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### 131I Radioconjugated Antibodies for the Locoregional Radioimmunotherapy of High-grade Malignant Glioma: Phase I and II Study

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# $^{131}\text{I}$ Radioconjugated Antibodies for the Locoregional Radioimmunotherapy of High-grade Malignant Glioma

## *Phase I and II Study*

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Locoregional radioimmunotherapy (LR-RIT) was administered to 111 patients (20 were recruited in a phase I and 91 in a phase II study) with malignant gliomas: 1 patient with oligodendroglioma, 7 patients with anaplastic oligodendroglioma, 2 with grade II astrocytoma, 10 with anaplastic astrocytoma and 91 with glioblastoma, amounting to 58 newly diagnosed and 53 recurrent tumours. The  $^{131}\text{I}$ -labelled monoclonal antibodies BC-2 and BC-4 were used in order to recognize stromal and intracellular glycoprotein tenascin, an antigen present particularly in glioblastoma. The patients were enrolled between February 1990 and December 1997 after conventional therapy. The radiopharmaceutical was injected directly into the tumour site. Sequential scintigraphies demonstrated a high and enduring uptake in the tumour. The mean irradiation dose in the tumour was 300 Gy per cycle. In the group of 74 phase II glioblastoma patients the clinical responses were as follows: 10 patients with stable disease (SD), 9 with partial responses (PR), 23 with no evidence of disease (NED) and 1 patient with complete response (CR). The median survival was 19 months. The response rate (CR + PR + NED) was 17.8% for those patients with bulky lesions, with a median survival of 17 months, but 66.6% for patients with small lesions, with a median survival of 25 months. Better outcomes were recorded in cases with less aggressive diseases: oligodendroglioma, anaplastic oligodendroglioma and anaplastic astrocytoma. We conclude that fractionated LR-RIT can be safely performed, with promising results especially in patients with minimal disease.

High-grade malignant glioma, and, in particular, glioblastoma (GLB), represents one of the most deadly and rapidly proliferating malignancies (1). There are no treatments for the prevention of gliomas and no instrumental techniques available for their early diagnosis. The doubling time of malignant gliomas is rapid. The current therapeutic procedures (surgery and radiotherapy) are unable to control the tumour progression for a significant duration. Although the results of surgery are apparently radical on visual inspection and on postoperative magnetic resonance imaging (MRI), the operation is almost always incomplete (2). In fact, a considerable number of occult, microscopic cell clusters located in the seeming normal brain close to the macroscopic neoplastic mass are left. In all cases these cytotypes cannot be entirely destroyed by radiotherapy and become the starting-point for a deadly relapse of the

disease. The median survival of the patients is short (12 months for GBL) and malignant gliomas may recur within a few months (3). Moreover, the majority of prior low-grade malignant gliomas can transform to a higher grade of histologic malignancy. Since this disease, at least during its initial stage, is circumscribed and does not invade the surrounding brain or spread throughout the rest of the body, a locoregional therapeutic approach can be useful in sterilizing the tumour bed after the traditional treatments in order to prevent, or reduce and slow down the local recurrence. For this reason the radioconjugated monoclonal antibodies (MAbs), topically infused into the postoperative crater, offer an appropriate theoretic rationale and have the potential to ameliorate the control of these malignancies (4–6). The MAbs are given in a solution form and are conveyed in the neoplastic zone by means of

a catheter. In this way the immunoglobulins find and bind their specific receptors and deposit the radioactivity in the neoplastic tissue. The glioma cells are reached both by the progressive penetration of MABs through the tissue and by the cross-fire of isotopes. We started our locoregional radioimmunotherapy (LR-RIT) study in 1990 and we report here the conclusive data of the phase I and phase II trials, which have taken 8 years to complete and which include 111 cases.

## MATERIAL AND METHODS

### Monoclonal antibodies

LR-RIT was carried out using two murine IgG monoclonal antibodies, BC-2 and BC-4, which react with two distinct epitopes on the tenascin (TN) molecule. TN is a glycoprotein present in the stroma and in cellular cytoplasm of glioma, but not in the normal brain tissue (7). TN belongs to an extracellular matrix found in developing tissue during the foetal period and it is uniformly expressed in viable neoplastic tissue: this feature leads to a strong and selective tumour targeting. Antitenascin MABs have already been used in preclinical studies (8) as well as in clinical trials (9).

### Isotope and labelling procedures

Both antibodies were labelled with I-131 by the Iodogen (Iodogen™: 1, 3, 4, 6-Tetrachloro-3 $\alpha$ ,6 $\alpha$ -diphenylglycouril) method. The labelled antibodies displayed radiochemical purity >98% in HPLC and immunoreactivity >65% in a direct antibody binding assay.

### Patients

*Phase I study.* Twenty cases were enrolled in this stage of the trial; their histological features, classified according to WHO recommendations, were: 2 astrocytomas grade II; 1 astrocytoma grade III (AA); 17 glioblastomas (GBLs) (12 males and 8 females, mean age 51.3; range 26–68). Six of these cases had newly diagnosed (ND) tumours whilst

**Table 1**

*Phase I study (20 patients)*

Histology	No. of patients	ND (s/b)	REC (s/b)
Astrocytoma grade II	2	1 (1/0)	1 (1/0)
Anaplastic astrocytoma	1	1 (0/1)	/
Glioblastoma	17	4 (0/4)	13 (3/10)

s = Small lesion (tumour volume <2.5 cm<sup>3</sup>, at the time of RL-RIT).

b = bulky lesion (tumour volume >3 cm<sup>3</sup>, at the time of RL-RIT).

Abbreviations: ND = newly diagnosed tumour; REC = recurrent tumour, following customary treatments.

**Table 2**

*Phase II study (91 patients)*

Histology	No. of patients	ND (s/b)	REC (s/b)
Oligodendroglioma	1	1(1/0)	–
Anaplastic oligodendroglioma	7	2 (1/1)	5 (1/4)
Anaplastic astrocytoma	9	6 (4/2)	3 (2/1)
Glioblastoma	74	38 (27/11)	36 (16/20)

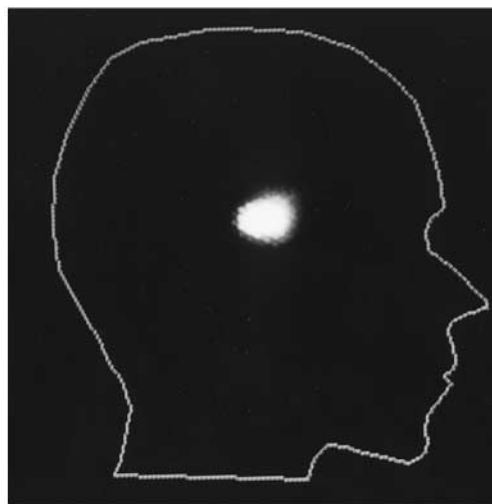
s = small lesion (tumour volume <2.5 cm<sup>3</sup>, at the time of RL-RIT).

b = bulky lesion (tumour volume >3 cm<sup>3</sup>, at the time of RL-RIT).

Abbreviations: ND = newly diagnosed tumour; REC = recurrent tumour, following customary treatments.

14 were suffering from recurrent lesions (REC). In 5 cases the tumour mass was small but in 15 cases the lesion volume was more than 3 cm<sup>3</sup> (see Table 1).

*Phase II study.* For this stage 91 cases were recruited, and more precisely these included: 1 oligodendroglioma (ODG) (ND, small); 7 anaplastic oligodendrogliomas (Ana ODG), of which 2 were ND and 5 REC, 1 with a small lesion and 5 with bulky disease; 9 anaplastic astrocytomas (AA) (6 ND, 3 REC, 6 small and 3 bulky); 74 GBLs (38 ND, 36 REC, 43 small and 31 bulky) (Table 2). The patients' median age was 51 years, ranging between 25 and 72 years. Karnofsky status was over 60% in all cases All



*Fig. 1.* A 64-year-old-man, with glioblastoma in the right temporal lobe was submitted to surgery on April 1997. He received three IL-RIT courses. A mean I-131 dose per cycle was 1 554 MBq. After injections, the uptake of MABs into the tumour remained high, ranging between 2.2% and 3.6% of injected dose calculated per gram of tissue during 24 h. Gamma imaging shows good targeting only in the postoperative cavity, at the site of the lesion without any further spread in normal brain. He is still alive 12 months after diagnosis, and 9 months from the first course of IL-RIT.

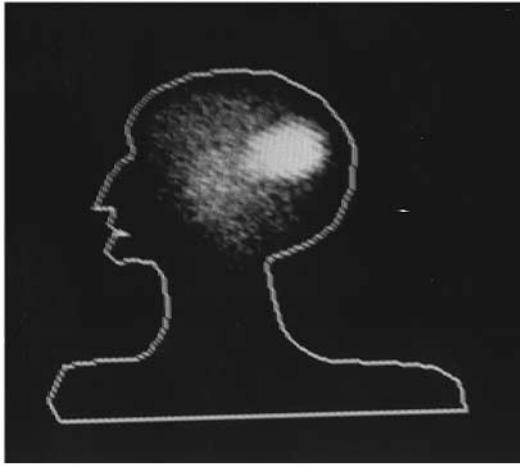


Fig. 2. This patient (female, 54 years old) had malignant glioma in the right parietal lobe, which relapsed after the first operation in October 1994 and subsequent external radiotherapy. She had repeat surgery on April 1995. A fistula between the surgical crater and the right lateral ventricle was produced. She received four LR-RIT treatments. The scintigraphy obtained 7 days after the injection of the first therapeutical dose (I-131 2257 MBq) shows a dispersion of radioactivity in the right lateral ventricle. However, considerable radioactivity remained in the site of the lesion, demonstrating a preferential targeting of the tumour by means of specific MAbs. Seven months later, the patient presented a second GBL mass in the temporal lobe, distant from the primary lesion. Her survival was 21 months from diagnosis and 14 months from the first course of LR-RIT.

the patients were surgically treated and diagnosis was confirmed by histology according to the WHO criteria. All had been given radiotherapy and 54 chemotherapy. The volume of primary tumours was large in most of these cases. At the time of the first IL-RIT, 52 patients had small or microscopic residual tumours (volume  $< 2 \text{ cm}^3$ ) and 39 had large bulky lesions with a tumour volume  $> 3 \text{ cm}^3$ . Both phase I and phase II patients were divided in two subgroups on the basis of the clinical course of their disease: 53 newly diagnosed tumours (ND) and 58 relapsed tumours (REC). The first subset of patients received, in turn, surgery, external radiotherapy, and 26 of these received chemotherapy. The volume of the lesion that remained after these therapies was an average  $15.8 \text{ cm}^3$  (range  $6.3\text{--}74 \text{ cm}^3$ ) in 19 patients. Conversely, in 34 cases, the neoplastic lesion was greatly reduced or not detectable by conventional imaging procedures, and was considered as a small or minimal disease. At that point, all patients were treated with an intralesional infusion of radioactive MAbs. The second group included 58 cases all of whom had surgery. All the patients were given external radiotherapy and 28 patients also received chemotherapy. Nevertheless, in all of these cases the malignant glioma relapsed or expanded during a median time, which was, in the GBL class, 5 months (range 2–15). All were submitted for a second operation before radioimmunotherapy. Surgery re-

sulted in microscopic disease in 23 cases. In these patients the radiological examinations (CT scan, MRI and brain SPECT (single photon emission computed tomography) with Tc-99m MIBI) demonstrated either a small abnormal area in the site of the previous lesion or complete negativity. In the remaining 35 cases, part of the recurrent tumour could not be surgically removed; the average volume of the remnant tissue was  $27.8 \text{ cm}^3$  (range  $6.3\text{--}110 \text{ cm}^3$ ). After surgery all the patients were given LR-RIT. In each case, liver, renal and cardiac function, and haematology were controlled before every course of LR-RIT, and always with normal results. A life expectancy of more than four months was required. Adequate presence of TN in glioma tissues (at least more than three plus) was confirmed by immunohistochemistry, which was carried out using the same BC-2 and BC-4 antibodies later employed for LR-RIT applications. All the patients gave their written informed consent to accept RIT according to the protocol approved by the Ethics Committee of 'M. Bufalini' Hospital, Cesena, Italy.

#### LR-RIT protocol

*Phase I.* First, the patients were submitted for surgery to reduce, if possible, the tumour mass and to place an indwelling (Rickam or Ommaya) catheter the internal tip of which was inserted in the core of the tumour or in the central part of the postoperative cavity (10). Before LR-RIT, the patients were given steroids, antiepileptic drugs and thyroid-blocking agents, as already described (11). Finally, they received intralesionally escalating doses (185, 370, 740, 110, 1480, 1850, 2220, 2590 and 2775 MBq) of I-131, labelled to 1–3 mg of antibody; 3 patients were studied at each incremental level. In these cases the radioiodine maximum tolerated dose (MTD), the local and systemic toxicity, the biodistribution, the pharmacokinetics and the dosimetry were assessed. In addition, possible clinical effects were recorded.

*Phase II.* Prior to receiving the antibody infusions, all of these patients underwent a complete treatment of their tumour by means of all established regimens: surgery, radiotherapy, chemotherapy (if judged beneficial) and a second operation was performed in cases of recurrent lesion. In both groups the antibodies were infused by using a shielded syringe, the needle being inserted into the reservoir of the indwelling catheter, which was previously surgically inserted. MAbs were administered if a small quantity of CSF was withdrawn by syringe aspiration, in order to be sure of the exact location of the needle. The mean volume of radioactive solution was 1.5 ml and its dispensation took less than one minute. The mean isotope dose was 2035 MBq (range 1295–2775). The patients were adequately isolated in a specially equipped ward, in accordance with the radioprotection rules, for 5–15 days (mean 7).

### Follow up

The patients were monitored continuously during their hospitalization in order to perceive any possible modifications of vital parameters. After hospitalization, they were clinically controlled every 30 days for one year, and by means of radiological examinations (CT scan, MRI, brain SPECT with Tc99m MIBI) every 3 months. After the first year, the clinical and instrumental investigations were performed every 3 and 6 months, respectively. Early or late side effects were actively controlled by means of ordinary procedures employed in clinical practice, such as physical examinations, blood analysis, and radiological investigations.

### Treatment responses

The parameters taken into account to evaluate the clinical response to LR-RIT were: a) the length of time free of disease from first cycle until the possible relapse; b) the median survival which was calculated from the time of the first operation to death or the latest follow-up in accor-

dance with the procedure of Kaplan & Maier (12); c) the objective response: in this case the WHO classification was used in assessing the treatment results: complete remission (CR), partial remission (PR), stable disease (SD) and progressive disease (PD). Those patients who had LR-RIT when their tumour mass was very small and not detectable but who remained free of disease from the start of therapy were classified as NED (no evidence of disease). This situation was considered as a favourable response since a relapse of the glioma had been hindered for a long period. In fact, recurrence of malignant glioma occurs rapidly in all cases treated by traditional procedures, and even in cases of minimal remnant tumours; d) the response rate which included PR, CR and NED. The haematological, hepatic, renal and cerebral toxicity was evaluated in agreement with WHO recommendations.

### Dosimetry

The radiation dose to the lesion and to normal parenchymas was calculated by means of a tracer dose, utilizing MIRD and Monte Carlo formalisms, as previously re-

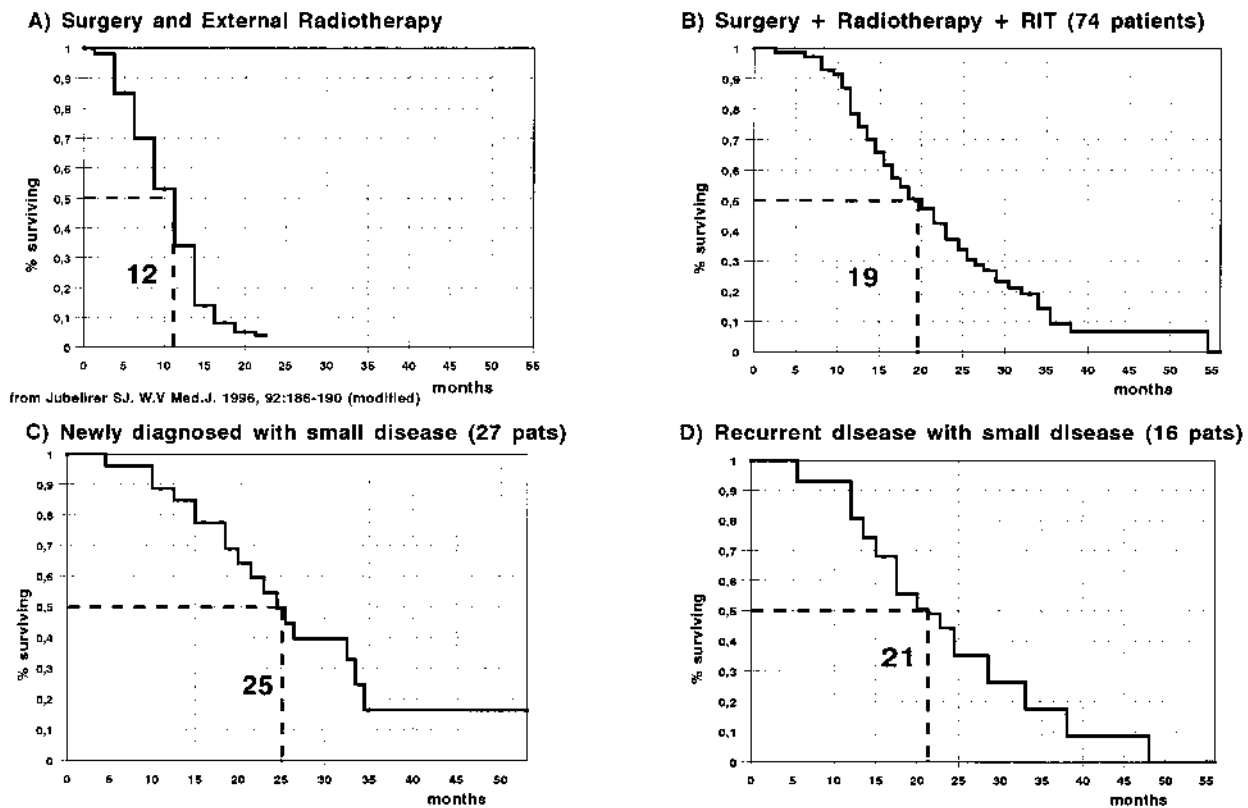


Fig. 3. A. Twelve months' median survival achievable by traditional methods (surgery and external radiotherapy) according to Jubelirer and co-workers (3). B. Nineteen months' median survival obtained in our GBL group (70 evaluable cases) by adding LR-RIT to conventional procedures. C. Twenty-five months' median survival obtained in 27 newly diagnosed GBL patients who received the radioactive antibodies intralesionally, following radical surgery and external radiotherapy. In all these cases the tumour burden was small or minimal as assessed by means of MRI, which was carried out immediately before the infusion of the radiopharmaceutical. D. Twenty-one months' median survival attained in 16 recurrent GBL patients, all of whom underwent surgery and external beam radiation; but their tumour recurred. They were operated on again and surgery succeeded in greatly reducing the disease, which resulted in a tiny remnant or not detectable at all by means of radiological examinations. Finally, they were submitted to LR-RIT.

**Table 3**  
Objective response to LR-RIT

Histology	PD	SD	PR	CR	NED
ODG (pat.n.1)	–	–	–	–	1 (24 m)
ANA ODG (pat. n. 7)	4 (6 m)	–	–	2 (18 m)	1 (15 m)
ANA ASTRO (pat.n. 7)	1 (7 m)	–	–	–	4 (39 m)
GBL (pat. n. 70)	27 (7 m)	10 (6 m)	9 (7 m)	1 (47 m)	23 (15 m)

Abbreviations: PD = progressive disease; SD = stable disease; PR = partial remission.

CR = complete remission; NED = not evidence of disease; ( ) = length, in months, of the duration of the outcome (median value); ODG = oligodendroglioma; ANA ODG = anaplastic oligodendroglioma;

ANA ASTRO = anaplastic astrocytoma; GBL = glioblastoma.

ported in detail (13). The main problem was the precise assessment of tumour volume. When dealing with a solid lesion with clearly defined edges, the lesion was easily measured by CT scan. However, if the patient was bearing a postoperative cavity, without radiological sign of neoplastic lesion, tumour volume was difficult to measure, and its shape was considered as a slim area with a depth of less than 1.5 cm located around the site of the primary lesion. In all cases the dose delivered to both the entire surgical hole and its rim, in which most of the occult malignant glioma cells are embodied, was calculated. Nevertheless, by these external computations, dosimetry variations within different areas of tumour mass (microdosimetry) could not be estimated. The assessment of the dosimetry was carried out before every LR-RIT, to confirm the behaviour of MAbs on the course of time and the possible changes caused by prior LR-RIT (necrosis) or by human anti-murine antibody (HAMA) (14).

#### Repeated courses of LR-RIT

As a rule, the patients underwent multiple, repeated administrations of monoclonal antibodies aimed at maximizing the neoplastic cytolysis (15). The first three cycles were given every 30–60 days and further applications were performed after 4–6 months with the same intervals. Twenty-four patient had three cycles, 18 were given four courses, 10 received five cycles and 6 patients were given six treatments. The patients who received 6 IL-RIT infusions had a radioiodine cumulative dose of 10345.2 MBq (mean).

## RESULTS

#### Adverse effects

No systemic, haematological, hepatic or renal side effects occurred as a result of LR-RIT. The I-131 MTD resulted in 2590 MBq but larger doses produced serious brain oedema. By contrast, normal cerebral tissue was not markedly damaged when doses below the MTD were given, as demonstrated by the lack of radiological signs of brain impairment. The complete sparing of normal CNS parenchyma was also assessed by external scintigraphy and

by dosimetry calculations (16). A transient, mild headache was recorded in 15% of our patients but it cleared up in few hours with NSAIDs. An expanded lesion arising from the original tumour area was demonstrated by CT scan in 17 patients and was believed to represent a PD. For this reason, further surgery was carried out and large areas of necrosis were found, neoplastic cells were not detectable in three cases and present in low numbers in the remaining ones. The appearance of HAMA was observed in 59% of the patients; their median titre was 1/64, ranging between 1/1 and 1/1024. Highest HAMA values were measured in patients who received more than three courses of RIT. Nevertheless, HAMA production did not modify the pharmacokinetics of MAbs, which was always evaluated by means of sequential scintigraphies, when their administration was repeated (17).

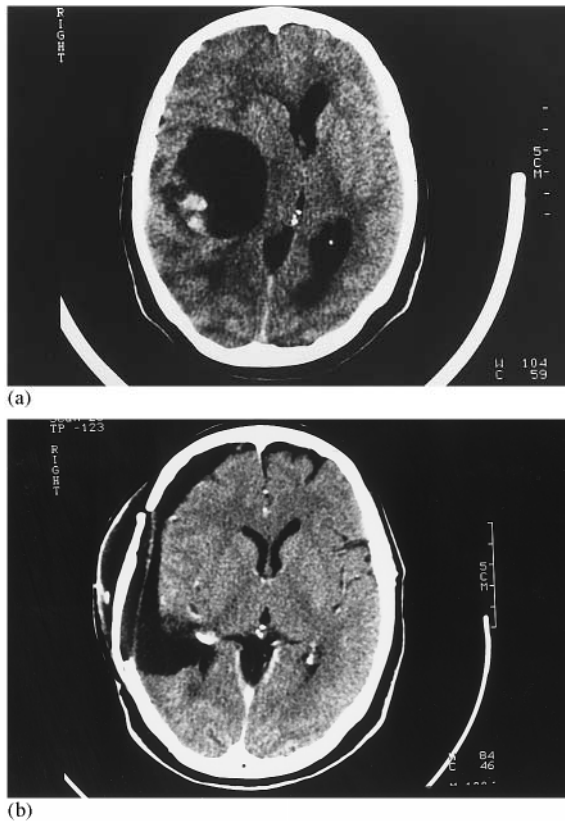
#### Biodistribution and dosimetry (phases I and II)

The pharmacokinetics and dosimetry data were commensurate in both the phase I and phase II studies. The average percentage of injected dose accumulated per gram of tumour was high, and, on average, resulted in 3.1% (range 0.18–7.2%) after 24 h. The residence time of the MAbs in the neoplastic tissue was remarkably long: a mean effective half-life of 57.1 h (range 17–166) was calculated. These biodistribution parameters did not change during subsequent courses. The radiation dose to the whole cavity and to its wall was high and the mean value per cycle exceeded 300 Gy (range 180–350) and 150 Gy (range 80–190), respectively. The liver, kidneys and bone marrow received a cumulative radiation dose of less than 0.5 Gy, while the radiation dose delivered to the thyroid did not exceed 4 Gy. Three different biodistribution patterns were recorded: a) diffusion into a postoperative cavity. In this case the radiopharmaceutical homogeneously spread throughout this space and gave rise to a scintigraphic image resulting in an intense hot spot (see Fig. 1); b) diffusion throughout a solid mass. In this situation the immunoglobulins were distributed, within 4–8 days, from the spot of injection inside the whole neoplastic mass which, in the end, was completely targeted; c) diffusion into the CSF. This happened when a

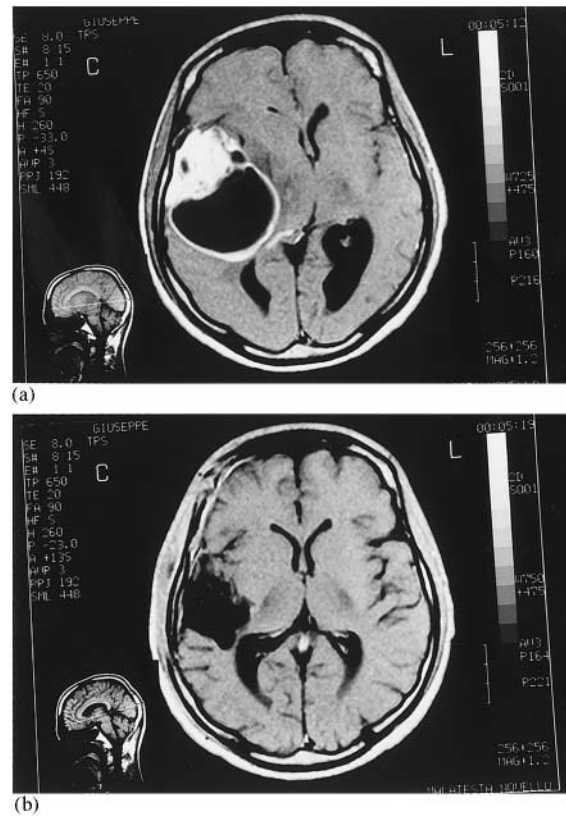
communication between the surgical cavity and the ventricles or cisterns was produced by operation procedures. In this instance part of the radiopharmaceutical circulated in the CSF and was then absorbed in the blood. Nevertheless, the greatest quantity of MABs was concentrated in the lesion and its accumulation increased with time (Fig. 2).

#### Clinical responses (phase II patients)

*Time disease-free until the relapse.* Thirty-one out of 38 cases with newly diagnosed GBL, who were previously treated with multiple courses of LR-RIT, presented a tumour relapse which occurred, on median, within 12



*Fig. 4.* A. This patient (female, 51 years old) had a glioblastoma in the right temporal lobe. The lesion was surgically removed on March 1996. This CT scan shows the primary tumour before the operation: a huge cystic lesion which presents, on the first external edge, two solid neoplastic masses. The median line is shifted left; the right lateral ventricle is constricted. This image gives clear evidence of the volume and the expansion of the initial lesion: on the basis of its size and its ominous histology, a recurrence in a few months could be anticipated. B. After surgery, the patient underwent external radiotherapy, followed by four LR-RIT treatments in the course of 12 months. Twenty-five months after diagnosis and 24 months after the first LR-RIT cycle, the patient is in good clinical condition and without any radiological indication of relapsing glioblastoma. This image concerns her latest CT scan obtained in February 1998. The postoperative cavity is well delineated; there is no evidence of any enhanced area. The internal tip of the indwelling catheter is imaged.



*Fig. 5.* A. A 38-year-old man, with glioblastoma in the right temporal lobe was submitted for surgery on July 1995. This MRI, performed before the operation, displays a remarkably bulky mass, partially cystic but with an extended solid area. There is radiological evidence of neoplastic infiltration of the neighbouring tissues. The space-occupying effect of the tumour is evident. This tumour could be destined to a rapid progression despite customary treatments which are unable to modify its grave, natural history. B. Following surgery, external radiotherapy was given and, afterwards, the patient also received chemotherapy. He had the first IL-RIT courses on November 1995. Later, he was given two further antibody infusions in January 1996 and March 1996. The I-131 cumulative dose was 6023.6 MBq. The patient is still disease-free, as shown in this latest MRI examination (June 1998), 33 months after diagnosis and 29 months after the beginning of LR-RIT.

months. In contrast, in 36 patients with glioblastoma and the same clinical features as former groups, who were referred to our department with a recurrent tumour following traditional regimens, the relapse took place within 5 months. The patients with less aggressive malignant glioma (1 patient with ND ODG, 2 with ND anaplastic ODG, and 7 with ND AA) who had prior infusions of radioactive antibodies and who later relapsed had a better behaviour in comparison with those (5 with anaplastic ODG, and 2 with anaplastic astrocytoma) in whom LR-RIT was given in a recurrent stage following traditional regimens. Nevertheless, their number is quite restricted, so these data cannot be considered statistically significant. In particular, so far, 1 ND oligodendroglioma does not

**Table 4***Objective response related to the histology, the pathological status and the clinical course of the disease*

Response and histology	Small	Bulky	ND small	ND bulky	REC small	REC bulky
PD (A ODG)	1	3	1	1	–	2
PD (A A)	–	1	–	1	–	–
PD (GBL)	8	19	2	9	6	10
SD (GBL)	6	4	4	1	2	3
PR (GBL)	4	5	2	1	2	4
CR (A ODG)	–	2	–	–	–	2
CR (GBL)	1	–	1	–	–	–
NED (A ODG)	1	–	–	–	1	–
NED (A A)	4	–	4	–	–	–
NED (GBL)	23	–	17	–	6	–

Abbreviations: PD = progressive disease; SD = stable disease; PR = partial remission; CR = complete remission; NE = not evidence of disease; s = small lesion (tumour volume  $<2.5 \text{ cm}^3$ , at the time of RL-RIT); b = bulky lesion (tumour volume  $>3 \text{ cm}^3$ , at the time of RL-RIT); ND = newly diagnosed tumour; REC = recurrent tumour, following customary treatments; ODG = oligodendroglioma; A ODG = anaplastic oligodendroglioma; A A = anaplastic astrocytoma; GBL = glioblastoma.

present recurrence after 31 months; 5 REC anaplastic oligodendrogliomas patients are free of disease after 36 months. Finally, 7 ND anaplastic astrocytomas patients remain without sign of relapsing tumour after 25 months.

*Median survival.* The median survival of patients was actually prolonged in comparison to the result so far achieved by means of standard regimens. Survival was 31 months in ODG (1 patient), 23 months in anaplastic ODG,  $>46$  months in anaplastic astrocytoma and 19 months in GBL. But in this last group, when the lesion submitted to LR-RIT was small or minimal, it was 25 months in cases with newly diagnosed GBL and 21 months in recurrent tumours (Fig. 3A–D).

*Objective response (Table 3).* In patients with ODG we observed 1 NED. In anaplastic ODG we have 4 PD, 2 CR, and 1 NED. In anaplastic astrocytoma we recorded 1 PD, 4 NED and, finally, in GBL we registered 27 PD, 10 SD, 9 PR, 1 CR and 23 NED (Figs. 4 and 5). Nevertheless, remarkable distinctions were encountered between cases with macroscopic or minimal lesions and, to a lesser extent, among patients with newly diagnosed or recurrent diseases (see Table 4).

*Response rate.* The global response rate of different histological groups was 100% in ODG patients, 42.8% in anaplastic ODG, 80% in anaplastic astrocytoma, and 47.2% in GBL patients. To give a more detailed account of this last subset of patients: the response rate was 66.6% in patients who had LR-RIT when their tumour burden was small ( $<2 \text{ cm}^3$ ), whilst the rate was 17.8% in those patients with large lesions ( $>3 \text{ cm}^3$ ) when they received radioactive antibodies. At the same time, this parameter was better in ND tumours (56.7%) (76.9% in small lesions and 9% in bulkier masses) in comparison with REC diseases (36.3%) (50% in small lesions and 23.5% in bulkier masses).

*Recurrences.* Seventy-three patients (5 anaplastic ODG, 3 anaplastic astro and 65 GBL) presented a recurrence of the tumour following one or more courses of LR-RIT. In 61 patients (83.5%) the malignant regrowing tissue was found in the bed of the primary lesion, while in the remaining 12 cases (16.4%) the new disease appeared in distant territories, whilst the local disease was kept under control with therapy.

*Reoperations.* Nineteen patients (1 A A and 18 GBL) who suffered a relapse after the antibodies infusion underwent a further operation, which had an apparently radical outcome in 5 patients. Eleven of the 19 patients were submitted to subsequent radioimmunotherapy cycles. The ANA Astro patient is still alive and apparently disease-free after 61 months. The median survival of this subgroup of GBL patients is 19 months.

#### Long surviving

A not negligible number of patients survived for a meaningful period of time. Three out of 7 cases with anaplastic oligodendroglioma survived 7 years from diagnosis and 2

**Table 5***Long surviving patients (still alive)*

Years	A ODG	A A	GBL
1	n.3 (42.8%)	n.4 (44.4%)	n.12 (16.2%)
2	n.3 (42.8%)	n.4 (44.4%)	n.7 (9.4%)
3	n.3 (42.8%)	n.3 (33.3%)	n.2 (2.7%)
4	n.3 (42.8%)	n.2 (22.2%)	n.2 (2.7%)
5	n.3 (42.8%)	n.1 (11.1%)	–
6	n.3 (42.8%)	–	–
7	n.3 (42.8%)	–	–
8	n.2 (28.5%)	–	–
9	n.2 (28.5%)	–	–

Abbreviations: A ODG = anaplastic oligodendroglioma; A A = anaplastic astrocytoma; GBL = glioblastoma.

are still alive after 9 years. Moreover, 3 out of 9 anaplastic astrocytoma patients survived for 3 years and one of them is still alive after 5 years. Finally, 7 out of 74 glioblastoma cases survived for 2 years and 2 are still alive and in good clinical condition after 4 years (Table 5).

#### Quality of life

The symptomatology and the quality of life of the patients were not prospectively recorded. However, our impression was that the patients' well-being improved, even though the treatment did not always control the course of the disease. Symptoms like headache, nausea and vomiting often improved. The amount of steroids was also reduced in many cases; all 28 patients who experienced a NED response completely discontinued steroids. The remaining patients continued the medication but in half of the cases with reduced doses.

#### DISCUSSION

We observed minimal toxicity during our IL-RIT regimen thus repeated treatments were possible allowing prolongation of the time of tumour remission or, in favourable cases, complete eradication of the neoplastic tissue. The major potentially critical normal organs (liver, kidneys and bone marrow) received a cumulative radiation dose of less than 0.5 Gy. Although HAMA production was recorded in many cases, no side effects occurred. No evidence could be found to support the hypothesis that anti-idiotypic HAMA could trigger an antitumour effect by suppressing the tumour growth directly or by activating the patient's immunoresponse via induction of a third antibody (18). The intralesional approach led to a remarkable accretion of specific MAbs in the neoplastic tissue, as demonstrated by examples of dosimetry data. Thus IL-RIT proved to be capable of circumventing the high interstitial pressure inside the neoplastic tissue as well as the poor blood supply to the tumour. Moreover, the residence time of radiopharmaceutical in the lesion was prolonged. This was due to a reduction of both deiodinating and catabolic phenomena of I-131 conjugated MAbs, which are always present when the immunoglobulins are intravenously injected.

The MAbs used demonstrated high immunoreactivity, i.e. the ability of antibodies to react with their specific antigens. This binding capacity could be reduced after labelling owing to steric variations following the radiochemical procedures. The 65% immunoreactivity represents a positive result, as methods evaluating the immunoreactivity do not have a sufficient sensitivity and they tend to underestimate the binding capacity of the immunoreagent.

All these factors explain the high radiation dose delivered to neoplastic tissue either by a single or by multiple IL-RIT courses, and, as a consequence, the possibility of achieving promising clinical effects. A widely used param-

eter to estimate the effectiveness of a specific treatment in malignant gliomas is the median survival time. The ability of a new treatment to prolong patients' survival is the most important indicator of efficacy. In our study, the median survival of the whole group of GBL patients was 19 months, which is substantially longer than the 12 months reported to be achievable by means of conventional types of therapy in these patients. Nevertheless, this value was 25 months in 27 ND patients and 21 months in 16 REC cases in whom LR-RIT was given when their lesions were small. In the literature, after intravenous RIT using I-131-labelled MAbs against epidermal growth factor receptor in brain tumours, a median survival of 15.6 months has been reported (19). In a recent intratumoral RIT trial using yttrium-90 labelled MAbs against neural cell adhesion molecule, preliminary dosimetric results in 15 patients with recurrent gliomas have been reported (20): in their heterogeneous patient population, median survival was only 6 months. Thus, our results are an improvement and the number of cases we treated is much larger for conclusions.

Most of our phase II patients had unfavourable characteristics for life expectancy and prognosis, as there were diagnoses of glioblastoma multiforme in 74 patients, a recurrence in 36 patients and a bulky large tumour in 31 patients (Table 2). Objective responses were recorded in some cases confirming the efficiency of our therapy: 33 out of 70 evaluable GBL patients experienced favourable outcomes (1 CR, 23 NED, 9 PR) and most of them have been free of disease for at least 20 months, and still are. We think that this therapeutic approach has promise in controlling malignant gliomas. The comparison of the different clinical and pathological features of the patients indicates that the determining factor for a positive response to RIT is the small volume of the disease (21). The response rate was 17.8% in GBL patients with a macroscopic lesions. By contrast, when the disease was small or not discovered at all by means of radiological tools, the response rate was 66.6%. It is worth pointing out that since malignant gliomas are always at high risk of relapse, a small or microscopic lesion cannot be regarded as an unimportant disease. Even when both surgical and external radiotherapy is successfully performed, many neoplastic cells have already spread beyond the rim of glioma mass through a narrow zone, and when the volume of the primary lesion is more than 2 cm<sup>3</sup>, a recurrence can be anticipated in this area. Thus when a small enhancing lesion disappears or the tumour does not reoccur until after many months following LR-RIT, the effectiveness of the therapy is demonstrated. In this respect, the patients who underwent LR-RIT without any radiological signs of neoplastic lesions and remained disease-free for a long time (NED result) represent a very successful outcome of this therapeutic application (22).

There were still patients who had a tumour recurrence following a PR or CR of RL-RIT, despite the large radiation dose delivered to the neoplastic tissue. This is perhaps due to the inability of radiolabelled MABs to reach neoplastic cells distant from the main lesion—owing to either histological barriers, e.g. haemorrhage or necrosis—in order to hinder the diffusion, or to the short range of the I-131 radiation not allowing effects beyond 1 mm of tissue.

We conclude that LR-RIT can produce favourable effects and can be used safely as a clinical therapy. The symptomatology improved even if the treatment did not succeed in controlling the disease. The study has now been concluded and the next clinical trial has begun with the use of a different isotope, Y-90, which penetrates deeper into tissue. In theory, Y-90 can reach more distant neoplastic cell clusters, and we hope to see more patients with longer survival. The current outcome of our nine years' study suggests that the IL-RIT approach has the potential to improve the control of malignant gliomas.

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#### REFERENCES

- Loeffler JS, Shrieve DC, Coleman CN. Chemoradiation and adjuvant chemotherapy: why does so much therapy yield so little improvement in survival? *Int J Radiat Oncol Biol Phys* 1995; 33: 531–3.
- Jeremic B, Antunovic V, Djuric L, Stojanovic M, Shibamoto Y. Influence of the extent of surgery and tumor location on treatment outcome of patients with glioblastoma multiforme treated with combined modality approach. *J Neurooncol* 1994; 21: 177–85.
- Jubelirer SJ. A review of the treatment and survival rates of 138 patients with glioblastoma multiforme. *W V Med J* 1996; 92: 186–90.
- Westlin JE, Snook D, Nilsson S, et al. Intravenous and intratumoural therapy of patients with malignant gliomas with <sup>90</sup>Yttrium labeled monoclonal antibody MUC 2-63. In: Epenetos A, ed. *Monoclonal antibodies: applications in clinical oncology*. London, New York: Chapman and Hall Medica, 1992: 17–25.
- Papanastassiou V, Pizer BL, Coakham HB, et al. Treatment of recurrent and cystic malignant glioma by a single intracavitary injection of I-131 monoclonal antibody: feasibility, pharmacokinetics and dosimetry. *Br J Cancer* 1993; 67: 144–51.
- Riva P, Arista A, Sturiale C, et al. Treatment of intracranial human glioblastoma by direct intratumoral administration of <sup>131</sup>I-labelled anti-tenascin monoclonal antibody. *Int J Cancer* 1992; 51: 7–13.
- Leprini A, Querzè G, Zardi L. Tenascin isoforms: possible targets for diagnosis and therapy of cancer and mechanisms regulating their expression. *Perspect Dev Neurobiol* 1994; 2: 117–23.
- Lee YS, Bullard D, Humprey PA, et al. Treatment of intracranial human glioma xenografts with <sup>131</sup>I-labeled anti-tenascin monoclonal antibody 81C6. *Cancer Res* 1988; 48: 2904–10.
- Zalutsky MR, Moseley RP, Coakham HB, Coleman RE, Bigner DD. Pharmacokinetics and tumor localization of <sup>131</sup>I-labeled anti-tenascin monoclonal antibody 81C6 in patients with gliomas and other intracranial malignancies. *Cancer Res* 1989; 49: 2807–13.
- Arista A, Sturiale C, Riva P, et al. Intralesional administration of I-131 labelled monoclonal antibodies in the treatment of malignant gliomas. *Acta Neurochir* 1995; 135: 159–62.
- P. Riva, A. Arista, C. Sturiale, et al. Intralesional therapy of glioma. In: *New antibody technology and the emergence of useful cancer therapy*. Begent R, Hamblin A, eds. London: The Royal Society of Medicine Press, 1995: 23–6.
- Kaplan EI, Maier P. Non parametric estimation from incomplete observations. *J Am Stat Assoc* 1958; 53: 457–81.
- Riva P, Arista A, Tison V, et al. Intralesional radioimmunotherapy of malignant glioma: an effective treatment in recurrent tumors. *Cancer* 1994; 73 (Suppl): 1076–82.
- Muto MG, Finkler NJ, Kassis AI, Lepisto EM, Knapp RC. Human anti-murine antibody responses in ovarian cancer patients undergoing radioimmunotherapy with the murine monoclonal antibody OC-125. *Gynecol Oncol* 1990; 38: 244–8.
- Sturiale C, Arista A, Lazzari S, et al. Intralesional radioimmunotherapy of malignant gliomas: clinical experiences with recurrent and primary tumours. *Tumor Targeting* 1995; 1: 163–76.
- P. Riva, A. Arista, C. Sturiale, et al. Radioimmunotherapy of CNS malignant gliomas by direct intralesional injection of specific I-131 radiolabeled monoclonal antibodies. In: *Cancer therapy with radiolabeled antibodies*. Goldenberg DM, ed. Boca Raton, Ann Arbor, London, Tokyo: CRC Press, 1995: 203–16.
- Riva P, Arista A, Sturiale C, et al. Glioblastoma therapy by direct intralesional administration of I-131 radioiodine labeled anti-tenascin antibodies. *Cell Biophysics* 1994; 24/25: 37–43.
- Baum RP, Niesen A, Hertel A, et al. Activating anti-idiotypic human anti-mouse antibodies for immunotherapy of ovarian carcinoma. *Cancer* 1994; 73 (Suppl): 1121–5.
- Brady LW, Miyamoto C, Woo DV, et al. Malignant astrocytomas treated with iodine-125 labeled monoclonal antibody 425 against epidermal growth factor receptor: a phase II trial. *Int J Radiat Oncol Biol Phys* 1992; 22: 225–30.
- Hopkins K, Chandler C, Bullimore J, et al. A pilot study of the treatment of patients with recurrent malignant gliomas with intratumoral yttrium-90 radioimmunoconjugates. *Radiother Oncol* 1995; 34: 121–31.
- Riva P, Arista A, Franceschi G, et al. Local treatment of malignant gliomas by direct infusion of specific monoclonal antibodies labelled with <sup>131</sup>I. Comparison of the results obtained in recurrent and newly diagnosed tumours. *Cancer Res* 1995; 55: 5952s–6s.
- Riva PG, Franceschi G, Arista A, et al. Local application of radiolabeled MABs for the treatment of high grade malignant gliomas: a six year clinical experience. *Cancer* 1997; 80 (Suppl): 2733–42.