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Clinical Trial

Intracerebral injection of CpG oligonucleotide for patients with *de novo* glioblastoma—A phase II multicentric, randomised study

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Abstract ***Background:*** Immunostimulating oligodeoxynucleotides containing unmethylated cytosine-guanosine motifs (CpG-ODN) have shown a promising efficacy in several cancer models when injected locally. A previous phase II study of CpG-ODN in patients with recurrent glioblastoma (GBM) has suggested some activity and has shown a limited toxicity. This multicentre single-blinded randomised phase II trial was designed to study the efficacy of a local treatment by CpG-ODN in patients with *de novo* glioblastomas.
Patients and methods: Patients with a newly diagnosed glioblastoma underwent large surgical resection and CpG-ODN was randomly administrated locally around the surgical cavity. The

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patients were then treated according to standard of care (SOC) with radiotherapy and temozolomide. The primary objective was 2-year survival. Secondary outcomes were progression free survival (PFS), and tolerance.

Results: Eighty-one (81) patients were randomly assigned to receive CpG-ODN plus SOC (39 patients) or SOC (42 patients). The 2-year overall survival was 31% (19%; 49%) in the CpG-ODN arm and 26% (16%; 44%) in the SOC arm. The median PFS was 9 months in the CpG-ODN arm and 8.5 months in the SOC arm. The incidence of adverse events was similar in both arms; although fever and post-operative haematoma were more frequent in the CpG-ODN arm.

Conclusions: Local immunotherapy with CpG-ODN injected into the surgical cavity after tumour removal and followed by SOC, although well tolerated, does not improve survival of patients with newly diagnosed GBM.

1. Introduction

Glioblastoma (GBM) is the most frequent malignant primary brain tumour in adults. Despite surgical resection and radiotherapy (RT), associated with chemotherapy, the prognosis in GBM patients remains poor, with a median time to relapse of around 7 months and a median survival below 15 months [1]. Oligodeoxynucleotides containing CpG motifs are derived from bacterial DNA and their biologic effects have been dramatically enhanced by stabilisation with phosphorothioate backbones. The immunogenic properties of CpG-ODNs have been successfully used in several experimental models of allergies [2], viral infections [3] and cancers [4].

Oligodeoxynucleotides containing unmethylated cytosine-guanosine motifs (CpG-ODN) display potent immunomodulatory effects through activation of the Toll-like receptor 9 (TLR9) [5]. In humans, TLR9 is mainly expressed by plasmacytoid dendritic cells and B cells [6]. TLR9 are also expressed in human microglial cells [7], and TLR9 expression has been shown in human glioblastoma samples [8].

The identification of tumour antigens is a limiting step for the design of cancer vaccines. To overcome this problem, CpG-ODNs alone can be directly injected into the tumour, with the expectation that the immune system will select by itself the most relevant antigens [8]. The validity of such an approach was shown in various cancer models, including malignant glioma [9,10]. Experiments in knock-out mice revealed that the efficacy of local CpG-ODN treatment *in vivo* required TLR9 expression by non-tumour cells [11]. In a murine glioma model, CpG-ODN appeared to enhance the antigen-presenting capacity of microglia, shift the immune response towards CD8+ T-cells and prolong the survival of mice with experimental tumours. Furthermore, regulatory T-cells (Treg) depletion was reported after treatment with CpG-ODN [12]. A phase I and a phase II clinical trial have been conducted in patients harbouring recurrent GBM. In these trials, the TLR9 agonist CpG-28 has been locally administered into the unresected tumour mass by convection-enhanced delivery. Both trials showed a good safety profile and some cases of minor responses [13,14].

This multicentre randomised phase II trial was designed to study the efficacy of a local treatment by CpG-28 in patients with *de novo* GBM.

2. Patients and methods

2.1. Trial design

This was a multicentre (seven academic centres in France), prospective, randomised (1:1), phase II trial. The trial was single-blinded, as patients were not informed of the results of randomisation. The physicians in charge of the patient after surgery were also blinded to the allocation arm. The trial protocol appears in Appendix 1.

2.2. Patient eligibility criteria

Eligibility criteria were defined as follows: brain magnetic resonance imaging (MRI) suggestive of GBM, eligibility to large or total surgical resection, age >40 years; Karnovsky performance status (KPS) score of 60% or higher; life expectancy >3 months and adequate bone marrow and hepatic function. Because histology was not available before surgery, patients were definitely included only if pre-operative histology confirmed the diagnosis of GBM.

Ineligibility criteria were pregnancy, past history of autoimmune disease, refractory epilepsy and MRI contraindication. Before being included in the study, patients signed an informed consent form, which was approved by the institutional review board (Registry number: NCT00190424).