was placed in the field of view. Symmetric 15% windows were used over both the

172- and 246-keV energy peaks. The data were stored digitally in a 256×1024 matrix. Directly after the recording of the ^{111}In -cG250 images at 4 days p.i., the patient was intravenously (i.v.) injected with 222 MBq (6 mCi) ^{131}I -cG250 (5 mg protein; total volume, 10 mL) over a 5-minute period. Again, vital signs were monitored up to 2 hours after injection. Two (2) hours postinjection (p.i.) and 4 days later, whole-body planar images were recorded (high-energy collimators, symmetric 15% window over 364 keV, scan speed 5 and 4 cm/minutes, respectively) and stored digitally in a 256×1024 matrix.

All whole-body scans were analyzed visually and quantitatively. When the tumor was visualized, regions of interest (ROI) were drawn over liver and the biliary tumor.

Tumor-to-normal liver uptake ratios and tumor-to-background for ^{111}In -cG250 and ^{131}I -cG250 were calculated.

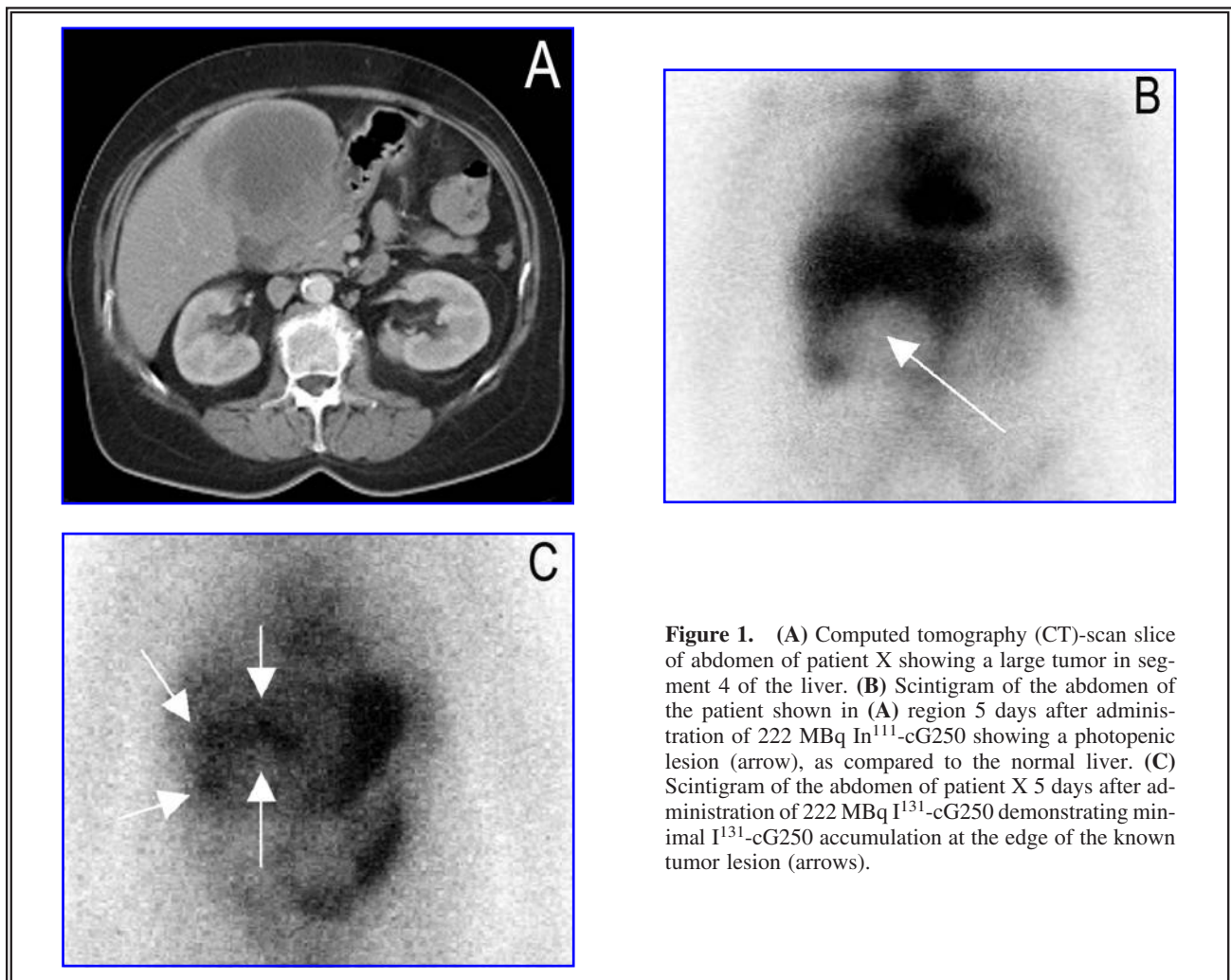


Figure 1. (A) Computed tomography (CT)-scan slice of abdomen of patient X showing a large tumor in segment 4 of the liver. (B) Scintigram of the abdomen of the patient shown in (A) region 5 days after administration of 222 MBq In^{111} -cG250 showing a photopenic lesion (arrow), as compared to the normal liver. (C) Scintigram of the abdomen of patient X 5 days after administration of 222 MBq I^{131} -cG250 demonstrating minimal I^{131} -cG250 accumulation at the edge of the known tumor lesion (arrows).

Blood samples were drawn up to 4 days after injection of each radiolabeled antibody preparation to estimate plasma clearance of the radiolabeled antibody preparations.

Immunohistochemistry

Tumor samples from included patients were collected retrospectively from pathology archives and processed for immunohistochemical analysis. Representative slides were stained with M75, recognizing CAIX using previously described methods.⁹ Briefly, slides were deparaffinized, rehydrated, microwave treated, and incubated with M75, washed, incubated with peroxidase conjugated rabbit-anti-mouse Ig (Dako A/S, Glostrup, Denmark), and developed using 3'3'-diaminobenzidine tetrahydrochloride (DAB; Sigma, St. Louis, MO) as chromogen, counterstained with hematoxylin, dehydrated, and mounted.

RESULTS

Three (3) patients (2 female, 1 male; age, 54–60 years) were included. Two (2) patients had stage IV gallbladder carcinoma, and 1 patient had stage II cholangiocarcinoma. One (1) patient with extensive disease underwent a cholecystectomy only, while a large portion of tumor mass proved to be nonresectable. The other 2 patients only had a biopsy of the tumor. None of the patients received any other therapy, such as chemotherapy, prior to cG250 imaging, assuring that antigen expression was not modified owing to systemic treatment. Two (2) patients showed slightly elevated liver enzymes owing to hepatic involvement and compression of the common bile duct. Administration of the radiolabeled antibody was well tolerated by all patients, and no clinical side-effects were observed. No significant changes in blood pressure, pulse rate, and body temperature were detected.

The initial plasma half-lives for both radiolabeled cG250 of ¹¹¹In-cG250 and ¹³¹I-cG250 were 45 ± 28 hours and 43 ± 23 hours, respectively. Overall, $t_{1/2}$ was 45 ± 23 hours for both labeled antibody preparations.

In only 1 of 3 patients (Fig. 1), a rim of the tumor (stage II cholangiocarcinoma) could be distinguished from normal liver activity. However, the tumor, as a whole, showed no significant antibody uptake and was photopenic, as compared to the normal liver (tumor-to-liver ratios of 0.4 for ¹¹¹In-cG250 and 0.77 for ¹³¹I-cG250). In the

other 2 patients, neither ¹¹¹In-cG250 nor ¹³¹I-cG250 showed any accumulation in known tumor lesions.

Immunohistochemical analyses revealed strong CAIX expression in 2 tumor specimens and moderate CAIX expression in the 3rd tumor specimen. Tumors consisted of epithelial components in a large field of connective tissue and/or large cysts (Fig. 2). CAIX expression was comparable to CAIX expression in normal larger bile ducts.

DISCUSSION

Although administration of radiolabeled cG250 was well tolerated without apparent clinical side-effects, this study shows that *in vivo* expression in biliary cancer is insufficient to delineate malignancies of the biliary tract, despite the documented presence of the G250 antigen on normal bile-duct epithelium. In 2 of 3 patients, the tumor was not visualized at all, and in the 3rd patient, the tumor was visualized because the lesion was photopenic, as compared to normal liver.

Additional single-photon emission computed tomography (SPECT) might have improved the readability of the images. However, this study was designed as a therapeutic clinical trial in which the imaging with low-dose ¹³¹I-cG250 and ¹¹¹In-cG250 served as an indicator for sufficient antigen expression *in vivo*, providing adequate tumor-to-normal tissue ratios that would allow for treatment with high-dose radiolabeled cG250. As all patients had large tumor deposits that failed to accumulate radiolabeled cG250 *in vivo*, SPECT was not performed. Although SPECT might have separated the tumors better from normal liver activity than the planar images, adequate tumor-to-liver ratios that provided a therapeutic window for radioimmunotherapy would not have been achieved. This finding is in sharp contrast with the observations in patients with RCC, in whom radiolabeled cG250 tumor uptake proved to be among the highest reported for any radiolabeled antibody.²² In RCC patients, ¹³¹I-cG250 accumulates in both primary tumors and metastases to allow accumulated delineation of malignancy in, and in the vicinity of, the liver.²³ As normal liver uptake of ¹¹¹In-cG250 is approximately three times higher than that of ¹³¹I-cG250,²⁴ ¹¹¹In-cG250 tumor uptake has to be high and persistent, as compared to normal liver, to provide any window for imaging and/or therapy.

The organ distribution, as observed on the

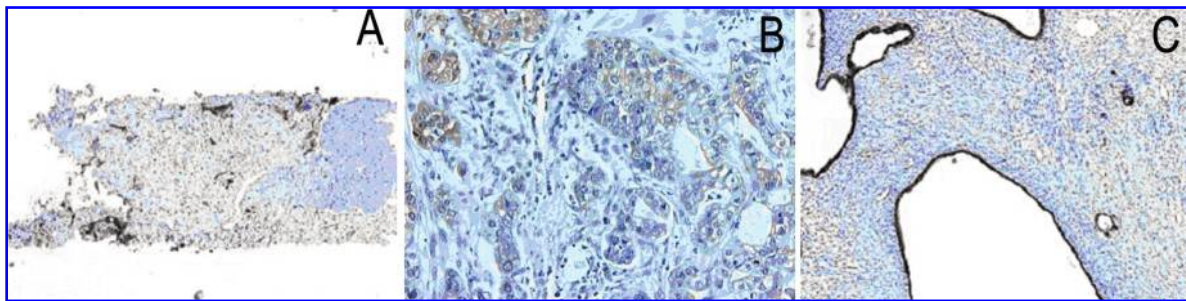


Figure 2. (A) Needle biopsy showing G250-positive cells in a field largely containing fibroblasts and supportive cells (50X magnification). Immunomicrographs of archival tumor tissues stained for CAIX expression: (B) Biliary tumor (gallbladder carcinoma) with low expression of CAIX (400X magnification) and (C) Biliary tumor (gallbladder carcinoma) with large cysts, demonstrating strong CAIX expression (50X magnification).

whole-body scans and the plasma clearance in biliary cancer patients, closely resembled those in RCC patients for both radiolabeled antibodies. Thus, distinct differences in these parameters cannot explain the lack of accumulation in biliary cancer, as compared to RCC patients.

Both ^{111}In -cG250 and ^{131}I -cG250 provided highly similar results in biliary cancer patients. Thus, the apparent lack of tumor uptake cannot be attributed to internalization and subsequent degradation of the radionuclide-antibody complex, as this would have resulted in a discrepancy between the ^{111}In (residualizing) and ^{131}I (non-residualizing) images (relatively high tumor uptake on the ^{111}In images, as compared to the ^{131}I images). This phenomenon played a role in RCC patients, in whom ^{111}In -cG250 showed generally better targeting of malignancy than ^{131}I -cG250.²⁵ Although the initial administration of 5 mg of ^{111}In -labeled cG250 might interfere with the targeting of 5 mg of ^{131}I -labeled cG250 4 days later, various trials with cG250 have convincingly demonstrated these doses do not interfere. For example, the accumulation of two radioiodinated cG250-preparations in primary RCC tumors was identical when the 2 injections were given 4 days apart.²⁶ Moreover, 5 mg of cG250 antibody followed by cG250 protein doses up to 25 mg did not affect tumor accumulation in patients.²⁷

Saarnio et al. showed that 57% of gall-bladder-invasive lesions and 78% of cholangiocellular malignant lesions expressed CAIX.²⁸ To exclude that the lack of tumor accumulation was owing to lack of CAIX expression; tumor tissues of all 3 patients were analyzed by immunohistochemistry. Strong CAIX expression was observed in 2 specimens, and moderate to low ex-

pression in the 3rd specimen. In none of the patients could an appreciable accumulation of radiolabeled cG250 in tumor tissue could be demonstrated, despite the presence of G250-positive tumor cells. Importantly, tumor areas consisted of large fields of stromal cells, with relatively few tumor cells or consisted of large cysts. Thus, the failure to adequately visualize these tumors is probably owing to an unfavorable tumor:nontumor ratio. Considering the former, the study was prematurely terminated after 3 patients, because it appeared that accumulation of radiolabeled MoAb cG250 in biliary cancer was insufficient to warrant successful development of a therapeutic approach.

CONCLUSIONS

This study shows that chimeric monoclonal antibody G250, whether labeled with ^{131}I or ^{111}In , shows insufficient tumor targeting in patients with biliary cancer, most likely owing to an intrinsic unfavorable tumor:nontumor ratio within liver tissue. Therefore, radioimmunotherapy of biliary cancer with radiolabeled cG250 seems unjustified.

REFERENCES

1. Jemal A, Murray T, Ward E, et al. Cancer statistics, 2005. *CA Cancer J Clin* 2005;55:10.
2. Vauthey JN, Blumgart LH. Recent advances in the management of cholangiocarcinomas. *Semin Liver Dis* 1994;14:109.
3. Hamrick RE, Jr., Liner FJ, Hastings PR, et al. Primary carcinoma of the gallbladder. *Ann Surg* 1982;195:270.

4. Oosterwijk E, Debruyne FM, Schalken JA. The use of monoclonal antibody G250 in the therapy of renal-cell carcinoma. *Semin Oncol* 1995;22:34.
5. Steffens MG, Boerman OC, Oosterwijk-Wakka JC, et al. Targeting of renal-cell carcinoma with iodine-131-labeled chimeric monoclonal antibody G250. *J Clin Oncol* 1997;15:1529.
6. Grabmaier K, Vissers JL, De Weijert MC, et al. Molecular cloning and immunogenicity of renal-cell carcinoma-associated antigen G250. *Int J Cancer* 2000;85:865.
7. Kaluz S, Kaluzova M, Opavsky R, et al. Transcriptional regulation of the MN/CA 9 gene coding for the tumor-associated carbonic anhydrase IX. Identification and characterization of a proximal silencer element. *J Biol Chem* 1999;274:32588.
8. Saarnio J, Parkkila S, Parkkila AK, et al. Transmembrane carbonic anhydrase, MN/CAIX, is a potential biomarker for biliary tumours. *J Hepatol* 2001;35:643.
9. Steffens MG, Boerman OC, Oosterwijk-Wakka JC, et al. Targeting of renal-cell carcinoma with iodine-131-labeled chimeric monoclonal antibody G250. *J Clin Oncol* 1997;15:1529.
10. Oosterwijk E, Debruyne FM. Radiolabeled monoclonal antibody G250 in renal-cell carcinoma. *World J Urol* 1995;13:186.
11. Steffens MG, Boerman OC, Oosterwijk-Wakka JC, et al. Targeting of renal-cell carcinoma with iodine-131-labeled chimeric monoclonal antibody G250. *J Clin Oncol* 1997;15:1529.
12. Velders MP, Litvinov SV, Warnaar SO, et al. New chimeric antipancarcinoma monoclonal antibody with superior cytotoxicity-mediating potency. *Cancer Res* 1994;54:1753.
13. Oosterwijk E, Ruiter DJ, Hoedemaeker PJ, et al. Monoclonal antibody G250 recognizes a determinant present in renal-cell carcinoma and absent from normal kidney. *Int J Cancer* 1986;38:489.
14. Pastorekova S, Parkkila S, Parkkila AK, et al. Carbonic anhydrase IX, MN/CAIX: Analysis of stomach complementary DNA sequence and expression in human and rat alimentary tracts. *Gastroenterology* 1997;112:398.
15. Steffens MG, Boerman OC, Oosterwijk-Wakka JC, et al. Targeting of renal-cell carcinoma with iodine-131-labeled chimeric monoclonal antibody G250. *J Clin Oncol* 1997;15:1529.
16. Uemura H, Nakagawa Y, Yoshida K, et al. MN/CAIX/G250 as a potential target for immunotherapy of renal cell carcinomas. *Br J Cancer* 1999;81:741.
17. Brouwers AH, Buijs WC, Oosterwijk E, et al. Targeting of metastatic renal-cell carcinoma with the chimeric monoclonal antibody G250 labeled with (131)I or (111)In: An inpatient comparison. *Clin Cancer Res* 2003;9:3953S.
18. Steffens MG, Boerman OC, Oosterwijk-Wakka JC, et al. Targeting of renal-cell carcinoma with iodine-131-labeled chimeric monoclonal antibody G250. *J Clin Oncol* 1997;15:1529.
19. Steffens MG, Boerman OC, De Mulder PH, et al. Phase I radioimmunotherapy of metastatic renal-cell carcinoma with 131I-labeled chimeric monoclonal antibody G250. *Clin Cancer Res* 1999;5:3268S.
20. Brouwers AH, van Eerd JE, Frielink C, et al. Optimization of radioimmunotherapy of renal-cell carcinoma: Labeling of monoclonal antibody cG250 with ¹³¹I, ⁹⁰Y, ¹⁷⁷Lu, or ¹⁸⁶Re. *J Nucl Med* 2004;45:327.
21. Lindmo T, Bunn PA, Jr. Determination of the true immunoreactive fraction of monoclonal antibodies after radiolabeling. *Meth Enzymol* 1986;121:678.
22. Steffens MG, Boerman OC, Oosterwijk-Wakka JC, et al. Targeting of renal-cell carcinoma with iodine-131-labeled chimeric monoclonal antibody G250. *J Clin Oncol* 1997;15:1529.
23. Brouwers AH, van Eerd JE, Frielink C, et al. Optimization of radioimmunotherapy of renal-cell carcinoma: Labeling of monoclonal antibody cG250 with ¹³¹I, ⁹⁰Y, ¹⁷⁷Lu, or ¹⁸⁶Re. *J Nucl Med* 2004;45:327.
24. Brouwers AH, Buijs WC, Oosterwijk E, et al. Targeting of metastatic renal-cell carcinoma with the chimeric monoclonal antibody G250 labeled with (131)I or (111)In: An inpatient comparison. *Clin Cancer Res* 2003;9:3953S.
25. Brouwers AH, Buijs WC, Oosterwijk E, et al. Targeting of metastatic renal-cell carcinoma with the chimeric monoclonal antibody G250 labeled with (131)I or (111)In: An inpatient comparison. *Clin Cancer Res* 2003;9:3953S.
26. Steffens MG, Boerman OC, Oyen WJ, et al. Intratumoral distribution of two consecutive injections of chimeric antibody G250 in primary renal-cell carcinoma: Implications for fractionated dose radioimmunotherapy. *Cancer Res* 1999;59:1615.
27. Steffens MG, Boerman OC, Oosterwijk-Wakka JC, et al. Targeting of renal-cell carcinoma with iodine-131-labeled chimeric monoclonal antibody G250. *J Clin Oncol* 1997;15:1529.
28. Saarnio J, Parkkila S, Parkkila AK, et al. Transmembrane carbonic anhydrase, MN/CAIX, is a potential biomarker for biliary tumours. *J Hepatol* 2001;35:643.