

Patterns of response to crizotinib in recurrent glioblastoma according to ALK and MET molecular profile in two patients

Emilie Le Rhun^{*1,2,3}, Marc C Chamberlain⁴, Fahed Zairi^{5,3}, Christine Delmaire⁶, Ahmed Idbaih^{7,8}, Florence Renaud^{9,10,11}, Claude Alain Maurage^{9,10,11} & Valérie Grégoire^{9,10,11}

PRACTICE POINTS

- The prognostic of glioblastoma remains limited.
- Novel targeted therapies have been recently developed for the treatment of glioblastoma.
- C-MET overexpression and MET gain are frequently detected in glioblastoma but c-MET gene amplification is rare, observed in only 5% of all glioblastoma.
- Anaplastic lymphoma kinase (ALK) overexpression and gain or amplification are seen in 18–48% of glioblastoma.
- Two patients with ALK or c-Met expression but no ROS1 expression, unmethylated MGMT promoter recurrent glioblastoma were treated with crizotinib. To one of both, a prolonged stabilization of disease was observed after initiation of crizotinib.
- These case studies suggest that patients with glioblastoma and ALK polysomy may derive clinically relevant benefit from novel targeted small molecular inhibitors such as crizotinib.

Two patients with an unmethylated *MGMT* promoter and IDH1 (R132H) wild-type recurrent glioblastoma were treated with crizotinib. Prolonged stabilization of the disease (17 months) was achieved in the first case. Interestingly, anaplastic lymphoma kinase (ALK) expression and c-MET protein overexpression was observed. Conversely, no response to crizotinib was obtained in the second case with MET protein overexpression and *c-MET* amplification but no *ALK* expression or *ALK* gene amplification. These case studies suggest that novel targeted ALK inhibitors may provide relevant clinical benefit in selected cases in which driver mutations are demonstrable.

¹Neuro-Oncology, Neurosurgery Department, University Hospital – CHRU Lille, France

²Neurology, Medical Oncology Department, Oscar Lambret Center, Lille, France

³Inserm, U1192, Lille, France

⁴Neurology & Neurological Surgery, University of Washington, Fred Hutchinson Research Cancer Center, Seattle, WA 98109, USA

⁵Neurosurgery Department, University Hospital – CHRU Lille, France

⁶Neuroradiology Department, University Hospital – CHRU Lille, France

⁷AP-HP, Groupe Hospitalier Pitié-Salpêtrière, Service de neurologie 2-Mazarin; Sorbonne Universités, UPMC Univ Paris 06, UM 75,

⁸Inserm, U 1127, CNRS, UMR 7225, ICM, F-75013 Paris, France

⁹Neuropathology Department, University Hospital – CHRU Lille, France

¹⁰Lille University, Lille, France

¹¹UMR-S, 1172 F-59000 Lille, France

*Author for correspondence: Tel.: +33 320 446 542; Fax: +33 320 446 555; emilie.lerhun@chru-lille.fr

KEYWORDS

- ALK • crizotinib
- glioblastoma • MET amplification • MET
- targeted therapy

Case report

A 39-year-old man was presented with progressive headaches and vomiting and was found by brain MRI to have a left frontal intra-axial mass. A gross total tumor resection was performed. Histopathology and immunohistochemistry revealed a giant cell glioblastoma (GB) with p53 expression and without IDH1 mutated R132H expression. The *MGMT* gene promoter was nonmethylated.

The patient was treated with conventional radiotherapy, concomitant and adjuvant temozolomide (TMZ) for 6 cycles. Following completion of the 6th cycle of post-RT TMZ, the patient manifested recurrent disease by MRI only.

Single agent bevacizumab was initiated and administered until a second asymptomatic radiographic disease recurrence 10 months later. A second salvage therapy including fotemustine and bevacizumab was started. Three months subsequently, a third asymptomatic disease recurrence was observed. Treatment was changed to lomustine plus bevacizumab. A fourth asymptomatic recurrence was seen 5 months later, for which the patient received carboplatin and bevacizumab. However, subsequently the patient was presented with a rapid and severe clinical deterioration leading to an ECOG-Performance Status of 3 (previously 0) in a few weeks. Clear radiographic disease progression was evident by both T1 postgadolinium and T2/FLAIR MRI sequences (Figure 1). Methylprednisolone was introduced at 100 mg per day.

Additional molecular analyses of the original tumor were then performed and demonstrated weak expression of anaplastic lymphoma kinase (ALK) protein in 25% of the tissue as well as polysomy of chromosome 2 (*ALK* locus) in 53% of neoplastic nuclei. MET or HGFR (HGF Receptor) analysis showed weak to moderate expression of protein in 70 and 20% of the tumor cells, respectively. Polysomy of chromosome 7 (*c-MET* locus) was revealed by FISH in 84.5% of tumor nuclei wherein 43% of nuclei demonstrated five or more *MET* copies and 36% of nuclei had six or more copies. The ratio gene/centromere was equal to one. ROS1 expression was not observed, and no amplification or gain of gene was observed by FISH (Figure 2). The *V600E BRAF* mutation was not observed (Table 1).

Bevacizumab was therefore discontinued and crizotinib (250 mg twice daily) was initiated after discussion with the patient and his family and after obtaining written consent.

Following 2 months of crizotinib therapy, clinical improvement was observed and the steroid dose could be reduced (prednisone 20 mg per day). Brain MRI showed stable disease. Adverse events ascribed to crizotinib were observed manifested as grade 3 thrombocytopenia and hepatotoxicity and required an interruption of crizotinib. Prednisone was maintained at 20 mg per day during this period. After a 4-month-cessation therapy, toxicities resolved and crizotinib (250 mg/day) was then reinitiated, after discussion with the patient and his

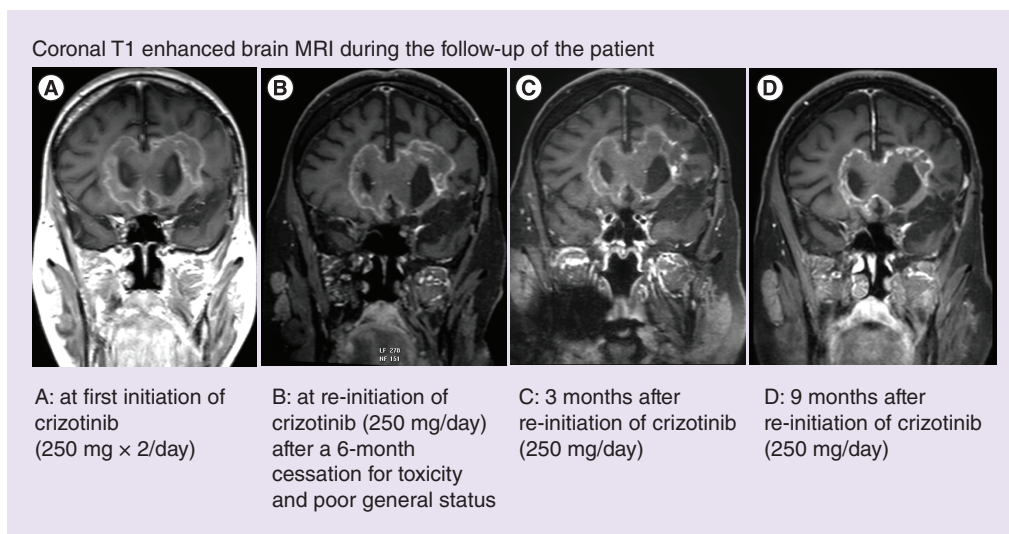


Figure 1. Coronal T1 enhanced brain MRI during the follow-up of the patient.

family, notwithstanding only minimal clinical and MRI progression (Figure 1). Following 3 months at reduced dose crizotinib therapy, a significant clinical improvement (personal autonomy and cognitive improvement) was observed as was the ECOG-PS (1). Additionally, prednisone was reduced to 10 mg per day. The patient remained independent in activities of daily living and with stable radiographic disease during the succeeding 9 months (Figure 1). The patient died of status epilepticus 12 months after the reintroduction of reduced dose crizotinib. The neurological condition was stable prior to the status epilepticus.

A 58-year-old woman was diagnosed with a right fronto-insular glioblastoma manifesting as partial left hemicorporal sensory-motor seizures and progressive headaches. A large but incomplete resection was performed. Histopathology and immunohistochemistry revealed a GB without IDH1 R132H expression. The *MGMT* gene promoter was unmethylated. Additional molecular analysis demonstrated strong expression of MET in 100% of tumor cells coupled with a high amplification of the gene (clusters) in 70% of tumor cells. Contrary to the first patient, the tumor did not express ALK and no amplification or gain of gene was observed by FISH. ROS1 expression was not observed, and no amplification or gain of gene was observed by FISH (Figure 3). The *V600E BRAF* mutation was not observed (Table 1).

The patient was initially treated with conventional radiotherapy and concomitant and adjuvant TMZ for 3 cycles. She then developed

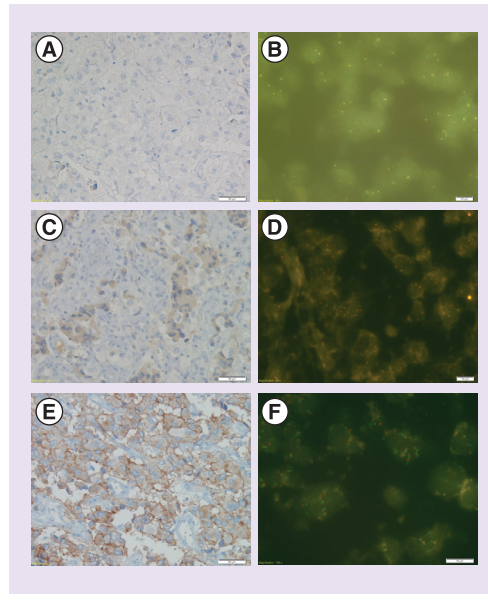


Figure 2. ROS1, ALK, c-MET immunohistochemical and FISH analysis for the first patient.

worsening and progressive left hemiparesis resulting in an ECOG-PS of 2. Brain MRI confirmed progression. Corticosteroids were initiated (Medrol 32 mg per day). Following the initial molecular analysis, crizotinib (250 mg twice daily) was initiated. Before drug treatment a discussion with the patient and family was initiated that included alternative treatments and the experimental nature of crizotinib for this indication. Also shared with the family was a soon to open French clinical trial (ACSE

Table 1. Association between crizotinib sensibility and molecular phenotypes.		
	Patient no. 1, man	Patient no. 2, woman
Diagnostic age	39-year-old	58-year-old
<i>MGMT</i> promoter	Nonmethylated	Nonmethylated
<i>IDH1</i> gene	Wild-type	Wild-type
ROS1 protein/gene	No expression No gain or polysomy	No expression No gain or polysomy
ALK protein/gene	Weak expression 25% Polysomy chromosome 2.53% and gain in 13%	No expression No polysomy no gain no amplification
MET	Weak + moderate expression 70 and 20%, respectively Polysomy of chromosome 7 and low amplification 36%	Moderate + strong expression 20 and 80%, respectively High amplification with clusters in 70%
Crizotinib sensibility	Strong benefit with stability Treatment at full dose: 2 months; break: 4 months, reduced dose: 12 months	No benefit Treatment during 4 months

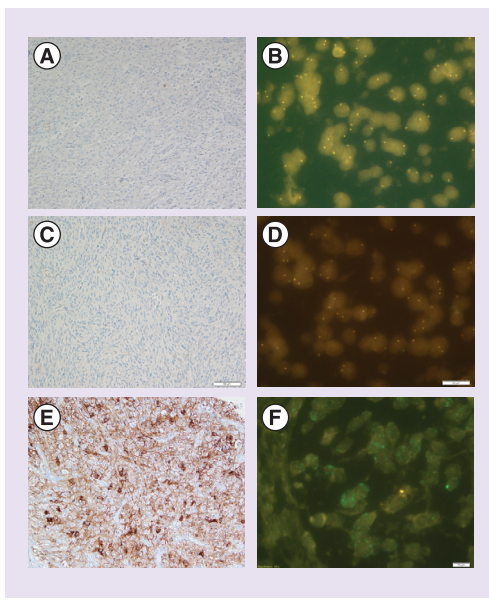


Figure 3. ROS1, ALK, c-MET immunohistochemical and FISH analysis for the second patient.

NCT02034981) in patients with recurrent high grade gliomas and MET amplified tumors that would be treated in an experimental manner with crizotinib.

Treatment was well tolerated without adverse events for 4 months at which time a second MRI revealed disease progression. Furthermore there was evidence of clinical deterioration with an ECOG-PS of 2. The treatment was changed to bevacizumab plus lomustine leading to a prolonged response and permitting cessation of corticosteroids.

Discussion

GB is the most common and aggressive malignant primary brain tumor in adults with a median overall survival of one year [1,2]. As GB is fatal despite multimodality treatment, new therapies for GB are an unmet need in neuro-oncology. Emerging data in the molecular characterization of GB have identified new rare but clinically relevant and actionable molecular alterations that are potentially druggable with targeted therapies. In the current case report, the tyrosine kinase receptors of the insulin receptor superfamily, ALK and MET, were investigated. Both are expressed in many cancers and appear to have a role in modulating mitosis, cell migration, tumor cell survival [3–5] and angiogenesis [6–8]. Additionally, ALK is highly expressed in the

nervous system during development [9] whereas c-MET regulates embryonic development and the immune response [3].

C-MET overexpression is detected in 29 to 88% of GB [10–12], but *c-MET* gene amplification has been observed in only 4–5% of all GB [13,14]. In a study of 70 GB, *MET* gain was present in 72%. In the majority of cases, the level of *MET* gain was low (2.7–5.0 copies). In this cohort, *MET* gain was more frequent in patients older than 40 years (54 vs 19%). The frequency of gain of *MET* was similar when comparing (IDH1 wild-type) and secondary GB (IDH1 mutated) respectively 47 and 44% [15]. Moreover, *MET* gain was more frequent in astrocytoma versus oligodendroglioma (38 vs 16%) and the appearance of *MET* gain appears to be associated with astrocytic progression [15]. C-MET overexpression has also been associated with multifocal lesions by MRI [10]. In the cohort of study by Pierscianek, *MET* gain was associated with significantly shorter patient survival and worse prognosis [15]. Overexpression or amplification of *c-MET* has been reported to be associated with a worse prognosis in most cancers as in glioblastoma [3,10,15–17]. It may be that c-MET expression affects prognosis due to the association with resistance to chemoradiation in GB [10,18].

Less data are available in the literature regarding ALK in GB. ALK overexpression and gain/amplification are seen in up to 18% of GB by immunohistochemistry and up to 48% by FISH, respectively [19,20]. The expression of ALK is more frequent in high-grade glioma as compared with low-grade glioma [5,19,21]. Oncogenic activation of the tyrosine kinase receptors c-ros oncogene 1 (ROS1) is rarely observed in glioblastoma [22,23]. ROS1 and ALK domains are partially homologous and consequently both are targeted by crizotinib.

Crizotinib is an orally available 1st generation ATP-competitive dual inhibitor of ALK and MET. Crizotinib has clinical activity in ALK translocation positive tumors and was approved for treating ALK positive nonsmall cell lung cancer [24,25]. Several additional ALK inhibitors are being tested in clinical trials, and of these, two with improved CNS penetration have been approved (ceritinib and alectinib). The role of crizotinib is currently being evaluated in several tumor types all of which have been demonstrated to express ALK, c-MET or ROS, all targets of crizotinib (ACSé trial: NCT02034981).

Generally after first recurrence of GB and with salvage therapy, 6-month progression-free survival varies from 17.5 to 52% and overall survival (OS) varies from 6 to 10 months. Survival postbevacizumab progression is very limited with an OS of 3–4.6 months regardless of subsequent treatment [1,2]. Nonetheless our first patient clearly benefited from crizotinib notwithstanding several prior clinical and MRI progressions.

The first patient required a 4-month cessation of crizotinib due to toxicity. The limited progression during this 4-month period without treatment and on a stable dose of corticosteroids cannot be clearly explained. Possibly there was a prolonged response effect of ALK inhibition despite interruption of therapy. We hypothesize that crizotinib has not only a cytostatic action but also a cytolytic action. *De facto* crizotinib induce cellular cycle stand by and cell death.

The respective roles of ALK, ROS1 and c-MET in the response to crizotinib in GB is unclear. Similar to the current case report, a previous case documented a rapid and prolonged clinical and radiological improvement when treated with crizotinib. In this patient with a recurrent *MET* gene amplified GB the expression of ALK was not reported [26]. In our second case c-MET protein overexpression was detected and was associated with high amplification of the *MET* gene but there was no benefit observed when treated with crizotinib. Interestingly ALK was not expressed nor was there evidence of gene amplification or gain observed. There was no evidence of a mutation in the kinase domain of ROS1 in our second case.

The main difference between our two cases of glioblastoma likely was the expression of the ALK, that probably explains the two differing patterns of response to crizotinib. We hypothesize that *MET* expression and *MET* amplification are not sufficient alone to determine a response to crizotinib. The *MET* gene may not be an oncogenic driver in GB. Indeed, although *MET* is important in the promotion of gliomagenesis, isolated dysfunction of *MET* may not be sufficient without corresponding loss of the *ALK* gene. In our second case, the inhibition of *MET* had no effect possibly because other genes like *AKT* or *ERK* are constitutively activated.

In conclusion, these case studies suggest that patients with GB and ALK polysomy may derive clinically relevant benefit from novel targeted small molecular inhibitors such as crizotinib. The respective roles of *ALK* and *c-MET* genes as predictors of response to crizotinib in GB need to be defined.

Ethical conduct of research

According to French law, no IRB/Ethical Committee approval is required for the publication of case reports. Nonetheless permission was obtained from the patient and family and they were informed of the manuscript.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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