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Original Paper

Multiple Infusions of Anti-epidermal Growth Factor Receptor (EGFR) Monoclonal Antibody (EMD 55 900) in Patients with Recurrent Malignant Gliomas

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In a prospective phase I/II trial, EMD 55 900, a murine monoclonal antibody (MAb) directed against EGF receptor, was administered at tumour recurrence to 16 patients previously treated with surgery, radiotherapy and chemotherapy for high grade supratentorial gliomas (11 glioblastomas, five anaplastic astrocytomas). Duration of treatment was planned for at least 4 weeks. The first 10 patients received 40 mg of MAb three times per week (median cumulative dose, 760 mg) and the last 6 patients received 200 mg three times per week (median cumulative dose, 2400 mg). Serum levels of EMD 55900 were proportional to the injected dose. Repeated infusions of EMD 55900 were well tolerated. In 13/16 patients, there were no adverse events. Among the 3 others, one had a grade IV neutropenia, one had a clinically asymptomatic hepatitis, and one had a skin rash. This last patient was the only one who had increased human antimouse antibodies (HAMA). After 4 weeks of therapy, 13 patients were evaluable for response. No measurable tumour regression was obtained with either schedule. 6 of the 13 patients (46%) showed evidence of progressive disease, while 7/13 (54%) had stable disease. All patients had progressive disease by 3 months. In this study, repeated infusions of EMD 55900 were well tolerated but no therapeutic benefit was demonstrated. **Copyright © 1996 Elsevier Science Ltd**

Key words: receptors, epidermal growth factor-urogastrone, murine monoclonal antibody, glioma, immunotherapy

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INTRODUCTION

MALIGNANT GLIOMAS have a poor prognosis despite aggressive and innovative techniques in surgery, radiotherapy or chemotherapy. In addition, there is an increased risk of toxicity to the normal brain parenchyma associated with intensive therapies. Alternative more specific treatments should be developed in order to improve outcome without jeopardising intact neurological functions. Immunotherapy of malignant gliomas with monoclonal antibodies (MAb) could be of value if the MAbs recognise tumour-associated antigens and are not retained in healthy tissues. The epidermal growth factor receptor (EGFR) is a potential target since it is expressed on plasma membranes of tumour cells in up to 80% of malignant gliomas, while it is virtually absent from normal brain [1–5]. A MAb raised against EGFR may be a useful treatment of malignant gliomas if a sufficient amount of the antibody reaches the target cells across the blood–tumour barrier, if tolerance is good and if the MAb can induce an antitumour effect. In a previous study, involving patients operated on for malignant gliomas, we found that pre-operative intravenous infusions of high doses (100–400 mg) of the anti-EGFR MAb EMD 55 900 were well tolerated and could "saturate" EGFR in surgical specimens of tumours [6]. Furthermore, a dose-dependent increase in the number of tumour infiltrating mononuclear cells was found, suggesting the initiation of an immunological cascade mediating cytotoxicity [7]. These results prompted us to start a prospective phase I/II study to evaluate the tolerance and therapeutic effects of repeated infusions of EMD 55 900.

PATIENTS AND METHODS

Monoclonal antibody

EMD 55 900 (MAb 425, E. Merck, Darmstadt, Germany), a murine IgG2a directed against the EGFR, was produced by hybridoma cells cultured in a serum-free medium. Hybridoma cells were obtained by immunising BALB/c mice with an intraperitoneal injection of A431 epidermoid carcinoma cells.

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Mice spleen cells were fused with myeloma cells as previously reported [8].

Patient characteristics

16 consecutive patients with recurrent malignant glioma were included in this trial. 11 patients had a glioblastoma and 5 had an anaplastic astrocytoma. There were 10 women and 6 men. The median age was 54 years (range 40–62 years) for the patients with glioblastoma and 48 years (range 36–59 years) for patients with anaplastic astrocytoma. The median Karnofsky performance scale was 70 (range 50–100). The median time from first surgery until entry into the study was 10 months in the glioblastoma group and 18 months in the anaplastic astrocytoma group. After surgery (resection or stereotactic biopsy, Table 1), all patients received external radiotherapy with a total dose of 55–60 Gy (1.8 Gy fractions, delivered five times per week) and chemotherapy (Table 1). Patients were treated with EMD 55 900 at tumour recurrence (clinical deterioration and tumour progression on computer tomography (CT) scan). Informed consent was obtained in all cases, and the study was approved by the ethical committees of the Salpêtrière Hospital.

Treatment procedure

The first 10 patients received 40 mg EMD 55 900 three times per week and the following 6 patients received 200 mg EMD 55 900 three times per week. The low dose of 40 mg was chosen because we found in a previous study [6] that it was the lowest dose required to induce significant saturation of the target receptor within the tumour (mean, 20% 2 days after a single i.v. infusion). For the dose of 200 mg of EMD 55 900, the mean intratumoral saturation of EGFR was much higher reaching 89% 2 days after a single i.v. infusion. Monoclonal antibody, diluted in 50 ml NaCl 0.9%, was given i.v. during 1 h by a perfusion pump. As a rule, patients were treated as inpatients during the first 2 weeks and afterwards as outpatients. One cycle of treatment lasted 4 weeks. MAb infusions were continued in patients with responding or stable disease until tumour progression. Patients with progressive disease received further chemotherapy after termination of MAb therapy (Tables 2 and 3).

Laboratory investigations

Blood samples were taken before EMD 55 900 infusion and every week during the study for blood cell count and

Table 1. Patients' characteristics prior to inclusion in the study

Case no.	Histology at diagnosis	Age/Sex	Surgery	Chemotherapy	Delay from diagnosis (months)	Karnofsky at inclusion
1	AA	38/M	Resected	3 VM26-CCNU-5FU	73	60
2	GBM	62/F	Biopsy	3 IA HeCNU 14 Carboplatin 3 PTV	36	70
3	GBM	49/F	Resected	1 BCNU 2 PCB	8	70
4	GBM	44/F	Resected	1 BCNU 2 PTV	8	90
5	GBM	54/M	Resected	3 BCNU 3 PTV	10	50
6	AA	57/F	Resected	4 BCNU	14	60
7	AA	48/F	Resected	3 IA HeCNU 2 BCNU 1 PTV 1 Carbo-VP16	13	90
8	GBM	53/F	Resected	6 BCNU	23	70
9	OA	40/F	Biopsy	3 IA HeCNU 2 BCNU 1 Carbo-VP16	37	100
10	GBM	56/F	Resected	5 BCNU	13	70
11	GBM	58/M	Resected	3 IA HeCNU 4 BCNU 5 PTV	29	80
12	GBM	40/F	Biopsy	1 BCNU 4 Carbo-VP16	8	90
13	GBM	61/F	Resected	4 BCNU 3 PTV	18	80
14	AA	59/M	Resected	3 IA HeCNU 5 BCNU 7 Carbo-VP16	32	70
15	AA	36/M	Resected	3 CCNU-VM26 1 Carbo-VP16	18	70
16	AA	55/M	Biopsy	2 BCNU 8 Carbo-VP16	17	70

GBM, glioblastoma; AA, anaplastic astrocytoma; OA, anaplastic oligodendroglioma; IA HeCNU, intra-arterial chemotherapy with HeCNU; PTV, procarbazine-thiotepa-vincristine; Carbo, carboplatin; PCB, procarbazine.

Table 2. Group I EMD 55 900: patients treated with 40 mg three times per week for 4 weeks

Case	Histology at recurrence	Number of infusions	Cumulated dose (mg)	Steroids (mg/d)*	Results†	Tolerance Karnofsky after 4 weeks tt‡	Treatment after MAb§	Survival (weeks) after MAb
1	GBM	19	760	SLM 160	1c: Stable 2c: Progression	Good KS 50	None	10
2	GBM	36	1440	SLP 45	1c: Stable 2c: Stable 3c: Progression	Good KS 20	3 PCB	55
3	GBM	24	960	SLP 80	1c: Stable 2c: Progression	Neutropenia KS 60	14 MTX-Cy	130
4	GBM	19	760	SLP 40	1c: Stable 2c: Progression	Good KS 50	1 MTX-Cy 1 Carbo-VP 16	12
5	GBM	10	400	SLM 120	Progression	Good KS 50	None	4
6	AA	12	480	SLP 60	Progression	Good KS 40 Septacaemia‖	14 Carbo-VP 16	108 AML‖
7	AA	7	280	SLP 60	Intolerance	Skin rash KS 90	2MTX-Cy	16
8	GBM	21	840	1c: None	1c: Stable 2c: Progression	Good KS 70	Operation coma post-op KS 10-20	48
9	OA	23	920	None	1c: Stable 2c: Stable	Increased liver enzymes KS 90	2 PCB 1 MTX-Cy 2 PCV	53
10	GBM	15	600	None	Progression	Good KS 60	2 Carbo-VP16 1 PCB	39

GBM, glioblastoma; AA and OA, anaplastic and mixed anaplastic astrocytoma. *Steroids during MAb treatment are as follows: SLM, solumedrol (methyl-prednisolone); SLP, solupred (prednisolone). †Results are detailed after the first cycle of 4 weeks (1c), 2 or more cycles (2c, 3c) in responding patients. ‡Adverse effects in 3 patients, and the neurological status: KS, Karnofsky score. §Further chemotherapy after MAb included MTX, methotrexate; Cy, cyclophosphamide; PCB, procarbazine; Carbo, carboplatin; PCV, procarbazine-CCNU-vincristine. ‖This patient had a septacaemia from the Port-a-cath, and died of acute myelogenous leukemia (AML).

Table 3. Group II EMD 55900: patients treated with 200 mg three times per week for 4 weeks

Case	Histology at recurrence	Number of infusions	Cumulated dose (mg)	Steroids (mg/d)*	Results†	Tolerance Karnofsky after 4 weeks tt‡	Treatment after MAb§	Survival (weeks) after MAb
11	GBM	12	2400	None	Progression	Good KS 80	3 Carbo-VP16 1 PCB	33
12	GBM	12	2400	SLP 30	Progression	Good KS 90	7 MTX-Cy 1 PCB	56
13	GBM	33	6600	SLP 20	1c: Stable 2c: Stable 3c: Progression	Good KS 70	3 Carbo-VP16	32
14	GBM	12	2400	SLP 20	Progression	Good KS 40	4 PCB	40
15	AA	9	1800	SLP 20	Progression	Good KS 90	1 PCB	20
16	AA	12	2400	SLP 50	Progression	Good KS 70	1 MTX-Cy	9

GBM, glioblastoma; AA and OA, anaplastic and mixed anaplastic astrocytoma. *Steroids during MAb treatment are as follows: SLM, solumedrol (methyl-prednisolone); SLP, solupred (prednisolone). †Results are detailed after the first cycle of 4 weeks (1c), 2 or more cycles (2c, 3c) in responding patients. ‡Adverse effects in 3 patients, and the neurological status: KS, Karnofsky score. §Further chemotherapy after MAb included MTX, methotrexate; Cy, cyclophosphamide; PCB, procarbazine; Carbo, carboplatin; PCV, procarbazine-CCNU-vincristine.

differentials, coagulation parameters, ionogram, liver enzymes, urea, creatinine and complement. Serum was taken before the first infusion, at the end of infusion, and 1, 2, 4, 8, 24 and 48 h after infusion for pharmacokinetic studies. Levels of human antimouse antibodies (HAMA) in the serum were measured before treatment and 1 week ($n = 16$), 2 weeks ($n = 15$), 3 weeks ($n = 13$) and 4 weeks ($n = 7$) after the onset of MAb therapy, using an ELISA test (Immustrip HAMA, Medac, Hamburg, Germany).

CT scan, with or without iodine contrast, was obtained prior to MAb treatment, and every 4 weeks thereafter, or in case of clinical deterioration. Response to treatment was determined clinically and on CT scan by the product of the two largest perpendicular diameters of contrast-enhanced lesion, according to the criteria of MacDonald and associates [9].

RESULTS

16 patients were treated with EMD 55 900. In the first 10 patients (40 mg three times/week), a median cumulative dose of 760 mg (range 280–1440 mg) of EMD 55 900 was administered. In the last 6 patients (200 mg 3 times/week), the median cumulative dose of EMD 55 900 was 2400 mg (range 1800–6600 mg). The median half-life of EMD 55 900 in the sera was 24 h in the low dose group (40 mg). Median serum concentrations of MAb for the low dose group were 10.8 μg/ml (± 4.9 standard deviation, S.D.) at the end of infusion, 5.1 μg/ml (± 2.3) after 24 h and 2.9 μg/ml (± 0.9) at 48 h. In the high dose group (200 mg), the median half-life was 30 h. The median serum concentrations were 70.9 μg/ml (± 13.9) at the end of infusion, 37.2 μg/ml (± 10) after 24 h and 24.7 μg/ml (± 11.6) after 48 h. Tolerance was good in 13/16 patients. 3 patients, all in the low dose group, had adverse events requiring discontinuation of treatment. Patient 3 (Table 2), who had been pretreated with BCNU and procarbazine, had a neutropenia grade IV (scale of the National Cancer Institute), during the second cycle of treatment (cumulated dose 960 mg); she was given granulocyte-colony stimulating factor support and improved. Patient 7, known for iodine allergy, developed a marked skin rash during the first week of treatment. The rash occurred at each infusion and treatment was stopped during the third week (cumulative dose 280 mg). A third patient (no. 9) had a 10-fold increase in liver enzymes during the second cycle of treatment (cumulative dose 920 mg), which normalised 2 weeks after discontinuation of MAb.

There was no HAMA response during the first 4 weeks of treatment except in the patient who developed a skin rash. In this patient, HAMA values were increased prior to treatment (201 ng/ml for a threshold of detection of 40 ng/ml) and they increased to 983 ng/ml after 1 week of treatment. None of the 16 patients experienced serum sickness.

13 patients were evaluable for response. Three patients were not evaluable because of early discontinuation of MAb therapy (skin rash in patient 7 and tumour progression before the end of the first cycle in patients 5 and 15). None of the 12 patients had a complete or partial response. 7 of the 13 patients (54%) had a stable disease lasting 1 month in 4 patients and 2 months in 3 other patients. 6 of the 13 patients (46%) had progressive disease after one cycle of treatment. None of the patients were stable at 3 months. After MAb failure, 13 patients received a second or third line chemotherapy, and 1 patient was re-operated upon. Median survival from the beginning of MAb therapy was 39 weeks (range 4–130 weeks) for patients with glioblastoma and 20 weeks (range 9–108 weeks) for patients with anaplastic astrocytoma. There was no difference between patients receiving high or low dose EMD 55 900. The cause of death was progression of the tumour, except one patient (no. 6) who died 2 years after MAb therapy from acute myelogenous leukaemia (AML).

DISCUSSION

A number of MAb recognising tumour-associated antigens in gliomas have been described [10–12]. High specificity as well as high antitumour cell activity have been shown *in vitro* and *in vivo* in animal models harbouring implanted brain tumours [13–15]. EGFR is a tumour-associated antigen that can be considered of "operational specificity" since it is overexpressed in 60–80% of malignant gliomas, while it is absent from normal brain. In addition, some evidence suggests that expression and gene amplification of EGFR is implicated in tumour progression of glioma and particularly glioblastoma [1–4]. An anti-EGFR MAb could inhibit tumour growth by blocking the access of EGF to its receptor or by inducing an immune-mediated cytotoxicity.

EMD 55 900 is a murine anti-EGFR immunoglobulin of IgG2a isotype. When bound to a protein determinant located on the external domain of the receptor, the MAb is internalised with its receptor, causing downregulation of the EGFR without stimulation of the tyrosine kinase pathway. This MAb also elicits a complement-mediated and an antibody-dependent cellular cytotoxicity in the presence of lymphocytes or monocytes [8]. Faillot and colleagues found that single injections of 100–400 mg of EMD 55 900 were well tolerated in humans and could "saturate" intratumoral EGFR *in vivo* in patients with malignant glioma [6]. The aims of the present study were to evaluate the toxicity and clinical response of repeated infusions of 40 and 200 mg EMD 55 900 in patients who had failed conventional treatment for malignant gliomas.

Tolerance of high cumulative doses of EMD 55 900 was good despite the expression of EGFR in extraneural tissues. Indeed, only 3/16 (19%) patients developed adverse effects requiring discontinuation of therapy; these adverse events were not life-threatening nor did they entail permanent morbidity. Skin rash occurred in one patient with increased HAMA levels prior to treatment, suggesting that he was already immunised against murine antigens. A transient increase of liver transaminases, found in 1 patient, had been noted previously after a single infusion of EMD 55 900 [6], and may be explained by EGFR expression in the liver. A single case of haematological toxicity (neutropenia) was seen in a patient who previously received repeated nitrosourea-based chemotherapy with poor haematological tolerance; the link between neutropenia and MAb treatment is not clear since EGFR is not expressed on leucocytes and is also absent in bone marrow. Previous studies of infusions of unconjugated murine MAb reported a wide span of side-effects, depending on the target recognised (reviewed in [16]). In haematopoietic diseases treated with MAb, 10–15% of patients experienced fever, increased transaminases and urticaria. In colorectal diseases, several MAb (mainly 17-1A) caused nausea and diarrhoea. The highest frequency of side-effects, reaching up to 100% of patients, was seen with anti-GD2 MAb in neuroblastoma, with severe pain, neurological symptoms, hyponatraemia and orthostatic hypotension [17, 18].

Although of great concern to most investigators using murine MAb, serum sickness and anaphylaxis were reported in less than 1% of cases. Good tolerance of EMD 55 900 injections may be due to three factors: first, 12/16 patients were taking corticosteroids, which have an immunosuppressive effect. Second, a general immune depression is a characteristic feature of malignant glioma [19]. Third, most of our patients had been pretreated with systemic chemotherapy, which may durably affect the immune status of the host.

HAMA developed at a significant level in only 1 patient within the first 4 weeks. This result is in contrast with most other studies during which HAMA were elevated usually by the second week after the first administration of murine MAb [16, 18]. A notable exception is chronic lymphocytic leukaemia, which is associated with immunodeficiency. The possible role of previous chemotherapy and radiotherapy or concomitant treatment with corticosteroids to lower the HAMA response has not been demonstrated [16]. In addition, repeated injections of murine MAb may induce a tachyphyllaxis, which could explain the absence of HAMA in our study.

Therapeutic response to repeated injections of EMD 55 900 was poor. No objective response was observed. Stabilisation of short duration, lasting 1–2 months, occurred in 54% of evaluable patients, but this cannot be interpreted as a positive result, because stabilisation is usually considered over a period of at least 3 months [9]. These results are in agreement with recent therapeutic trials of unconjugated MAb for haematological and solid tumours, even if isolated cases of objective response to MAb have been described in follicular lymphoma or melanoma [16]. The reasons for the absence of therapeutic effect may be the same as those accounting for good tolerance. An impaired T cell response is present in patients with malignant glioma. *In vitro*, this inhibition is reproduced by TGFβ2, a cytokine secreted by glioma cells [19, 20]. Altered T cell response may in turn prevent the development of an appropriate antibody-dependent cellular toxicity. Complement-mediated cytotoxicity has been reported to be much weaker with IgG subclass as compared to IgM MAb [16] and indeed concentrations of complement components measured in the sera of our patients were almost unaltered during treatment (data not shown). Concomitant treatment with corticosteroids could also interfere with antibody-mediated antitumour effect. However, it should be noted that failure of MAb therapy was also encountered in 4 patients off steroids. Finally, EGFR amplification is only one of several genetic alterations found in malignant gliomas and it is not surprising that blocking access to the EGFR alone is not sufficient to reverse the malignant phenotype.

In summary, repeated infusions of high doses EMD 55 900 for recurrent malignant glioma are well tolerated but there is no objective therapeutic value. Nevertheless, an intriguing finding is that 8/11 glioblastoma responded favourably to a second or third line chemotherapy after MAb failure, with a median survival time of 54 weeks from recurrence in responding patients, an unusually high figure. Whether this finding is related to a chemosensitisation of the tumour cells by MAb, as reported in experimental models [21], or is a mere coincidence in this small series, remains to be demonstrated.

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