

Clinical Trial

Treatment of High-Grade Glioma Patients with the Humanized Anti-Epidermal Growth Factor Receptor (EGFR) Antibody h-R3

Report from a Phase I/II Trial

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monoclonal antibody, epidermal growth factor receptor, high-grade glioma, glioblastoma, clinical trial, anaplastic astrocytoma, immunotherapy

ABSTRACT

The poor prognosis of patients with high-grade glioma has led to the search for new therapeutic strategies. More than half of these tumors overexpress Epidermal Growth factor Receptor (EGFR). h-R3 is a humanized monoclonal antibody that recognize the EGFR external domain with high affinity, inhibiting tyrosine kinase activation. In order to evaluate safety, immunogenicity and preliminary efficacy of h-R3 in newly diagnosed high-grade glioma patients, we conducted a Phase I/II trial. Patients received six weekly infusions of h-R3 at the dose of 200 mg in combination with external beam radiotherapy. Twenty-nine patients (mean age, 45 years and median KPS 80) were entered into the study. Tumor types were: glioblastoma (GB) (16 patients), anaplastic astrocytoma (AA) (12 patients) and anaplastic oligodendroglioma (AO) (1 patient). All patients underwent debulking surgery or biopsy before entering the trial. The antibody was very well tolerated. No evidences of grade 3/4 adverse events were detected. None of the patients developed acneiform rash or allergic reactions. One patient developed a positive anti-idiotypic response. Objective response-rate was 37.9% (17.2% complete response, 20.7% partial response) while stable disease occurred in 41.4% of the patients. With a median follow up time of 29 months, the median survival is 22.17 months for all subjects. Median survival time (MST) is 17.47 months for GB, whereas MST is not reached for AA patients.

INTRODUCTION

The poor prognosis of patients with high-grade gliomas, in spite of aggressive conventional anticancer therapies, has led to the search for new therapeutic strategies.

For these patients, irradiation remains the main postoperative treatment,¹ whereas the role of radiochemotherapy is still discussed controversially.²

These patients are appropriate candidates for clinical trials designed to improve local control by adding newer forms of treatment to standard therapy.

More than half of astrocytomas of higher grade, especially of the de novo type, overexpress Epidermal Growth Factor Receptor (EGFR), and 50–70% of these, express ΔEGFR, that lack exons 2 to 7 and remain constitutively activated.³ EGFR signal transduction pathways have been correlated with various processes that contribute to the development of malignancy, such as cell cycle progression, inhibition of apoptosis, angiogenesis, tumor cell motility and metastasis. EGFR overexpression has also been associated with chemo and radioresistance.⁴

Since EGFR is frequently amplified, overexpressed, or mutated in glioblastomas, but only 10 to 20 percent of patients have a response to EGFR kinase inhibitors, the mechanism of responsiveness of glioblastomas to these inhibitors is unknown.⁵

h-R3 is a humanized monoclonal antibody (MAb) (IgG1 isotype) that recognize an epitope located in the extracellular domain of the EGFR.⁶

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 modeling.⁶

Previously, nine patients with histologically confirmed gliomas, who had active or recurrent disease after receiving conventional treatment, received 4 intravenous doses of the murine antibody ior egf/r3. Total dosages ranged from 160 to 480 mg. 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. Despite no major objective antitumor responses, eight out of the nine patients had stable disease after six months of treatment.⁷

To study safety, pharmacokinetic and biodistribution of h-R3, 12 patients with advanced epithelial tumours received single intravenous infusion of the MAb, at four dose levels: 50, 100, 200 and 400 mg.⁸ Then, to evaluate efficacy and safety of h-R3 at multiple doses in combination with radiotherapy, 24 patients with advanced locoregional squamous cell carcinomas of the head and neck received six weekly intravenous doses of h-R3, at the same dose levels.⁹

h-R3 was very well tolerated after single or multiple administrations.^{8,9} The optimal biological dose, associated to the systemic saturation of clearance was equivalent to 200 mg.⁸ The most frequent h-R3 related adverse events consisted of fever, chills, nausea and hypotension. No skin toxicity or allergic reactions were detected. Seventeen of 22 evaluable patients showed partial or complete response after receiving h-R3 plus radiotherapy. Overall survival significantly increased after the use of the antibody doses of 200 and 400 mg.⁹

In order to further evaluate the safety, immunogenicity and the preliminary efficacy of h-R3 as a radiosensitizer, we conducted a Phase I/II trial in newly diagnosed high-grade glioma patients.

MATERIAL AND METHODS

This was a multicenter, open-labeled, Phase I/II study in patients with histologically verified glioblastoma (GB) and anaplastic astrocytoma (AA) who had undergone biopsy alone or debulking surgery. All subjects were suitable candidates for radical radiation therapy at the time of inclusion.

Other selection criteria were as follows: age more than 18 years, a Karnofsky performance status (KPS) >70, absolute neutrophil count $\geq 1.5 \times 10^9/L$, platelet count $\geq 100 \times 10^9/L$, serum creatinine level \leq the upper limit of normal, and serum bilirubin level less than two times the upper limit of normal. The most important exclusion criteria consisted of previous treatments with murine anti-EGFR antibodies, pregnancy or lactation, serious chronic diseases and active infections. EGFR overexpression in primary tumors was not mandatory for enrollment. Radiation therapy had to begin maximum 28 days after surgery.

Patients received 6 weekly infusions of h-R3 at the 200 mg level. Total MAb cumulative dose was 1200 mg. The antibody was administered by intravenous (IV) infusion, diluted in 250 ml of sodium chloride, over 1 hour. Whole-brain radiation was delivered in doses of 1.8–2 Gy given once daily, five days per week, to a total dose of 50 Gy to 60 Gy. Radiotherapy treatment planning and simulation was performed on the basis of recent CT scans. The planned overall treatment time was five to six weeks.

The clinical trial was done under the principles embodied in the Declaration of Helsinki. The protocol was approved by the Institutional Review Board of the 4 clinical sites and by the National Regulatory Agency. All patients signed a written consent form, prior to their inclusion in the clinical trial.

Toxicity was assessed using the National Cancer Institute's Common Toxicity Criteria (NCI-CTC), version 2.0.

Contrasted computer tomography (CT) or magnetic resonance imaging (MRI) scan was done before inclusion in the trial and then, monthly. Tumor response was classified according WHO modified criteria.¹⁰ 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: $\geq 50\%$ 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: $\geq 25\%$ 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.¹⁰ A MRI or CT with contrast within 48 hours post-surgery was used to assess the primary response to therapy.

Radio-immunoscintigraphy. Radio-immunoscintigraphy using the murine MAb ior egf/r3 was done to confirm the antitumor response and to evaluate EGFR expression in the lesions. The murine antibody was considered suitable for the imaging procedure taking into consideration its slight higher affinity for the receptor as compared to the humanized MAb and the low probability of patients' sensitization after a single exposure to the murine molecule.

Three milligrams of the anti-EGFR MAb were labeled with 50 mCi (1.85 GBq) of pertechnetate from a ^{99m}Molybdenum (^{99m}Mo)/^{99m}Technetium (^{99m}Tc) generator (Amersham, London, UK). The eluate was obtained from a sterile generator eluted within the previous 24 hrs. Planar scans were performed on 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 4 hrs post injection using acquisition times of 15 minutes.

Anti-idiotypic response. Blood samples were collected at 0, 7 days and then monthly up to 6 months to detect antibodies that react with the murine ior egf/r3 idiotype. A qualitative direct ELISA was performed by first coating high binding ELISA plates (Maxisorb, Costar, Cambridge, MA, USA) with ior egf/r3 (10 μ g/ml) overnight at 4°C. One hundred microliters of patient serum at 1: 100 dilution was added. Goat anti-human IgG or IgM conjugated to alkaline phosphatase (Sigma Chemical Co, USA) was then added to the wells. After incubation, 100 μ L substrate (para-nitro-phenyl-phosphate, 1 mg/mL) was added. Absorbance was measured at 405 nm. Pretreatment serum samples of each patient were used as controls. The assay was considered positive, when the post-treatment/pretreatment ratio was higher than 2.

Survival analysis. Survival time was calculated from the date of the first treatment until the date of death. Survival data were analyzed using the Kaplan-Meier method and the log-rank test.

RESULTS

Between February 2002 and February 2004, 29 patients (13 men and 16 women), with newly diagnosed high-grade glioma were entered into the study.

Mean age was 45 years (range 20 to 68 years) and the median Karnofsky performance status (KPS) was 80. Tumor types were: glioblastoma (16 patients), anaplastic astrocytoma (12 patients) and anaplastic oligodendroglioma (1 patient). Prior to radiotherapy, partial (n = 21), complete resection (n = 3) or a biopsy (n = 5) of the tumor was performed. Table 1 describes patients' individual characteristics.

Safety was assessed in the 29 patients who received at least one dose of the investigational drug. The antibody was very well tolerated. No evidences of grade 3 or 4 adverse events were detected. Eight patients showed grade 1 or 2 adverse events consisting of chills, nausea, fever, asthenia, anorexia, somnolence, cephalgia and increase of transaminases or alkaline phosphatase. The most frequent unrelated adverse event consisted in cephalgia. Radiation related grade 3/4 alopecia was seen in all patients. None of the patients developed acneiform rash or allergic reactions. A single patient had a positive anti-idiotypic response (IgG subclass) as per the ELISA method. We did not find any association between the immune response against h-R3 and safety or antitumor response. h-R3 possibly, probably or definitively associated adverse events are depicted in Table 1.

Antitumor response was evaluated in all patients, albeit three discontinued or interrupted antibody therapy before completing the six doses and one subject had a primary diagnose of anaplastic oligodendroglioma.

Objective response-rate [complete response (CR) + partial response (PR)] in our series was 37.9% (17.2% CR, 20.7% PR), stable disease (SD) occurred in 41.4% of patients and 20.7% had progressive disease (PD). Two patients among the five that showed complete remission had a total resection of the primary tumor while two subjects showing a PR had only a biopsy. The remaining patients evidencing complete or partial remission had a partial surgery of their tumors. Stabilization of the disease was seen in 12 patients, among which ten had a partial resection of the tumor while two subjects had merely a biopsy.

Table 1 Patients individual characteristics and h-R3 related adverse events

| Inclusion Number | Age | KPS | Diagnose | Extent of Surgery | Mental Status | Total Cumulative MAb Dose | Survival Since Inclusion (Months) | Best Response | h-R3 Related Adverse Events |
|------------------|-----|-----|----------|-------------------|---------------|---------------------------|-----------------------------------|---------------|--|
| 1 | 42 | 70 | GB | Biopsy | Normal | 1200 mg | 14,50 | PR | |
| 2 | 52 | 70 | ODA | Complete | Normal | 1200 mg | 2,30 | PD | |
| 3 | 27 | 90 | AA | Partial | Normal | 1200 mg | 44,63 | CR | |
| 4 | 34 | 70 | AA | Partial | Normal | 1200 mg | 15,07 | PR | Increased SGPT (G2), SGOT (G2), and Alkaline Phosphatase (G2) |
| 5 | 20 | 90 | GB | Partial | Normal | 1200 mg | 11,80 | PD | Increased SGPT (G2), SGOT (G2) and Alkaline Phosphatase (G2) |
| 6 | 64 | 70 | AA | Partial | Normal | 1200 mg | 12,97 | SD | |
| 7 | 68 | 70 | AA | Biopsy | Abnormal | 1200 mg | 1,47 | PD | Asthenia (G1), Dizziness (G1) |
| 8 | 50 | 90 | GB | Partial | Normal | 1200 mg | 29,93 | CR | |
| 9 | 49 | 70 | AA | Partial | Abnormal | 800 mg | 3,43 | PD | |
| 10 | 45 | 80 | GB | Partial | Normal | 1200 mg | 13,80 | SD | |
| 11 | 56 | 90 | GB | Partial | Normal | 1200 mg | 12,60 | SD | |
| 12 | 37 | 80 | GB | Partial | Normal | 1200 mg | 18,07 | SD | Increased SGPT(G1) |
| 13 | 46 | 70 | GB | Partial | Normal | 200 mg | 5,80 | PD | |
| 14 | 34 | 90 | GB | Biopsy | Normal | 1200 mg | 31,33 | SD | |
| 15 | 55 | 80 | GB | Partial | Normal | 1200 mg | 9,33 | SD | |
| 16 | 49 | 80 | AA | Biopsy | Normal | 1200 mg | 29,23 | PR | |
| 17 | 51 | 80 | GB | Partial | Normal | 1200 mg | 8,47 | SD | |
| 18 | 26 | 100 | AA | Complete | Normal | 1200 mg | 27,17 | CR | |
| 19 | 47 | 70 | GB | Partial | Normal | 1200 mg | 26,53 | CR | |
| 20 | 51 | 80 | AA | Partial | Normal | 1200 mg | 26,17 | PR | |
| 21 | 57 | 100 | GB | Complete | Normal | 1200 mg | 26,10 | CR | |
| 22 | 28 | 90 | AA | Partial | Normal | 1200 mg | 25,00 | PR | |
| 23 | 52 | 90 | GB | Partial | Normal | 1200 mg | 23,17 | PR | |
| 24 | 44 | 70 | GB | Partial | Normal | 1200 mg | 22,30 | SD | Chills (G1), Nauseas (G1), Increased SGPT(G1), asthenia (G1), anorexia (G1), somnolence (G1) |
| 25 | 39 | 90 | GB | Partial | Normal | 1200 mg | 24,07 | SD | Nauseas (G1), fever (G1), chills (G1) |
| 26 | 41 | 90 | AA | Partial | Normal | 1200 mg | 22,17 | SD | Cephalaea (G1), chills (G1) |
| 27 | 55 | 90 | GB | Partial | Normal | 1200 mg | 17,47 | SD | Chills (G1) |
| 28 | 61 | 80 | AA | Partial | Normal | 800 mg | 5,20 | PD | |
| 29 | 41 | 90 | AA | Biopsy | Normal | 1200 mg | 22,20 | SD | |

GB, glioblastoma; AA, anaplastic astrocytoma; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; SGPT, serum glutamic-pyruvic transaminase; SGOT, serum glutamic-oxaloacetic transaminase; G1, grade 1; G2, grade 2.

Among the glioblastoma patients, clinical responses were as follows: three patients with CR (18.8%), two patients with PR (12.5%), nine patients with SD (56.3%) and two patients (12.5%) with PD, while imaging evaluation of tumor size in the anaplastic astrocytoma subset showed two CR (16.7%), four PR (33.3%), three SD (25%) and three PD (25%). Four patients who relapsed or had disease progression received salvage surgery. None of the patients received chemotherapy due to their poor performance status after progression and the limited efficacy of the cytotoxic drugs in the relapsing setting.¹¹

In one of the clinical sites, radio-immunoscintigraphy with the parental murine MAb (ior egf/r3) was done to contrast with the CT or MRI assessment and to further characterize EGFR expression status after finishing the combined therapy.

Two patients showing complete response according standard imaging methods evidenced no MAB uptake by this in vivo functional scan, while patients showing partial, stable or progressive disease showed positive uptake of the murine anti-EGFR antibody in the known tumor areas. Figure 1 shows planar images obtained at 4-h post-administration of ^{99m}Tc-labelled ior egf/r3, illustrating a selective accumulation of the radioactivity at the site of the primary tumor in comparison with the corresponding MRI findings in a patient that achieved disease stabilization as best response.

With a median follow up time from treatment initiation to the closeout date of 29 months (range 22 to 45 months), the mean and median survival is 24.09 and 22.17 months, respectively for all subjects. At the time of analysis, 18 patients (62.06%) had died and 11 patients (37.93%) remain alive.

This therapeutic strategy yielded a median survival time (MST) of 17.47 months for glioblastoma whereas MST is not reached for anaplastic astrocytoma patients. Overall survival rates at 12 and 18 months were 75 and 53.6%. For GB patients, survival rates after combined h-R3 + radiation were 75% at 1 year and 50% at 18 months, while survival rates in the AA group were 75% and 58.3% at 12 and 18 months, respectively.

DISCUSSION

Despite aggressive therapy, the prognosis for patients with malignant glial tumors remains dismal.¹² These neoplasms are resistant to all currently used treatment modalities including surgery, radiation and chemotherapy.¹³ Current research focuses on novel targeted molecular therapy. Immunotherapy is one such novel approach since a number of potential antigens have been identified in gliomas. EGFR has been studied extensively as a target for direct immune attack via specific antibodies.¹⁴

h-R3 is a novel neutralizing humanized monoclonal antibody against EGFR, that recognizes the receptor with high affinity, inhibiting tyrosine kinase activation.⁶ The antibody showed a synergistic effect when combined with radiotherapy in patients bearing squamous carcinomas of the head and neck.⁹ The selective expression of EGFR by gliomas of higher degree and the potential of sparing normal brain tissue, also suggest the possibility of successful radiosensitization in this setting.

This manuscript shows for the first time the results of combining a humanized EGFR blocking MAb and radiation in patients bearing high grade gliomas.

The trial was designed to assess safety and immunogenicity of h-R3 in combination with radiation in high-grade astrocytoma patients. Secondly, the preliminary impact of the dual therapy on patients' survival was evaluated.

The results showed good tolerability of the schedule. Radiotherapy was safe and its toxicity was not exacerbated by the addition of h-R3. h-R3 related adverse events were classified as infusion reactions, while the increase of the liver function enzymes in four patients could be associated with the high EGFR expression in the hepatocytes.

h-R3 did not provoke anaphylactic reactions, that were indeed seen after treating glioma patients with repeated doses of its murine version (ior egf/r3), in relation with previous sensitization to the MAb and the development of human anti-mouse antibodies (HAMA) response.⁷ After 6 doses of h-R3, only 1 patient developed immune response against the humanized molecule, with no deleterious clinical effect upon the patient.

Skin rash, which had been detected in roughly 80% of patients treated with other EGFR blocking drugs,^{15,16} was not seen after repeated doses of h-R3. We hypothesize that some intermediate affinity antibodies like h-R3 ($K_d = 10^{-8}$ M), which recognize ubiquitous antigens expressed at lower levels on normal tissues, might show a preferential uptake by those tumors that overexpress the target, avoiding undesirable toxicity and increasing the therapeutic index.⁹

Although the blood-brain barrier (BBB) may hamper the delivery of monoclonal antibodies to brain malignancies, agents such as surgery, radiation and the tumour itself disrupt its integrity,¹⁷ allowing drug uptake by the tumours. Previously, our group found that immunoscintigraphy with ^{99m}Tc-labelled ior egf/r3 showed a very high sensitivity, specificity and accuracy for the in vivo detection of astrocytomas in patients.¹⁸

In this trial, immunoscintigraphy after the dual therapy, showed positive MAb uptake by patients with residual lesions, while subjects

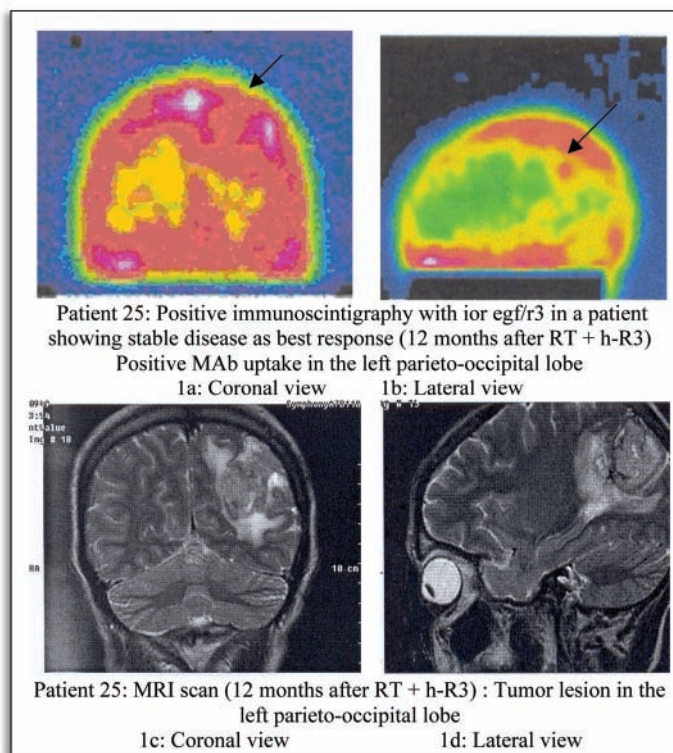


Figure 1. Immunoscintigraphy images obtained at 4-h post-administration of ^{99m}Tc-labelled ior egf/r3, illustrating a selective accumulation of the radioactivity at the site of the primary tumor in comparison with the corresponding MRI findings in a patient that achieved disease stabilization as best response.

with complete responses showed no antibody accumulation at the previously known site of tumours. However, these results should be interpreted with carefulness, since paired scintigraphy studies to compare MAb uptake before and after therapy were not performed and EGFR expression was not evaluated in the pretreatment specimens. Other factors like disordered vasculature and increased hydrostatic pressure in tumours, loss of expression of the EGFR or the emergence of mutated variants of the EGFR after the oncospecific therapy might also obstruct the specific MAb accumulation.

Even though the trial was not designed to assess the drug efficacy, a preliminary evaluation of the overall survival was done in our small patient subset.

Overall survival times [MST = 22.17 months (all patients), 17.47 months (GB) and not reached (AA)] were relatively long compared to survival times reported for radiotherapy alone.²

In our trial, whole brain irradiation was used. This modality of radiotherapy has demonstrated to induce more late side effects than localized field irradiation, but not to be less efficacious.¹⁹⁻²¹ At the National Institute of Neurology and Neurosurgery, a control group that received whole brain irradiation after surgery yielded an overall median survival of 17.8 months: 15.0 months for glioblastoma patients (n = 31) and 20.2 months for anaplastic astrocytoma patients (n = 36).^{22,23}

These results also compare very favorably with the figures reported for several radiochemotherapy schemes that have yielded negative or inconclusive results.²³

Other groups have used anti-EGFR monoclonal antibodies, either naked or labeled with radioisotopes to treat patients bearing

newly diagnosed or recurrent high-grade astrocytomas. No significant clinical improvement was found in any of the trials using murine antibodies, either naked or labeled with radioisotopes.²⁴⁻²⁷

Other small tyrosine kinase inhibitors targeting EGFR, gefitinib (Iressa, Astra Zeneca, London, UK) and erlotinib (tarceva, Genentech, South San Francisco, CA) were evaluated in the treatment of 57 and 45 patients with recurrent malignant gliomas, respectively.^{28,29} The median overall survival time from Iressa treatment initiation was 39.4 weeks, while adverse events were mild and consisted mainly of skin reactions and diarrhea.²⁸ After treating patients with tarceva, there were only 2 PR among the 45 patients and no prolongation of the progression free survival at six months.²⁹

In summary, combination of h-R3 plus radiation therapy is feasible and safe. The relatively long median survival time is promising but a further randomized controlled clinical trial should be performed to confirm this result. The increased survival and long-term duration of response seen after six weeks of therapy with h-R3 could be associated with its potent anti-angiogenic effect. h-R3 downregulates Vascular Endothelial Growth Factor (VEGF), after abrogating EGFR activation.³⁰ As gliomas are highly vascularized and their growth is angiogenesis-dependent, direct or indirect inhibition of pro-angiogenic factors is a very appealing therapeutic approach.³¹

A phase III trial comparing h-R3 + radiation vs. temozolomide + radiation in GB patients is being planned. Pharmacogenomic studies encompassing the evaluation EGFR gene amplification as well as mutations in PTEN and EGFR or in EGFR-regulated downstream signaling pathways including RAS, phosphatidylinositol 3-kinase (PI3-K), mitogen-activated protein kinase (p44/p42) are also intended to predict patients' response to h-R3.

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