

Short Communication

Phase I Clinical Evaluation of a Neutralizing Monoclonal Antibody Against Epidermal Growth Factor Receptor in Advanced Brain Tumor Patients: Preliminary Study

T. CROMBET,¹ O. TORRES,¹ V. RODRÍGUEZ,² A. MENÉNDEZ,² A. STEVENSON,² M. RAMOS,¹
F. TORRES,¹ R. FIGUEREDO,² I. VEITÍA,² N. IZNAGA,¹ R. PÉREZ¹ and A. LAGE¹

ABSTRACT

High levels of growth factors and their receptors have been demonstrated in human tumors. Gliomas and meningiomas are characterized by overexpression of epidermal growth factor receptor (EGF-R). Ior egf/r3, is a neutralizing murine monoclonal antibody (MAB) against EGF-R, and was generated at the Cuban Institute of Oncology. The antibody recognizes EGF-R with high affinity, inhibiting tyrosine kinase activation. A clinical trial was conducted in brain tumor patients to evaluate toxicity, immunogenicity, and clinical benefit of escalating doses of the antibody. Nine patients with histologically confirmed gliomas or meningiomas, who had active or recurrent disease after receiving conventional treatment, received four intravenous doses of ior egf/r3. Total dosages ranged from 160 to 480 mg. As inclusion criteria, radioimmunoscintigraphy with the same MAB labeled with ^{99m}Tc was performed. Immune response against the murine antibody was also evaluated. After four doses of ior egf/r3 MAB, no significant toxicity was found, except in one patient who developed a grade 4 allergic adverse event. This reaction was probably related with previous sensitization to the same MAB and the development of human anti-mouse antibodies (HAMA) response. Despite no major objective antitumor responses, eight patients had stable disease on the 6-month evaluation, and two patients remain alive after four years of MAB therapy.

INTRODUCTION

THE INCIDENCE OF BRAIN TUMORS is worldwide increasing,⁽¹⁾ and despite advances in neurosurgery and radiotherapy, limited progress has been made in the treatment of patients with high-grade gliomas.⁽²⁾

Amplification and rearrangement of the epidermal growth factor receptor (EGF-R) have been found in primary high-grade astrocytic tumors⁽³⁻⁶⁾ and in meningiomas.⁽⁶⁾ Expression of these molecules seems to increase with progressive grade. A subset of glioblastoma multiforme also expresses a truncated, constitutively activated receptor (EGF-R vIII).⁽⁷⁾ On the contrary, EGF-R is not found in normal brain tissue.⁽⁴⁾

For primary brain tumors, overexpression of EGF-R has been

associated with poor survival due to growth advantages and increased invasiveness.^(7,8) Furthermore, it has been proposed that oncogenes like EGF-R may contribute indirectly to tumor development and growth, by facilitating angiogenesis after inducing or, up-regulating the expression of pro-angiogenic factors like vascular endothelial growth factor (VEGF).^(9,10)

The murine anti EGF-R monoclonal antibody ior egf/r3, is an anti EGF-R antibody generated at the National Institute of Oncology, Havana, Cuba.⁽¹¹⁾ This monoclonal antibody (MAB) recognizes an epitope located in the extracellular domain of the human EGF-R with high affinity and blocks the EGF binding to the receptor, inhibiting the activation of the receptor-associated tyrosine kinase.^(11,12)

Ior egf/r3 was able to recognize human EGF-R in fresh tu-

¹Center of Molecular Immunology, PO Box 16040, Havana 11 600, Cuba.

²National Institute of Neurology and Neurosurgery, Calle 29 and D, Havana 10 400, Cuba.

mor samples as shown by immuno-histochemistry⁽¹²⁾ and inhibited the proliferation of a variety of cultured tumor cell lines, which over-express EGF-R.⁽¹³⁾ This antibody, as well as other EGF-R blocking agents, have proved to exert its antitumor activity—at least in part—by the suppression of tumor angiogenesis.^(14,15)

^{99m}Tc-labeled MAb ior egf/r3 was previously evaluated in 148 patients for the detection of epithelial-derived tumors, its metastases and recurrences. Overall sensitivity and specificity of radioimmunoscinigraphy was 84.2 and 100.0%, respectively, but for brain tumors the sensitivity rate was 100%.⁽¹⁶⁾

Safety, pharmacokinetic and immunogenicity of ior egf/r3 was previously evaluated in a therapeutic phase I clinical trial in 19 patients with advanced epithelial lung and digestive tract tumors (Manuscript accepted in *Cancer Biotherapy & Radiopharmaceuticals*). In the present paper we show the results of a clinical trial with MAb ior egf/r3, in nine patients carrying unresectable or recurrent brain tumors.

MATERIALS AND METHODS

Study design

This was an open, uncontrolled, not randomized, single center clinical trial intended to obtain information about safety and toxicity of the antibody. The measurement of the therapeutic response was included as a secondary objective.

The trial was conducted at the Institute of Neurology and Neurosurgery (Havana, Cuba) and the protocol was approved by the Hospital Ethic Committee, and also by the National Regulatory Authority of the Republic of Cuba: the State Center of Drug Quality Control (CECMED, Havana, Cuba).

Patient selection

Patients with histologically confirmed astrocytomas or meningiomas, who had active or recurrent tumors after receiving conventional treatment, were eligible.

Other inclusion criteria were adequate liver and renal function, hematological parameters within normal limits, no previous history of hypersensitivity to this product, normal respiratory function, life expectancy higher than 3 months and Karnofsky Performance Status over 50. Written informed consent was received from all patients entered into the trial.

Monoclonal antibody

Ior egf/r3 is a highly specific murine monoclonal antibody, IgG_{2a} isotype. The MAb is secreted by the hybridoma A24/15/128 derived from the fusion of a mouse myeloma with the splenocytes from Balb/c mice immunized with a partially purified fraction of the EGF-R from human placenta. Its generation and characterization have been previously described.^(11,12)

Treatment schedule. As the trial was designed to study the toxicity of the antibody, a modified Fibonacci scheme was applied. Three different treatment cohorts were established.

Each patient received four doses of this antibody, every 4 days. Single doses were 40 mg for the first cohort of treatment, 80 mg for the second, and 120 mg for the last treatment group. Previously, it had been reported that doses over 40 mg of an-

other murine anti-EGF-R-MAb, EMD 55 900 (Merck KgaA, Darmstadt, Germany), induced significant saturation of the target receptor within brain tumors.⁽¹⁷⁾ The cumulative administered doses in the three different cohorts were 160, 320, and 480 mg, respectively. Ior egf/r3 was administered by intravenous (i.v.) infusions, diluted in 200 mL of sodium chloride solution for injection over 120 min in the loading as well as in the three maintenance doses.

Patient monitoring and response evaluation. Patients vital signs and symptoms were monitored every 6 h during and up to 3 days after each antibody injection. Patients also underwent complete cell blood count, glutamic oxalacetic transaminase, glutamic-pyruvic transaminase, alkaline phosphatase, and seric creatinine measurements prior to the inclusion in the trial. These parameters were tested weekly during the first month and then, monthly. Adverse reactions were classified in mild, moderate, severe, or very severe according the World Health Organization (WHO) Toxicity Scale.⁽¹⁸⁾

Magnetic resonance (MR) or computer tomography (CT) scans were performed prior inclusion in the trial and then every 3 months, to evaluate the antitumor response. Objective responses were classified according to WHO criteria.⁽¹⁹⁾

Radioimmunoscinigraphy. Before treatment with the naked antibody, radioimmunoscinigraphy studies were done to evaluate the capacity of the antibody to reach and accumulate into the brain tumors.

Three mg of the anti-EGFR MAb were labeled with 50 mCi (1.85 GBq) of pertechnetate from a ^{99m}Mo/^{99m}Tc generator (Amersham, London, UK). The eluate was obtained from a sterile generator eluted within the previous 24 h. Planar scans were performed on a Sophy DS-7 gamma camera (Sophia Medical Systems, Canada), fitted with a low-energy collimator. Images were acquired using a 20% window centered on the 140-KeV emission from the technetium radiation. Planar anterior, posterior and lateral images were purchased at 2, 4, 6 h and 24 h postinjection using acquisition times of 5 min each.

Human anti-mouse antibodies (HAMA) response

The antibody titers against murine MAb ior egf/r3 were determined by an enzyme-linked immunoadsorbent assay (ELISA) technique. Briefly, microtiter plates were coated overnight with 10 µg/well of the antibody in bicarbonate buffer (pH 9.6) at 4°C. Then, the wells were blocked with the same buffer containing 1% bovine serum albumin (BSA) during 1 h at room temperature. At that moment, the plates were incubated for 2 h at 37°C with two dilutions of the patients serum samples (1:50 and 1:100). The plates were then washed with PBS/Tween-20 and incubated with 50 µL/well of 1:1000 diluted alkaline-phosphatase conjugated antihuman polyvalent immunoglobulin at 37°C for 1 h. The reaction was developed by adding 50 µL/well of 1 mg/mL para-nitro-phenylphosphate (PNPP) diluted in diethanolamine buffer (pH 9.8). The absorbance at 405 nm was read in an ELISA plate reader (Organon Teknika, Denmark). HAMA response was measured before treatment, 15 days after, and monthly thereafter until 6 months. Pretreatment serum samples of each patient were employed as individual controls. The HAMA assay was considered positive, when post-treatment/pretreatment ratio was higher than 1.5.

TABLE 1. PATIENTS' DEMOGRAPHIC CHARACTERISTICS AND TOXICITY

Patient no.	Total MAb dose (mg)	Age	Sex	KPS	Histopathologic diagnosis	Adverse reaction (Grade)
1	160	48	F	90	Hemangiopericytic meningioma	No
2	160	40	M	90	Glioblastoma multiforme	No
3	160	28	M	90	Anaplastic astrocytoma	No
4	320	59	F	70	Anaplastic astrocytoma	Visual darkness (grade 1)
5	320	54	F	90	Anaplastic oligodendroglioma	No
6	80	28	F	90	Glioblastoma multiforme	Anaphylactic shock (grade 4)
7	320	23	F	90	Glioblastoma multiforme	No
8	480	52	F	90	Anaplastic oligodendroglioma	No
9	480	19	F	90	Fibrilar astrocytoma	No

RESULTS

Eight high-grade glioma patients and 1 meningioma patient, 2 men, and 7 women received treatment with ior egf/r3. Their ages ranged from 19 to 59, with a mean of 39 years. All patients had prior surgery consisting in debulking resection followed by postoperative irradiation. At the moment of inclusion all the subjects had recurrent or unresectable brain tumors. Patients characteristics are described in Table 1.

As inclusion criteria, radioimmunoscintigraphy using ^{99m}Tc labeled ior egf/r3 was performed to candidates. All subjects had definitive diagnoses established by cytohistologic findings. In all patients, selective accumulation of radioactivity was observed at the site of the primary tumor. Positive antibody uptake in histologically proven anaplastic astrocytoma is shown in Fig. 1.

All patients received four doses of ior egf/r3, except Patient 5 who interrupted treatment after the first dose. This patient exhibited significant toxicity consisting in an anaphylactic shock. Apart from the rest, 5 months before the inclusion in the ther-

apeutic trial, this woman was in contact with the murine antibody. At that time, she was enrolled in a diagnostic protocol with ^{99m}Tc labeled ior egf/r3, after being suspicious of a malignant brain tumor. A big brain mass was visualized using radioimmunoscintigraphy, which was confirmed by CT scan. Patient received standard therapy (surgery and external beam irradiation) but shortly after, new neurological symptoms suggested tumor recurrence. At that point, this patient was included in the trial with the naked antibody, being assigned to the second cohort of treatment. After administering the first dose, the anaphylactic shock appeared. The allergic reaction was controlled with standard therapy, and treatment with ior egf/r3 was interrupted. HAMA response against the murine anti-EGF receptor antibody was demonstrated in this case. In the rest of the subjects no positive HAMA response was detected.

Patients have been followed for 4 years. In the 6-month evaluation, eight patients out of nine had stable disease, and 1 year after MAb therapy four patients exhibited tumor stabilization. Four years after ior egf/r3 antibody administration, 2 patients (Patients 7 and 9) were alive.

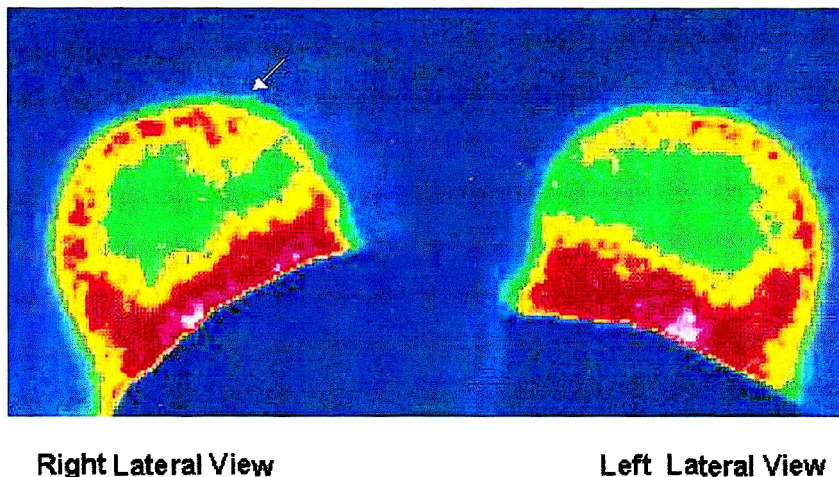


FIG. 1. ^{99m}Tc -labelled MAb ior egf/r3 uptake showing positive immunoscintigraphic image in a right temporal anaplastic astrocytoma.

DISCUSSION

Primary high-grade gliomas are characterized by several genetic alterations like EGF-R overexpression, PTEN mutations, p16 deletions, and MDM2 (Murine Double Minute 2) amplification.^(20,21) Consequently, several EGF-R blocking or radiolabeled antibodies have been evaluated in the management of advanced brain cancer.^(17,22–34)

This report summarizes the results of using another neutralizing EGF-R MAb in nine patients carrying unresectable or recurrent brain tumors.

Even though the blood–brain barrier and blood–tumor barrier are obstacles to the delivery of any agent to brain malignancies, ior egf/r3 was able to accumulate into the brain tumors, as was demonstrated by radioimmunosciintigraphy. Positive tumor uptake also confirmed EGF-R overexpression in the studied lesions. Other authors had also reported that anti-EGF-R MABs could reach the brain tumors after intravenous or intra-arterial administration.^(23–28)

EGF-R detection in brain tumors largely depends on the analytical technique used.^(7,24,26,35–38) In our trial, selective accumulation of ^{99m}Tc labeled ior egf/r3 was observed in all the evaluated subjects. The small number of patients tested and the increased vascularization of intracranial neoplasms could favor the high sensitivity of radioimmunosciintigraphy. Similar results had been obtained in other immunosciintigraphy trials using anti EGF-R MABs. The localization of gliomas using 111 Indium anti EGFR 425 (mouse IgG_{2a}, EMD 55900, Merck KGaA) in 28 patients, had a sensitivity of 0.96, a specificity of 0.60 and an accuracy of 0.90.⁽²⁶⁾ In a different study, positive tumor uptake was seen in eight of nine patients, using the same radioimmunoconjugate in patients bearing malignant gliomas.⁽²⁴⁾

Safety was the endpoint of the trial. In our study, the maximal ior egf/r3 cumulative dose was 480 mg. Despite the high EGF-R expression in the liver, clinical and laboratory findings did not show any sign of hepatic injury. After MAB administration, 1 patient presented a serious allergic adverse event. This was the only patient that was in contact with the same antibody, at a low dose, 5 months before entering the trial. We conclude that the toxicity was low and appeared primarily related with preceding sensitization to the MAB and the development of HAMA response. This result agrees with previous reports that confirmed the lack of toxicity of high intravenous doses of murine anti EGF-R antibodies.^(17,28)

In three previous clinical trials, the naked anti EGF-R antibody 425, was injected by the intravenous route or directly into the brain tumors, through a catheter in single or multiple infusions.^(17,28,34) In 1996, Stragliotto et al.¹⁷ described that after several cycles of 425, 7 of the 13 patients were stable for 1–4 months and the median survival from MAB treatment was 39 weeks for the glioblastoma multiforme patients and 20 weeks for patients bearing astrocytomas with anaplastic foci. In the intratumoral injection trial, 2 of 8 patients developed signs of massive tumor necrosis and the total median survival after the first MAB administration was 18.5 weeks.⁽³⁴⁾ In our small patients set, despite no objective tumor regression, eight patients out of nine had stable disease on the 6-month evaluation, and four patients exhibited stable disease 1 year after treatment completion. Two of these patients were alive after 4 years of MAB

treatment. We hypothesize that the long lasting disease stabilization achieved after treatment with ior egf/r3 in some patients, could be somewhat attributed to its anti-angiogenic property in addition to the direct antiproliferative effect. Brain tumors are among the most vascularized of all human neoplasms⁽³⁹⁾ and signal transduction inhibitors like EGF-R blocking antibodies can inhibit blood vessels formation by down-regulating the expression of pro-angiogenic factors.^(9,14,15) Angiogenesis suppression could significantly support the use of such new cancer drugs that inhibit tumor growth by blocking oncogenes cellular signals, especially in neoplasms with high rates of invasion and recurrence such as gliomas largely dependent on neovascularization.

Ior egf/r3 has recently been humanized in order to increase its clinical utility, by decreasing the potential generation of human anti-mouse antibodies.⁽⁴⁰⁾ The humanized MAb h-R3 (IgG₁ isotype) was obtained by transplanting the complementary determining regions (CDRs) of the murine MAb ior egf/r3 to a human framework. CDR-grafting dramatically reduced its binding capability and then the antibody was reshaped. A variant, in which three murine residues were retained, exhibited a similar capacity to inhibit the binding of EGF to its receptor as compared with the original antibody. The humanized antibody had already proved to be less immunogenic in African green monkeys in comparison with the murine version.⁽⁴⁰⁾

Three clinical trials are currently ongoing to study the toxicity, biodistribution, and pharmacokinetic of the humanized antibody in patients with advanced epithelial cancer.

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Address reprint requests to:
Tania Crombet-Ramos, M.D.
Center of Molecular Immunology
Division of Clinical Immunology
P.O. Box 16040
Havana 11600, Cuba.

E-mail: Taniac@ict.cim.sld.cu or taniacrombet@hotmail.com

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