Eight-year survival of a recurrent glioblastoma patient treated with molecularly tailored therapy: a case report

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Abstract
Treatment options for recurrent glioblastoma are scarce; targeted therapy trials were disappointing, probably due to enrollment of patients without molecular selection. We treated with bevacizumab and erlotinib a 66-year-old male suffering from recurrent glioblastoma, IDH-wildtype and MGMT unmethylated, after three neurosurgeries. Treatment was tailored on molecular profile of recurrent tumor—namely, EGFRvIII positivity, VEGF overexpression, normal PTEN, low total VEGF and VEGF-121 mRNA—and resulted in complete, exceptionally durable response (51-month progression-free survival). Notably, histology of further recurrence after therapy was reminiscent of sarcoma. We suggest a thorough molecular screening for personalization of targeted therapy in recurrent glioblastoma.

Keywords Glioblastoma · Targeted therapy · Tailored therapy · Bevacizumab · Erlotinib

Introduction
Recurrent IDH-wildtype glioblastoma (GBM) after standard-of-care surgery and chemoradiation carries a particularly unfavorable prognosis, with a median overall survival of approximately 9 months [21]. Targeted therapies using inhibitors of vascular endothelial growth factor (VEGF) including bevacizumab, and inhibitors of epidermal growth factor receptor (EGFR) pathway including erlotinib, have yielded disappointing results in trials [10, 14, 21] despite exciting preclinical and early clinical data [5, 8]. However, trials have been conducted on unselected populations of recurrent GBMs, whereas ideally a targeted drug should be administered only to molecularly selected GBM patients expressing specific targets [19]. In previous papers from our group, we showed that (a) bevacizumab is effective in recurrent GBM with immunohistochemical overexpression of VEGF [3] and low expression of the diffusible VEGF isoform, VEGF-121 [2] and (b) erlotinib is effective in GBMs expressing EGFRvIII, a constitutively activated EGFR variant [3].

In the present paper, we report on a patient suffering from recurrent GBM who was treated with bevacizumab and erlotinib tailored to the molecular profile of the tumor. This patient experienced an exceptionally long survival, the longest reported in literature for a recurrent GBM.
Case report

Standard treatment

A 66-year-old male farmer was operated at our Department in February 2008 for gross total removal of a 5-cm right temporal GBM, IDH-wildtype (Fig. 1). Molecular analyses showed unmethylated MGMT promoter and expression of EGFRvIII. Post-operative course was uneventful and adjuvant chemoradiotherapy according to Stupp protocol was given. The MR scan performed after 2 cycles of temozolomide showed a local tumor recurrence, for which gross total resection was performed (September 2008). The patient then completed adjuvant temozolomide (12 cycles overall). One year after the second surgery, local recurrence involving the temporal uncus was diagnosed. Again, the patient was operated on for tumor removal (October 2009). Post-operative course was characterized by a left leg deep venous thrombosis, recovered with transient anticoagulation.

Histological examination of the specimen obtained at the third procedure confirmed the diagnosis of GBM. Molecular testing showed expression of EGFRvIII, normal PTEN expression, and VEGF overexpression [3, 9]. Semiquantitative RT-PCR for mRNA of VEGF isoforms [2] showed low values of total VEGF and VEGF-121 (total VEGF, 5,408; VEGF-121, 5655 × 10^{-5}; VEGF-121/total VEGF ratio, 1046 × 10^{-3}%). Two-month post-operative scan showed a further tumor recurrence close to the surgical field (Fig. 2(a)).

Experimental treatment

In January 2010, based on the molecular profile of recurrent tumor, we started treatment with bevacizumab (10 mg/kg iv every 2 weeks in 6-week cycles) plus erlotinib (300 mg/day orally; the patient was taking phenobarbital for seizure prophylaxis) [2, 3]. A brain MR scan was scheduled at the end of every cycle. Treatment was well tolerated (a grade 2 rash was treated using topical emollients). Brain MR scan after the first two therapy cycles showed a complete response [20] (Fig. 2(b)); treatment was therefore continued. By the end of 2012, i.e., 3 years after treatment start, the patient had completed 12 cycles of therapy. Clinically, he was alert and able to walk with minimal assistance. Brain MR scan was persistently negative for any sign of recurrent disease (Fig. 2(c)). We thus decided to loosen the therapeutic schedule, administering bevacizumab every month for the following 6 months and then every 2 months. Moreover, due to the onset of blepharitis, erlotinib was tapered to 100 mg/day (phenobarbital had been replaced with levetiracetam). Brain MR scans every 4 months were performed.

Recurrence

Despite the lack of neurological worsening, brain MR scan performed in April 2014 (51 months after treatment start) showed a hemorrhage in the previous surgical field and a new lesion on the floor of the middle cranial fossa, consistent with disease progression. We therefore decided to resume

Fig. 1 Histological appearance of the GBM at diagnosis. a H&E staining (× 10 magnification) showing the hallmarks of glioblastoma (high cellularity, pleomorphism, microvascular proliferation, pseudopalisading necrosis). b–d On immunohistochemistry (× 20 magnification), tumor cells display strong positivity for GFAP (b) and lack IDH1R132H expression (c). Mitotic index (Ki-67) is 40–50% (d)
bevacizumab with the initial schedule continuing erlotinib. Follow-up MR showed a stable disease until December 2014 when progression was noticed (Fig. 2(d)). In January 2015, 5 years after the start of therapy, the patient underwent further neurosurgery. The tumor appeared as a firm, fibrous, sarcoma-like lesion infiltrating the right temporal lobe and extending to the dura and bone of middle cranial fossa. Due to bony infiltration, the tumor was sub-totally removed. Postoperative course was characterized by a left jugular vein thrombosis without clinical sequelaes. At discharge, the patient was alert, oriented, with no focal neurological deficits; however, he experienced a profound fatigue and needed assistance for activities of daily life. Histologically, the recurrent tumor after bevacizumab was reminiscent of sarcoma (Fig. 3).

Fig. 2 Radiological timeline of recurrent tumor and experimental treatment. (a) Post-Gd T1-weighted (upper) and FLAIR (lower) axial images 2 months after neurosurgery performed in October 2009. The contrast-enhancing mass close to the surgical field, with an irregularly hyperintense FLAIR appearance, is consistent with recurrent tumor. (b) Post-Gd T1-weighted (upper) and FLAIR (lower) axial scans after two cycles of bevacizumab plus erlotinib, showing disappearance of contrast enhancement and reduction of nonenhancing alterations. Since the patient was well without steroids, this picture is consistent with complete response. (c) Post-Gd T1-weighted (upper) and FLAIR (lower) axial scans after 12 cycles of bevacizumab plus erlotinib, showing persistent complete response. (d) Post-Gd T1-weighted axial (upper left) and coronal (upper right), FLAIR axial (lower left) and T2-weighted coronal (lower right) scans of MR performed in December 2014, showing infiltrative recurrent tumor involving temporal lobe and the dura of middle cranial fossa floor, and extending into petrous bone: picture consistent with progressive disease.

Fig. 3 Histological appearance of recurrent GBM after experimental treatment with bevacizumab and erlotinib. a H&E staining (× 100 magnification). The tumor shows a sarcomatous component consisting of fascicles of spindle cells separating nodules of round tumor cells with moderate amounts of surrounding cytoplasm. Moreover, a greater number of hyalinized vessel walls was noticed. Interestingly, we found less necrotic areas and inflammatory infiltrate than in the original tumor. b Magnified picture (× 200) of a hyalinized tumor vessel as an effect of prolonged bevacizumab treatment, surrounded by GBM sarcomatous-like cells. c Immunohistochemical staining with anti-VEGF antibody (× 200 magnification) showing diffuse VEGF expression.
Therapy effects, consisting mainly of hyalinized vessel walls (Fig. 3b), were prominent. VEGF immunostaining was strongly positive (Fig. 3c); mitotic index (Ki-67) was 10%. Moreover, the tumor retained EGFRvIII expression.

**Salvage treatment**

Two months after surgery, clinical conditions had slightly improved. Post-operative MR confirmed the subtotal removal. In order to preserve the patient’s quality of life, and based on the molecular profile of the recurrent tumor, we opted for a re-challenge with bevacizumab and erlotinib, that the patient had well tolerated, with an adapted schedule (bevacizumab 10 mg/kg every month plus erlotinib 100 mg/day). Five bevacizumab administrations could be performed, resulting in a stable disease on follow-up MR. However, eventually, the patient developed an acute kidney failure requiring hospitalization. We therefore decided to stop every aggressive treatment. The patient died for complications of renal failure, almost 8 years after diagnosis and 6 years after bevacizumab and erlotinib start.

**Discussion**

The main findings of this report can be summarized as follows: (a) the extremely long survival of a patient harboring a recurrent, IDH-wildtype GBM treated with a tailored therapy using bevacizumab and erlotinib; (b) the importance to obtain a detailed molecular profile of the recurrent tumor; (c) the temporal link between tapering of bevacizumab therapy and tumor recurrence; and (d) the infiltrating, sarcomatous-like pattern of the recurrent tumor.

To the best of our knowledge, the case here described has the longest PFS (51 months) and OS (71 months) from treatment start among recurrent GBM patients. In fact, though young patients harboring GBM with hypermethylated MGMT promoter who survived 20 years since diagnosis are known [6, 16], we were unable to find cases of recurrent GBM with such long survival [15, 17, 21]. The prolonged survival of our patient is thus hard to explain [21], mostly for an IDH-wildtype recurrent GBM with unmethylated MGMT promoter. One possible explanation is that the patient’s tumor profile—VEGF overexpression, EGFRvIII expression, normal PTEN expression, low expression of mRNA of total VEGF and VEGF-121 isoform—rendered it sensitive to the targeted therapy [2, 3, 8]. Consistent with this assumption, when treatment schedule was loosened, tumor recurred. Noteworthy, EGFRvIII expression was retained by the patient’s tumor throughout the clinical history. GBMs that are EGFRvIII positive at diagnosis have been reported to lose EGFRvIII expression after standard chemoradiation in about 60% of cases [18], and this loss has been related to a worsened prognosis [9].

There is a remarkable paucity of data on the predictive factors for response to targeted therapies in GBM [1, 4, 7, 8, 13, 21] and biomarker analysis has been usually conducted on GBMs at the time of the initial diagnosis, whereas treatments have been delivered on recurrent GBMs [14]. Such evidence reinforces the need to obtain a pathological sample of the recurrent tumor for a reliable molecular analysis, which can have both prognostic and therapeutic implications.

Notably, histological appearance of recurrent tumor after tailored therapy differed from the classic GBM and was reminiscent of sarcoma, with a marked tendency to infiltrate the dura and the bone of the cranial base. This event is consistent with the preclinical evidence of a mesenchymal shift as a key mechanism of escape to anti-angiogenic therapy [11, 12].

In conclusion, the present case suggests that in recurrent GBM, a thorough molecular screening, performed on a tumor sample taken at surgery for recurrence, can identify patients who are more likely to benefit from targeted therapies.

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**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

**Informed consent** Informed consent was obtained from all individual participants included in the study.

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