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Clinical Study

# Phase I single-dose study of intracavitary-administered Nimotuzumab labeled with $^{188}$Re in adult recurrent high-grade glioma

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**Key words:** gliomas, nimotuzumab, anti-EGF-R monoclonal antibody, h-R3, radioimmunotherapy, rhenium, brain tumor



**Radioimmunotherapy (RIT) may improve the management of malignant gliomas. A Phase I clinical trial was performed to evaluate, for the first time, the toxicity and clinical effect of an intracavitary administration of a single dose of Nimotuzumab (h-R3) labeled wit $^{188}$Re. Nimotuzumab is a humanized monoclonal antibody directed against epidermal growth factor receptors. Three patients with anaplastic astrocytoma (AA) and 8 with glioblastoma multiforme (GBM) were intended to be treated with 3 mg of mAb labelled with 10 or 15 mCi of $^{188}$Re. In patients treated with 10 mCi (n = 6) transitory worsening of pre-existing neurological symptoms were observed. Two patients treated with 15 mCi (n = 4) developed early severe neurological symptoms and one also developed late severe toxicity (radionecrosis). In the group treated with 10 mCi, 1 GBM patient died in progression 6 months after the treatment, 2 patients (1 GBM and 1 AA) developed stable disease during 3 months. One GBM patient had partial response for more than 1 year and 2 patients (1 GBM and 1 AA) were asymptomatic and in complete response after 3 years of treatment. Maximal tolerated dose of the radioimmunoconjugate $^{188}$Re-Nimotuzumab was 3 mg of the h-R3 labelled with 10 mCi of $^{188}$Re. The radioimmunoconjugate showed a high retention in the surgical created resection cavity and the brain adjacent tissues with a mean value of 85.5% of the injected dose one hour post-administration. This radioimmunoconjugate may be relatively safe and a promising therapeutic approach for treating high grade gliomas.**

## Introduction

The life expectancy of patients with high-grade gliomas, in particular glioblastoma multiforme (GBM) is still very poor. Standard treatments including surgery, radiation and, if suitable, systemic chemotherapy, result in median survival times ranging from 1 year for GBM to 3 years for anaplastic astrocitomas (AA).[1,2]

Standard treatment is not able to control tumor progression for a long-lasting period since in most cases; microscopic tumor cell clusters located in the peritumoral brain tissue are let and usually become the starting point for early tumor recurrence.[3,4]

Complete surgical resection or eradication of the tumor by subsequent radiation therapy is usually impossible.

The median overall survival for patients with recurrent GBM is 25 weeks, and 47 weeks for those with recurrent AA.[5] These patients are appropriate candidates for clinical trials designed to improve local control by adding newer forms of treatments to the standard therapy.

To overcome these limitations, more specific approaches for brain tumor treatment in the form of locoregional radioimmunotherapy (RIT) have been introduced.[6-10]

The infusion of radiolabelled monoclonal antibodies directly into the postsurgical resection cavity has enabled the delivery of high radiation doses to the affected area without important harm to the surrounding normal brain tissue or distant organs.

More than half of astrocytomas of high grade, especially of the novo type, overexpress the epidermal growth factor receptor (EGF-R).[11]

EGF-R signal transduction pathways have been correlated with various processes that contribute to the development of malignancy, such as cell cycle progression, inhibition of apoptosis, tumor cell motility and metastasis. EGF-R overexpression has also been associated with chemo and radioresistance.[12]

Nimotuzumab (h-R3) is a humanized monoclonal antibody (MAb), IgG1 isotype, that recognizes an epitope located in the extracellular domain of the EGF-R. The antibody was obtained by transplanting the complementary determining regions (CDR) of the murine MAb ior egf/r3 (IgG2a) to a human framework, assisted by computer modelling.[13]

Nimotuzumab has shown a synergic effect when combined with external bean radiotherapy and it has been well tolerated through the

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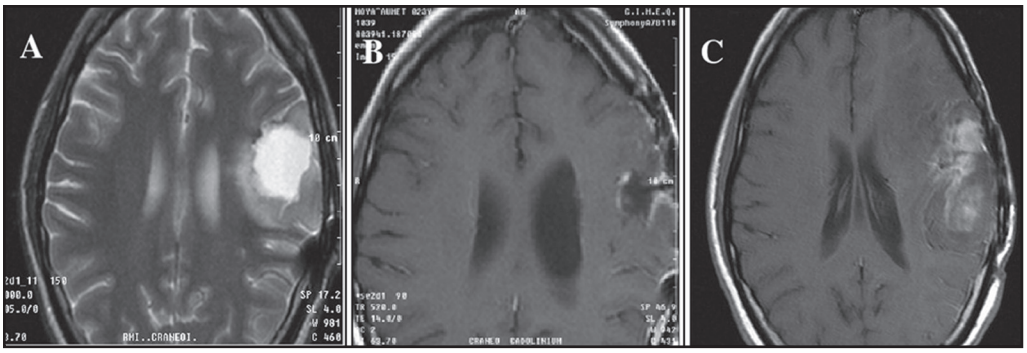


Figure 1. MRI, axial views from patient number 4. (A) T2 pre-RIT view showing post-surgical tumoral cavity of the tumoral nucleus with perilesional edema. (B) T1 post-gadolinium view, 15 months after RIT showing the post-surgical tumoral cavity without evidences of tumor or edema. (C) T1 post-gadolinium view, 26 months after RIT showing an increased gadolinium uptake zone and perilesional edema with mass effect at the same place of primary tumor. The patient died 1 year later.

intravenous route for treating the advanced epithelial-derived cancer patients including high-grade glioma patients.[14-17]

Recent data from a phase I/II clinical trial, with 200 mg/week of Nimotuzumab for 6 weeks, administered by IV infusion, combined with external radiotherapy in 24 patients with high grade astrocytoma showed no grade 3/4 adverse events. Four patients achieved complete response, 5 a partial response and 11 stable disease, while the median survival time was 22.17 months for all subjects.[17]

Preclinical toxicological studies in relevant animal species have also demonstrated the low toxicity effect of Nimotuzumab when it was administered intravenously to monkeys or intracerebrally to rats.[18-22]

Rhenium 188 (Re-188) from a tungsten-188/rhenium-188 ($^{188}$W/$^{188}$Re)-radionuclide generator system, represents an attractive alternative radionuclide. Rhenium-188 ($t$1/2 = 16.9 h) is produced after the beta decay of the tungsten-188 parent ($t$1/2 69.0 d). In addition to the emission of high-energy electrons (Eb = 2.118 MeV), $^{188}$Re also decays with emission of a gamma photon with an energy of 155 keV, 15% abundance. Besides the therapeutic usefulness of $^{188}$Re in different diseases, the emission of 155 keV gamma photon is a second advantage since the biodistribution of $^{188}$Re-labeled antibodies can be evaluated in vivo with a gamma camera. In addition, since Re-188 and technetium 99 have similar chemical properties, both can be conjugated to antibodies using similar chemistry methods.[23-25]

A recent intracavitary RIT study in a few number of patients bearing malignant gliomas and using a $^{188}$Re-labelled anti-tenascin antibody has shown a substantially prolonged survival.[26]

The aims of the present study were to evaluate the safety and the maximal tolerated dose (MTD) of $^{188}$Re-labeled antiepidermal growth factor receptor monoclonal antibody Nimotuzumab (h-R3) administered by the intracavitary route, in adult patients with recurrent high grade glioma. The assessment of antitumor effect was a secondary end point of the study. A detailed biodistribution and internal dosimetry study was previously reported.[27]

## Results

Eleven patients with recurrent high-grade glioma were enrolled in the study, 8 had GBM, and 3 had AA. One GBM patient was excluded after surgery due to an important tumor progression 4 weeks after surgery, which occluded the indwelling catheter and precluded the radioimmunoconjugate administration.

All, but one patient received one dose of the radioimmunoconjugate. Patient number 4 had a recurrent AA and was included in the first group (10 mCi), after treatment, the patient achieved a complete response lasting for more than 15 months but, 26 months after treatment the tumor recurred at the same place. Another surgery was carried out and the new pathological study revealed the presence of a GBM tumor. On a compassionate use basis, and after the approval of the Cuban Regulatory Agency, the patient received a second dose of the radioimmunoconjugate. The patient died 1 year after the second treatment (Fig. 1).

Patient's characteristics are listed in Table 1.

Regarding safety, in patients treated with 10 mCi of $^{188}$Re (n = 6), a transitory worsening of pre-existing neurological symptoms was observed, even though they had no severe or very severe toxicity related to the study drug .

Two patients treated with 15 mCi (n = 4) developed severe or very severe neurological symptoms. Patient 8 developed a worsening of his pre-existing neurological symptoms with significant brain edema, patient 5 developed radionecrosis 6 months after RIT as a late treatment complication (Table 2).

In spite of the main objective of the study was to monitor side effects and to determine the maximal tolerated dose (MTD), 2 patients developed complete responses, 1, partial response and 2, stable diseases (Table 1).

After a median follow-up of 46 months (28.7–62.8 months), the mean and median overall survival was 16.76 and 6.07 months respectively.

For those patients that were treated with 10 mCi, the mean and median survival have corresponded to 25.14 and 18.7 months respectively.

No antiidiotypic IgG or IgM response was induced in any patient.

**Antibody radiolabeling and immunoreactivity assay.** All radiolabeling procedures were performed under aseptic conditions in a shielded laminar flow hood. All glassware, plastics and solutions were sterile and pyrogen free. The purified humanized MAb h-R3 was labeled with a specific activity range from 5.00–6.67 mCi/mg protein. A mean of 94.0 ± 2.3% of $^{188}$Re was bound to the $IgG_1$ and 2.68 ± 0.62% to glucoheptonate as determined by paper chromatography.

Instant paper chromatography of labeled MAb in acetone showed that about 2.41 ± 0.70% or less free perrhenate ran at the Rf = 1.0.

The radiocolloid determination was 1.50 ± 0.27%.The immunoreactive fraction (IRF) of $^{188}$Re-h-R3 measured by flow cytometry analysis and by Lidmo method on the H-125 human lung adenocarcinoma cell line was 0.78, with a correlation coefficient of $r$ = 0.9984.

**Biodistribution and dosimetric calculations.** The biodistribution results showed that liver, kidneys and urinary bladder presented the

Table 1 **Patient's individual characteristics**

| Inclusion number | Sex | Age (years) | Diagnosis | IHQ (SI) | Tumor localization | Dose level | Best response | Survival since RIT (months) |
|---|---|---|---|---|---|---|---|---|
| 1 | M | 53 | GBM | 3 | Right temporo-occipital | I | ED | 6.1 |
| 2 | M | 32 | GBM | 3 | Right temporo-parietal | - | PD | [a]2.9 |
| 3 | M | 20 | GBM | 3 | Right parieto-occipital | I | CR | [b]61.0 |
| 4 | F | 25 | AA/GBM | 2/3 | Left fronto-parietal | I | CR | [c]38.4 |
| 5 | M | 44 | AA | 3 | Left fronto-parietal | II | PD | 9.4 |
| 6 | F | 65 | GBM | 2 | Left fronto-parietal | II | PD | 2.4 |
| 7 | M | 54 | GBM | 1 | Left parieto-occipital | II | PD | 3.8 |
| 8 | M | 62 | GBM | 2 | Right parieto-occipital | II | PD | 1.2 |
| 9 | M | 53 | GBM | 3 | Right parietal posterior | I | PR | 20.8 |
| 10 | M | 35 | AA | 1 | Right frontal | I | ED | 18.7 |
| 11 | M | 51 | GBM | 3 | Left temporo-occipital | I | PD | 5.9 |

GBM = Glioblastoma multiforme; AA = Anaplastic astrocitoma; IHQ = Immunohistochemical study of the EGF-R expression. SI = Staining intensity from 0 to 3; I = 3 mg of h-R3 labelled with 10 mCi of $^{188}$Re; II = 3 mg of h-R3 labelled with 15 mCi of $^{188}$Re; a = Patient number 2 was not treated due to an Ommaya camera obstruction; The survival time was calculated since the intention to RIT; b = Patient number 3 is still alive; c = Patient number 4 had an AA which evolved to GBM (see text); CR = Complete response; PR = Partial response; ED = Stable disease; PD = Progressive disease.

higher Nimotuzumab uptakes. Liver and kidneys reached maximum values of 5.6% the injected dose (ID) and 4.6% ID, respectively at 48 hours post injection, meanwhile the urinary bladder accumulated a maximum value of 3.3% ID, 24 hours after de radioimmunoconjugate administration. The $^{188}$Re-Nimotuzumab uptake in SCRC and BAT correspond to of 85.5 ± 10.3% ID, 1 hour post injection with a biological half life of approximately 22.7 ± 8.9 hours. About 6.2 ± 0.8% ID was excreted during the first 48 hours post-administration by the urinary pathway. Figure 2 shows planar views of the $^{188}$Re-Nimotuzumab distribution in the total body of patient number 6, one hour after the radioimmunoconjugate administration.

Table 2 **Treatment related-toxicity**

| Inclusion number | Dose level | Adverse reaction (Grade) |
|---|---|---|
| 1 | I | Headache (moderate); confusion (moderate). |
| 2 | - | - |
| 3 | I | Increased level of SGPT (moderate); headache (moderate); seizures (moderate); objective muscle weakness (moderate). |
| 4 | I | Increased level of SGPT (mild); seizures (moderate). |
| 5 | II | Increased level of SGPT (mild); increased level of SGOT (mild); headache (moderate); seizures (moderate); confusion (moderate); aphasia (severe); radionecrosis (severe). |
| 6 | II | Increased level of SGOT (mild); aphasia (mild); headache (mild). |
| 7 | II | Seizures (moderate). |
| 8 | II | Aphasia (severe); confusion (very severe); brain edema (severe). |
| 9 | I | Increased level of SGPT (mild). |
| 10 | I | Increased level of SGPT (moderate); seizures (moderate). |
| 11 | I | Confusion (moderate); aphasia (moderate). |

SGPT = Serum glutamic pyruvic transaminase. SGOT = Serum glutamic oxalacetic transaminase; Patient number 2 was not treated due to an Ommaya camera obstruction.

The dosimetric studies showed that the average of the mean absorbed doses received in the tumour region were 24.1 ± 2.9 Gy and 31.1 ± 6.4 Gy, in groups I and II respectively, while the maximum doses in these VOIs were 58.9 Gy and 79.3 Gy. Dosimetric results in the tumour region showed a high variability among all patients; it seem to be due to the great differences found between the analyzed subjects regarding the cavities volumes and the $^{188}$Re kinetics in the VOIs; similar results have been reported by other authors.[35]

As expected, kidneys, liver and urinary bladder received the higher mean absorbed doses (0.754, 0.223 and 0.604 mGy/MBq, respectively), all these values were lower than the reported limits inducing radiotoxicity. Figure 3 shows the absorbed dose received by the main target organs.

These results showed that the use of locoregional RIT of high grade gliomas with $^{188}$Re-Nimotuzumab allow the delivery of a high tumoricidal dose to the tumour region without a significant irradiation of normal organs.

## Discussion

Local radioimmunotherapy for treating high-grade brain gliomas using anti-EGF-R mAb is an attractive modality because this target expression is enhanced on malignant brain tissues as compared with the normal counterparts. On the other hand, high quantities of the radiolabeled mAb can penetrate into brain tissues and can kill those antigen-negative tumor cells, which have no specific radiolabeled antibody localized on their surface.[36,37]

It has been previously described that Nimotuzumab is a potent anti-cancer agent both, in vitro and in vivo by exerting a combined antiproliferative, antiangiogenic and proapoptotic activity in tumors overexpressing EGF-R.[38]

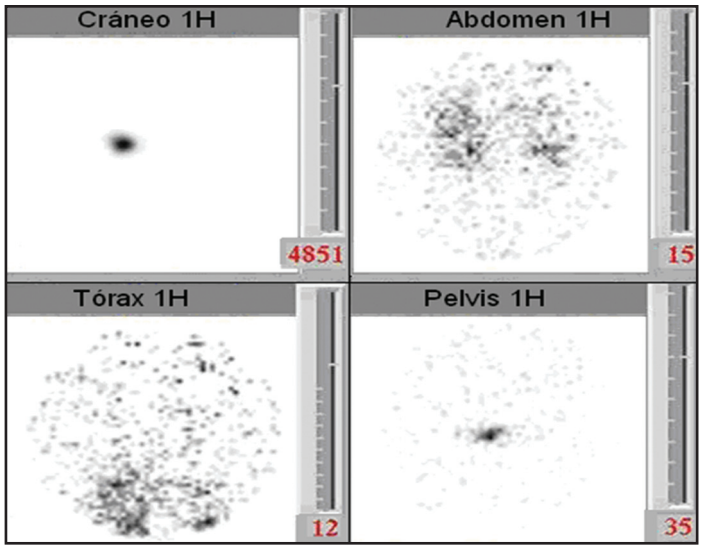


Figure 2. Geometric mean images of head, chest, abdomen and pelvis from patient number 6. Images were acquired 1 hour post-injection.

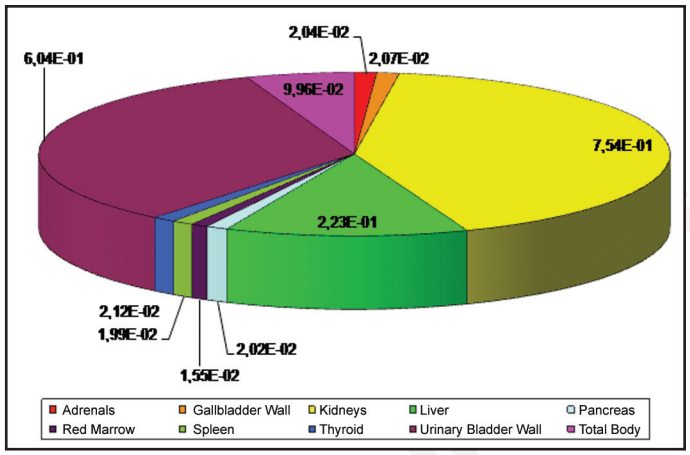


Figure 3. $^{188}$Re-Nimotuzumab normal organ dosimetry (mGy/MBq).

This manuscript shows for the first time the results of brain tumor intracavitary radioimmunotherapy using an anti-EGF-R mAb. Previous studies using anti-EGF-R mAb administered by systemic routes have not given satisfactory treatment results, perhaps due to normal cells; mainly hepatocytes and epithelial cells, also expressing EGF-R which makes it inappropriate for treating malignant gliomas by the intravenous or intra-arterial routes.[39]

In an attempt to overcome the low uptake of radiolabelled mAb by the tumor and improve the tumor to normal tissues ratio, various studies have proposed the concept of tumor pre-targeting.[40]

In 1997 Wersall et al reported the toxicity and therapeutic effects of intratumoral infusion of 4 to 120 mg of the naked anti-EGF-R 425 mAb in 8 patients with advanced malignant glioma. The murine 425 mAb induced a severe local inflammatory reaction, and 3 patients had tumor necrosis. HAMA response was detected in 6 patients. Part of the effect was probably mediated by the activation of various immune functions with infiltration, in the tumor lesion, of macrophages, granulocytes, CD4 and CD8 T cells.[41]

In our study, a unique intracavitary administration of 3 mg of Nimotuzumab labeled with 10 or 15 mCi of $^{188}$Re elicited a dose-dependent cerebral edema and in 3 cases (CR or PR) an unexpected beneficial effect.

In our trial, the effects were also more pronounced than those previosly reported from other intratumoral radioimmunotherapy studies using other non-anti-EGF-R mAb.[8-10] As Nimotuzumab is an IGg1 humanized mAb, it could activate more efficiently immune effector cells in the tumor area than murine mAbs.[42]

The visual inspection of the processed scintigraphic images and the biodistribution calculations revealed that $^{188}$Re-Nimotuzumab was retained in tumour cavity and adjacent malignant tissues for a long period. One hour after the injection approximately 85.5 ± 10.3% of Nimotuzumab was accumulated in the tumour cavity and adjacent tissues with a biological half life of approximately 22.7 ± 8.9 hours.

Even though, the number of patients was small, the results of this study showed that locoregional administration of $^{188}$Re-Nimotuzumab to patients bearing high-grade gliomas could have potential effectiveness for the treatment of this malignant disease. This result should be confirmed in a larger trial designed to property evaluate the drug-efficacy.

However, the high radiation dose achieved locally may also cause inflammatory infiltrates and radiation necrosis more frequently than the standard treatment modalities.

We hypothesize that the antitumor effect of the radioimmunoconjugate h-R3-$^{188}$Re is primarily due to the association between the monoclonal antibody immune effect and the DNA damage elicited by the radioisotope.

The tumor of patient number 2 occluded the Ommaya catheter in the post-surgical period of 4 weeks and was impossible to administer the radioimmunoconjugate. It is known that GBM is a very aggressive tumor. He died 2.9 months later in progressive disease.

There was no apparent correlation between the immunohistochemistry expression of EGF-R and the clinical output, but a larger series of patients is needed for a final conclusion. Similar results have been obtained with cetuximab in colorectal cancer.[43]

The absence of antiidiotypic response was an anticipated result since the humanized mAb was intracavitary administered to the majority of the patients only once.

This intracavitary radioimmunotherapy using 3 mg of Nimotuzumab labelled with 10 mCi of $^{188}$Re could be relatively safe and a promising therapeutic approach for treating high grade gliomas.

A phase II trial studying the therapeutic effect of 3 mg of Nimotuzumab labelled with 10 mCi of $^{188}$Re, in a multiple administration regimen, in patients with high-grade glioma is being planned.

## Material and Methods

We performed an open, uncontrolled, dose escalation Phase I clinical trial to evaluate the safety and the maximal tolerated dose (MTD) of a single local administration of 3 mg of Nimotuzumab labelled with increasing dose of $^{188}$Re, in a dose range between 10 and 30 mCi, increasing 5 mCi, every 3 patients. If 2 patients, in any dose-scaling group, experience severe or very severe toxicity according to the Common Toxicity Criteria of the National Cancer Institute (NCI-CTC) in a 3 months period after RIT then, the previous dose level was considered as the maximal tolerated dose. On

the contrary, if only 1 develops severe or very severe toxicity then, 1 to 3 new patients were included in the same dose cohort.

**Patients.** Eleven patients (2 females, 9 males) with recurrent tumors after conventional treatment (surgery plus external radiotherapy) with a mean age of 44.9 years and histopathologically confirmed anaplastic astrocytoma (AA) or glioblastoma multiforme (GBM) and immunohistochemically proven overexpression of the EGFR were included in the study. The EGF-R immunostaining intensity was semi-quantitatively classified as negative (0), weak (1), moderate (2) or intense (3).

An Ommaya reservoir was implanted into the resection cavity and patients were allowed to recover from surgery for 2–4 weeks before treatment with the radioimmunoconjugate. Writing informed consent was obtained from all patients.

The study was approved by the CIREN's Ethics Committee and the National Regulatory Authority of Cuba (CECMED).

Other selection criteria were: age older than 18 years, a Karnofsky performance status (KPS) ≥70, absolute neutrophil count ≥1.5 x $10^9$/L, platelet count ≥100 x $10^9$/L, serum creatinine level ≤ the upper limit of normal, and serum bilirubin level less than two times the upper normal limit. The most important exclusion criteria consisted of previous treatments with murine anti-EGF-R antibodies, pregnancy or lactation, serious chronic diseases and active infections.

**Imagenological studies.** Computer tomography (CT) or magnetic resonance imaging (MRI) scan was done before inclusion in the trial and then every 3 months. Unscheduled CT or MRI scans were also done after symptoms of progressive disease. Tumor response was classified according to WHO modified criteria.[28] Complete response was defined as disappearance of all enhancing tumor on consecutive CT or magnetic resonance, off steroids and neurologically stable or improved; partial response: ≥50% reduction in size of enhancing tumor on consecutive CT or MRI scans at least 1 month apart, steroids stable or reduced and neurologically stable or improved; progressive disease: ≥25% increase in size of enhancing tumor or any new tumor on CT or MRI scans or neurologically worse and steroids stable or increased. Stable disease was attributed to all other situations.

**Safety.** All patients were monitored after the radioimmunoconjugate injection for any adverse event. Vital signs were also evaluated and registered at different time intervals. Special attention was given to the detection of neurological symptoms and signs that could be related to neurological radiotoxicity. The Common Toxicity Criteria of the National Cancer Institute (NCI-CTC) were used to classify the adverse events being scaled as mild, moderate, severe or very severe. Complete blood cell counts with differential and platelet examination, chemistry panel evaluation, liver function tests and urine analyses were performed weekly for the first 8 weeks post-injection. Data were analyzed in order to identify signs of haematological or normal tissue toxicity. Antiidiotypic response was measured during the first 6 months post-injection using an indirect ELISA method as previously reported;[16] briefly, the murine mAb ior egf/r3 was coated on microtiter plates at 5 μg/mL diluted in carbonate buffer at pH 9.6 and was incubated overnight at 4°C. After washing, serial dilutions of patient' sera starting at 1:400 were incubated for 1 h at 37°C. After washing, bound human immunoglobulin was detected both by alkaline phosphatase conjugated goat anti-human IgG and anti human IgM (Sigma Chemical, A-3188 and A-9794, USA, respectively). After washing, a chromogen solution (paranitrophenil phosphate 1mg/mL in diethanolamide buffer pH 9.8) was added and incubated for 30 minutes at room temperature. Plates were read on an ELISA reader at 405 nm (Organon Teknika, Netherlans). Positive response was considered if the ratio between pre/post administration readings was >1.5.

**Biodistribution and dosimetric calculations**. More than 90 scintigraphic images were acquired from all patients, including emission, scatter and attenuation studies at 1 h, 4 h, 24 h and 48 h; planar and SPECT acquisitions were combined in order to compute the biodistribution of the radioimmunoconjugate in the total body, as well as the three-dimensional activity accumulation in the tumor region and brain adjacent tissues (BAT). The studies were processed according to well established procedures and the biodistribution results were computed and reported as percent of injected activity.[29,30]

The absorbed doses received by normal organs and the tumor region were estimated using the MIRD methodology at organ and voxel level, respectively. Residence times were computed from the time-activity curves of the main source organs and provided as input parameters to the MIRDOSE 3 software in order to estimate the absorbed doses in the normal tissues.[31] Blood and urine samples were also collected and processed to complement the dosimetric calculations in red marrow and urinary bladder. Mean absorbed doses were reported for the nine normal organs receiving the highest radiation doses and the total body.

A dedicated software package was developed and used to estimate the three-dimensional dose distributions in the tumor and the BAT.[32] Volumes of interest (VOIs) were defined by mean of isocontours or manual sequential in regions of interest (ROIs), which included the surgical created resection cavity (SCRC) and the BAT, using magnetic resonance images (MRI), previously corregistered with the SPECT studies, as references. It was necessary to estimate the mean dose per unit of cumulated activity ("S" values) for the $^{188}$Re radionuclide, according to the voxel size of the SPECT acquisitions.[33] Mean and maximum absorbed doses were calculated and reported from all patients in the tumoral region and BAT; the volume of VOIs, the estimated percent of injected dose on the tumour region at one hour post-injection (without decay correction) and its effective half life were also computed and reported.

Safety, efficacy, biodistribution and dosimetric analysis were performed on all patients who received a single intracavitary dose of the radioimmunoconjugate.

**Antibody radiolabeling.** The antibody developed at the Center of Molecular Immunology (Havana, Cuba) and concentrated to 5 mg/mL was reduced with 2-ME at a molar ratio of 2000:1 (2-ME:mAb) at room temperature for 30 min. The reduced antibody was purified to eliminate the excess of 2-ME through a PD-10 Sephadex G-25 M gel filtration column (Pharmacia, Biotech, Uppsala, Sweden) using a pH 8.2, 0.1 M phosphate buffered saline (PBS) solution purged with nitrogen as mobile phase as previously described by Iznaga-Escobar et al;[23] briefly, following reduction of intrinsic disulfide bonds, aliquots containing 3 mg mAb were dispensed into 10 mL vials and added to the above solution. Two mL of the glucoheptonate solution purged with nitrogen (containing 900 mg of glucoheptonate (Sigma, USA), 90 mg of ascorbic acid (Merck, England) and 9 mg of SnF (Sigma, USA)) were added and were labelled with 555–740 MBq (15–20 mCi) of perrhenate ($^{188}ReO_4^-$) eluted from 188W/$^{188}$Re generator (MAP Medical Technologies Oy, Finland). Activity was measured in

a Radioisotope Dose Calibrator (CAPINTEC CRC-15R, Ramsey, NJ, USA).

For the quality control of the radiolabeled product, ascending paper chromatography on Whatman 3MM paper as stationary phase and 0.9% saline and acetone as mobile phase to separate free perrhenate and $^{188}$Re-glucoheptonate was run. Human Serum Albumin (HSA, 1%)-impregnated ITLC-SG (Gelman Science Inc, Ann Arbor, USA) strips were used as stationary phase and ethanol: $NH_4OH$: Water (2:1:5 v/v) as the mobile phase to separate radiocolloids which remained at the base while the radiolabeled mAb and free perrhenate moved away (colloid, $R_f$ = 0.0, labeled $^{188}$Re, $R_f$ = 1.0.[34]

**Survival analysis.** Survival time was calculated from the data of the first treatment until date of death using the Kaplan-Meier method.

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