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Phase 1 trial of a CpG oligodeoxynucleotide for patients with recurrent glioblastoma[1]

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Oligodeoxynucleotides containing CpG motifs (CpG ODNs) display a strong immunostimulating activity and drive the immune response toward the Th1 (T helper type 1) phenotype. These ODNs have shown promising efficacy in preclinical studies when injected locally in several cancer models. We conducted a phase 1 trial to define the safety profile of CpG-28, a phosphorothioate CpG ODN, administered intratumorally by convection-enhanced delivery in patients with recurrent glioblastoma. Cohorts of three to six patients were treated with escalating doses of CpG-28 (0.5–20 mg), and patients were observed for at least four months. Twenty-four patients entered the trial. All patients had previously been treated with radiotherapy, and most patients had received one or several types of chemotherapy. Median age was 58 years (range, 25–73) and median KPS was 80% (range, 60%–100%). Adverse effects possibly or probably related to the studied drug were moderate and consisted mainly in worsening of neurological conditions (four patients), fever above 38°C that disappeared within a few days (five patients), and reversible grade 3 lymphopenia (seven patients). Only one patient experienced a dose-limiting toxicity. Preliminary evidence of activity was suggested by a minor response observed in two patients and an overall median survival of 7.2 months. In conclusion, CpG-28 was well tolerated at doses up to 20 mg per injection in patients with recurrent glioblastoma. Main side effects were limited to transient worsening of neurological condition and fever. *Neuro-Oncology 8, 60–66, 2006 (Posted to Neuro-Oncology [serial online], Doc. 05-047, November 29, 2005. URL http://neuro-oncology.mc.duke.edu; 10.1215/S1522851705000475)*

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Glioblastoma is the most frequent malignant glioma in adults. Despite surgical resection and radiotherapy, the prognosis in these patients remains poor, with a median survival of around 12 months. When glioblastoma recurs, the efficacy of chemotherapy is very limited and the median survival is around six months (Brada et al., 2001; Yung et al., 2000).

Synthetic phosphorothioate oligodeoxynucleotides (ODNs) containing unmethylated CpG dinucleotides

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Dr. Antoine Carpentier holds a patent position on cancer immunotherapy with CpG ODNs. The Assistance Publique—Hôpitaux de Paris is co-owner of a patent on CpG.

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[3]Abbreviations used are as follows: DLT, dose-limiting toxicity; MTD, maximum tolerated dose; ODN, oligodeoxynucleotide; SS, Sjögren's syndrome; Th1, T helper type 1.

(CpG ODNs) are strong activators of both innate and adaptive immunity. They drive the immune response toward the Th1 (T helper type 1) phenotype, which promotes CD8+ cellular cytotoxicity (Klinman, 2004; Krieg, 2004). Given their potent Th1 adjuvant properties, CpG ODNs have been introduced into clinical trials in vaccine protocols combined with the hepatitis B surface antigen or with Fluarix influenza vaccine (GlaxoSmithKline Biologicals, Rixensart, Belgium) (Cooper et al., 2004a, b; Halperin et al., 2003). In cancer research, the identification of tumor antigens is a limiting step for the design of therapeutic vaccines. To overcome this problem, CpG ODNs alone can be directly injected into the tumor, with the expectation that the immune system will select the most relevant antigens. In addition, CpG ODNs activate innate immunity (natural killer cells and macrophages), which can directly kill tumor cells. The validity of such an approach was shown in a neuroblastoma model, in which peritumoral injections of a synthetic ODN containing a CpG motif induced complete tumor rejection in the majority of mice and triggered a long-term immunity (Carpentier et al., 1999). Further studies have confirmed the antitumor effects of CpG ODNs in various cancer models, including malignant glioma (Carpentier et al., 2003). In an intracranial model of syngeneic glioma (CNS1), more than 85% of the rats treated five days after tumor inoculation with a single intratumoral injection of CpG ODNs showed long-term survival and tumor eradication. Rats that were cured by CpG ODN injections were further protected against new tumor challenge, showing that a long-term immunity was primed (Carpentier et al., 2000).

On the basis of our preclinical data, we initiated a phase 1 trial to assess the feasibility and safety of local injections of CpG ODNs in patients with recurrent glioblastoma. CpG-28, the CpG ODN that gave the best results in preclinical glioma models (Meng et al., 2005), was selected for this trial. Direct infusion of CpG-28 into brain tumors was achieved by implanted catheters and high-flow microinfusion. This technique allows fluids to be distributed by bulk flow (convection) through the interstitial spaces and spread throughout distant areas of the brain (Broaddus et al., 1998).

Patients and Methods

Patient Eligibility Criteria

Eligibility criteria were defined as follows: histologically proven glioblastoma, disease progression documented at least three months after surgery and radiotherapy, measurable contrast enhancement (>1 cm) on MRI, KPS score of 50 or higher, four-week interval from last chemotherapy (six-week interval for nitrosourea), and adequate bone marrow (platelet count >100,000) and hepatic function (plasma aspartate aminotransferase and alanine aminotransferase levels <3 times the normal limit). Ineligibility criteria were pregnancy, past history of autoimmune disease or multiple sclerosis, and recurrent tumor considered resectable by the neurosurgeon.

All patients signed an informed consent form before being included in the study, which was approved by the institutional review board. Concurrent systemic chemotherapy was not allowed until tumor progression.

Treatment Design and Dose Escalation

CpG-28 (sequence 5'-TAAACGTTATAACGTTATGACGTCAT-3') (Carpentier et al., 2003; Meng et al., 2005) was synthesized with a wholly phosphorothioate backbone by Avecia (Milford, U.K.) and was supplied by the Agence Générale des Equipements et Produits de Santé (Paris, France) in vials containing 10 mg of CpG-28. When needed, the drug was further diluted in 0.9% sodium chloride, so that the total drug volume injected was 1 ml per implanted catheter. Catheters were placed by using stereotactic guidance through small twist-drill holes with patients under local anesthesia. No surgical resections of the tumors were performed. The tips of the catheters targeted the contrast-enhanced areas, and they had to be at least 2 cm deep within the brain. After surgery, a CT scan was performed to verify the position of the catheters and the absence of any hemorrhage, and the externalized catheters were connected to a 5-ml syringe filled with CpG-28. The infusion began 1 h after implantation at an infusion rate of 0.2 ml/h (DPS-orchestra electric syringe; Fresenius Vial, France) for 6 h (1 h for the dead volume of the catheter and 5 h for drug infusion). In addition to their usual treatment, patients were premedicated with 1 mg diazepam to prevent seizures. The catheters were removed 1 h after completion of drug administration.

The dose of CpG-28 was escalated from 0.5 mg (level 1) to 1.0 mg (level 2) to 2.0 mg (level 3) to 5.0 mg (level 4) to 10.0 mg (level 5) to 20.0 mg (level 6). One implanted catheter was used for levels 1 to 4 and for the first three patients of level 5. The protocol was then amended to infuse CpG-28 through two catheters instead of one. Three additional patients were then treated at the same dose (level 5) before the dose was escalated to level 6.

Follow-up

Patients were assessed two days, 15 days, and monthly for four months after injection. At each follow-up visit, a neurological and a general examination, a KPS rating, and an MRI with contrast media were performed (except on day 2, when a CT scan was allowed). Complete blood count, serum biochemistry, and liver function tests were repeated at each visit. Antinuclear antibody and anti-SSA/SSB (Sjögren's syndrome) antibody titers were measured at baseline and day 30. Toxicity was graded according to the NCI expanded Common Toxicity Criteria (NCI CTC 2.0). Radiographic response was evaluated by using the Macdonald criteria (Macdonald et al., 1990). MRI images were centrally reviewed by the same radiologist (N.M.-D.).

Patients were allowed to receive chemotherapy if evidence for tumor progression was seen after day 60. Even in those cases, the patients were monitored for four months after drug administration.

Study Design and Quality Insurance

This phase 1 trial was designed as an open-label, nonrandomized study, in which groups of three to six patients were treated with escalating doses of CpG-28. The first three patients at a dose level were observed for four weeks after drug administration. If no dose-limiting toxicity (DLT) was observed among those three patients, the dose would be escalated to the next level. If one instance of DLT was observed among the initial three patients treated at a dose level, an additional three patients had to be treated at that dose level with no further DLT for dose escalation to proceed. If two instances of DLT were observed at a dose level, the maximum tolerated dose (MTD) was surpassed, and a total of six patients had to be treated at the previous level to ensure its tolerability. The MTD was the highest dose to cause DLT in no more than one of six patients at that dose level.

DLT was defined as severe peritumoral edema resistant to steroids and leading to intracranial hypertension; neurological deterioration with a Rankin score decrease of more than one point that was unrelated to tumor progression and that lasted more than 15 days; or grade 4 nonhematopoietic or hematologic toxicity occurring within one month after administration of CpG-28. Evidence for severe autoimmune diseases or multiple sclerosis was also considered to be DLT, irrespective of the delay after drug administration.

All recorded data were monitored by an independent scientific committee that approved each dose escalation. (This scientific committee was composed of one neurosurgeon [H. Loiseau], one neuro-oncologist [O. Chinot], one neuroradiologist [F. Lafitte], one oncologist [E. Raymond], and one statistician [B. Asselain]).

Statistical Analysis

Demographic and baseline characteristics were recorded as medians (with ranges) for continuous variables and proportion for categorical variables. All adverse events occurring within the first 30 days after drug administration were analyzed by dose group. A Kaplan–Meier method was used to estimate overall survival after study enrollment.

Results

Patient Characteristics

Twenty-four patients were enrolled in this single-center trial between January 2003 and June 2004. Nine patients (37%) were included at the time of first recurrence, eight (33%) at the time of second recurrence, and seven (29%) at the time of third recurrence. Most patients were treated with steroids at the time of study enrollment (prednisone equivalent dose: >60 mg/day, two patients; 30–60 mg/day, nine patients; 5–30 mg/day, 10 patients; and no steroids, three patients). The clinical characteristics of patients are outlined in Table 1.

Dose Escalation and Dose-Limiting Toxicity

One adverse event was considered to be a probable DLT (patient 21 at a 20-mg dose). This patient was rapidly deteriorating at the time of inclusion, experienced a transient worsening after drug injection (increased aphasia, hemiparesis, and visual disorders), and did not fully recover despite increased steroid levels. Although it was unclear whether this incomplete recovery was related to tumor progression or injection of CpG-28, these symptoms were considered a DLT. Consequently, three additional patients were treated at this dose level, but no further DLTs were observed.

Safety and Tolerability

There were 120 adverse events reported, and 63 were considered as possibly related to the investigated treatment (Table 2). Adverse events (≥grade 2) possibly or probably related to CpG-28, according to the investigator in charge of the patient, are summarized in Table 3.

Table 1. Characteristics of patients treated with CpG ODN

Characteristic	No. of Patients*	Percent
Sex		
Male	14	58
Female	10	42
Age, years		
Median	58	
Range	25–73	
KPS		
Median	80%	
Range	60–100%	
100%	2	8
90%	5	21
80%	6	25
70%	6	25
60%	5	21
Tumor histology, glioblastoma	24	100
Time from first diagnosis to treatment, months		
Median	11	
Range	6–62	
Prior therapy		
Surgery**	23	96
No. of interventions before enrollment		
1	18	75
2	5	21
Radiotherapy	24	100
Chemotherapy	21	88
No. of regimens before enrollment		
1	10	42
2	6	25
3	5	21

Abbreviations: KPS, Karnofsky performance status; ODN, oligodeoxynucleotide.

*Medians, ranges, and percentages as indicated.

**Except biopsies.

Table 2. Reported adverse events (grade >2)

Adverse Event	Probably Related	Possibly Related	Probably Unrelated
Clinical AEs			
Partial seizures	0	5	7
General seizures	0	0	2
Fever (grade 2)	0	4	0
Fever (grade 3)	0	1	0
Worsening of previous neurological condition	1	4	15
Somnolence (grade ⩾2)	0	2	4
Nausea (grade 2)	0	1	3
Fatigue (grade 2)	1	5	0
Other clinical symptoms (grade ⩾2)	0	0	9
Biological AEs*			
Lymphopenia (grade 2)	0	20	6
Lymphopenia (grade 3)	0	9	1
ALT (grade ⩾2	0	6	1
AST (grade 2)	0	1	0
Other biological alterations (grade ⩾2)	0	3	9

Abbreviations: AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

*Counted several times if they lasted over several follow-up visits.

Except for the patient with DLT, the adverse events were generally mild and well tolerated, the most frequent being lymphopenia.

Transient worsening of baseline neurological symptoms was seen in one patient on dose 4 and in three patients on level 6. In patient 21, the worsening was considered as a DLT (see above). In patients 11, 22, and 23, this worsening was moderate and resolved within two weeks. Patient 4 experienced nausea and somnolence four days after injection, but the relationship with CpG-28 is unclear because the patient had similar symptoms before inclusion. When a neurological worsening did occur, although no increase in peritumoral edema was seen on MRI, steroid dosage was increased. In addition, five patients complained of grade 2 fatigue for a few days after injection (two patients on level 5 and three patients on level 6).

Increased body temperature (>38°C) was noted in five patients (one patient in level 3, two patients in level 5, and two patients in level 6). Fever peaked on day 3 (maximum of 39.3°C in one patient), was well tolerated, and disappeared within five days without antibiotics.

In the month following administration of CpG-28, five patients had short partial seizures. In patient 19, a relationship with the treatment is possible because the seizure occurred just after administration of CpG-28. The other patients had occasional seizures before enrollment, and the relationship with the studied drug is unclear.

Two adverse events were considered as probably related to the procedure. Patient 1 was on long-term anticoagulant therapy for a past history of pulmonary embolism diagnosed six years before inclusion. This treatment was discontinued by the time of enrollment into this trial. A pulmonary embolism and right-leg deep-vein thrombosis were diagnosed 37 days after administration of CpG-28. The anticoagulation treatment was then resumed, and the patient slowly recovered. Patient 11 experienced neurological worsening and a partial seizure on day 5 after drug administration. MRI on day 15 showed a T1 hypersignal suggesting a small hemorrhage at the site of catheter implantation. Although the exact date of onset cannot be determined, it is likely that this hemorrhage was related to catheter implantation. The patient recovered within two weeks.

Seven patients experienced grade 3 lymphopenia ($<500/mm^3$), three of whom already had a grade 2 lymphopenia at the time of inclusion. All these patients had chemotherapy between one and four months before study enrollment. The decrease in lymphocyte counts on day 30 (when compared to the day of preinclusion screening) was not statistically significant (mean ± SD, 982 ± 596 vs. 1367 ± 1191/mm^3; $P = 0.38$). This lymphopenia resolved in all cases by day 60 and was not associated with any infectious diseases. The relationship with the studied drug is possible, although no dose-response relationship was found.

Grade 3 nonhematological toxicities were limited to reversible alanine aminotransferase elevation in two patients and hyponatremia in one patient treated at the highest dose. These biochemical alterations were reversible, and their relationship with the studied drug is unclear. A moderate elevation of antinuclear antibody titers at day 30 was seen in patient 4 (nondetectable to 1:320) and patient 18 (1:160 to 1:320) without symptoms associated with autoimmune disease. Anti-SSA/SSB antibodies were undetectable in all patients.

There were no treatment-related deaths, and we found no evidence for drug-induced edema, increased mass effect, or autoimmune disease after administration of CpG-28.

Table 3. Adverse events (grade ⩾2) possibly or probably related to administration of CpG-28

Patient	Dose (mg)	Fever >38°C	Clinical Symptoms Within 30 Days	Lymphopenia	Liver Toxicity	Other Events
1	**0.5**			Grade 3, day 30	Grade 2, day 15	Pulmonary embolism, day 37
2			Nausea, somnolence on day 4	Grade 3, day 30		
3						
4	**1**			Grade 3, day 30		
5						
6						
7	**2**			Grade 2, day 30		
8						
9				Grade 3, day 15		
10	**5**					
11		38.3, day 1	Transient neurological worsening Partial seizure, day 5	Grade 3, day 15		Local bleeding (catheter track)
12						
13	**10**			Grade 2, day 60		
14			Partial seizures, days 13 and 23	Grade 2, day 30		
15						
16			Transient fatigue (grade 2)			
17		38.3, day 4		Grade 2, day 15		
18		38.4, day 3	Transient fatigue (grade 2)		Grade 2, day 15	
19	**20**		Partial seizure, day 0. Transient fatigue (grade 2)			
20		39.3, day 3	Transient fatigue (grade 2). Partial seizure, day 24			
21		38.1, day 3	Partially regressive neurological worsening Partial seizure, day 12	Grade 3, day 60	Grade 3, day 30	Hyponatremia grade 3, day 2 Thrombopenia, grade 2, day 60
22			Transient neurological worsening			
23			Transient neurological worsening			Thrombopenia, grade 2, day 60
24			Transient fatigue (grade 2)	Grade 3, day 30	Grade 3, day 30	

Antitumor Response

All patients but one were assessable for tumor response: Patient 12 wanted to be taken off protocol in order to be treated with temozolomide one month after administration of CpG-28.

Two patients (patients 9 and 17), whose tumors were growing at the time of inclusion, showed minor response (29% and 20% reduction, respectively, in the product of the largest perpendicular diameters) at the injection sites (Figs. 1 and 2). These local responses were associated with reduced mass effect and decreased surrounding edema. Two other patients had stable disease for more than four months (progression-free survival at four months, 9%).

At the time of analysis, 20 patients had died. One-year survival was 28% (Fig. 3). Median survival time for all patients was 7.2 months from time of enrollment (95% confidence interval, 4.8–12.7 months). The progression-free survival at six months was 4.5%.

Discussion

CpG ODNs are exciting new immunostimulating agents that are currently under clinical trials in cancer patients, either as single agents or combined with monoclonal antibodies or chemotherapy. In the present study, we report phase 1 results of a new CpG ODN, administered locally by convection-enhanced delivery in patients with recurrent glioblastoma. This is the first report of CpG ODN in cancer as a stand-alone approach.

Intracranial administration of CpG-28 was generally well tolerated. Given the immunostimulating properties of CpG ODN, the main concern was that CpG-28 might induce local inflammation or trigger an autoimmune disease. Indeed, it was recently reported that intracerebral injection of CpG ODN can induce experimental allergic encephalitis in mice previously immunized against a neural antigen (Conant and Swanborg, 2004). Increased edema or evidence of autoimmune disease was

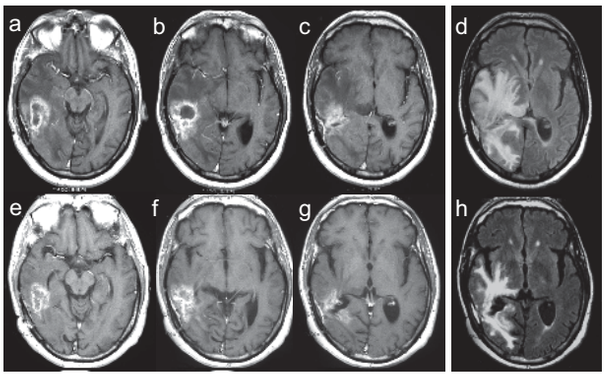

Fig. 1. Brain MRI studies of patient 9. Before administration of CpG oligodeoxynucleotide (ODN), gadolinium-enhanced sections (a–c) and fluid-attenuated inversion recovery (FLAIR) sequence (d) showed a recurrent tumor in the right temporal lobe with surrounding edema. Thirty days after administration of CpG ODN, the area of contrast enhancement, the surrounding edema, and the mass effect were reduced at the site of injection (e–h).

not observed in any of our patients, which suggests that this risk may be minimal. However, we cannot exclude the possibility that our patients were protected by the concomitant use of steroids, and careful monitoring of future patients treated with CpG ODNs is warranted.

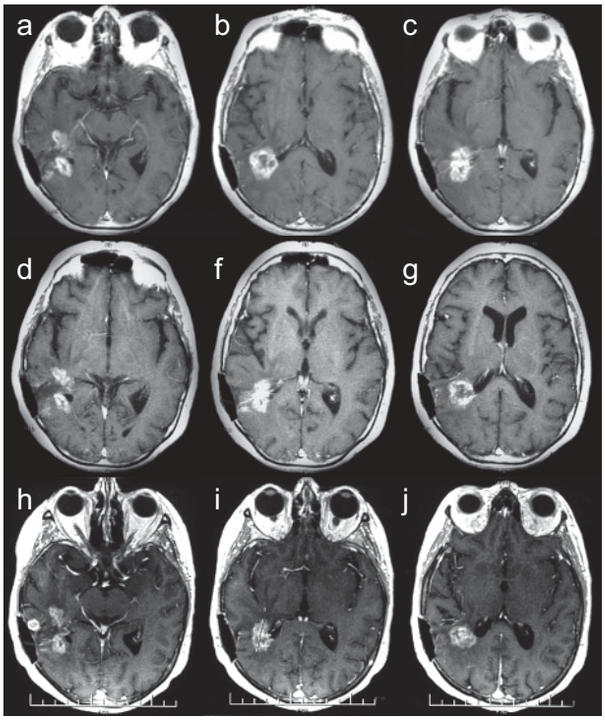

Fig. 2. Brain MRI studies of patient 17. At time of inclusion, gadolinium-enhanced sections (a–c) showed recurrence in the right temporal lobe. MRI performed 30 days (d–f) and 60 (g–i) days after administration showed a minor response at the site of injections. Reduction of surrounding edema was also noted (not shown). A small recurrence occurred on the external part of the temporal lobe (g).

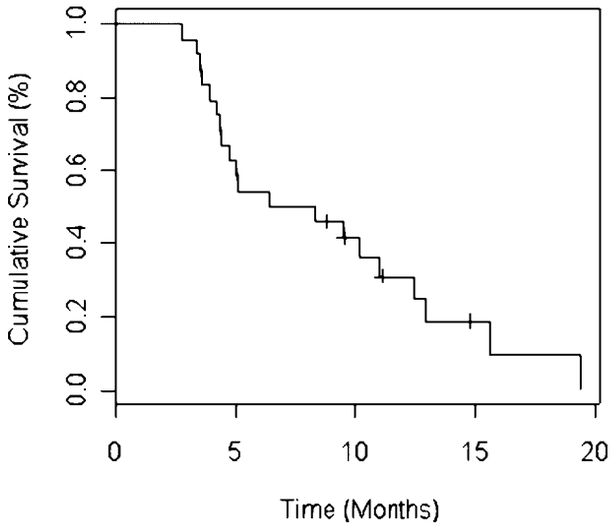

Fig. 3. Kaplan–Meier estimate of overall survival.

Transient neurological worsening was the most significant toxicity observed at the 10- or 20-mg dose level, an adverse event that was not related to increased edema. In organotypic brain cultures, CpG ODNs were shown to be cytotoxic to neurons, through secretion of inflammatory mediators such as nitric oxide and tumor necrosis factor alpha by microglial cells (Iliev et al., 2004). Such inflammatory cytokines might partly explain the neurological worsening seen in some of our patients. However, this worsening regressed spontaneously and was considered to be a potential DLT in only one patient.

Lymphopenia was the most frequent adverse event. This was unexpected, as CpG ODNs stimulate B-lymphocyte proliferation. We did not observe any dose-response relationship, and it remains unclear whether this lymphopenia is related to the studied drug or to previous treatments with chemotherapy. Preliminary analysis did not suggest that a particular subset of lymphocytes was more affected, but this point is currently under study.

Altogether, a DLT was observed in only one patient at the highest dose, and the MTD was therefore not reached. However, as all patients in the 20-mg dose level experienced either neurological worsening or fatigue, the independent scientific committee recommended the dosage of 20 mg for the phase 2 clinical trial.

Efficacy was not the primary objective of this phase 1 trial, conducted on previously heavily treated patients and with escalating doses. Unfortunately, no immunological surrogate markers are available in clinical trials for patients with recurrent glioblastoma who do not undergo surgical resection. Median survival was 7.2 months, and one-year survival was 28%. These figures compare favorably with previous trials using temozolomide, in which median survival was around six months, and one-year survival was less than 15% (Brada et al., 2001; Yung et al., 2000). It should be emphasized that, in those temozolomide trials, patients were treated at the time of first recurrence of glioblastoma, whereas our population for the most part was treated at the time of

second or third recurrence and therefore carried a worse prognosis. A minor radiological response was observed in two patients. This response rate may be underestimated because contrast enhancement might be induced by local immunostimulation triggered by CpG-28 as well as tumor progression.

Further improvements can be suggested for subsequent trials. Convection-enhanced delivery is subject to a number of variables, such as backflow along the catheters or marked heterogeneity of drug distribution within tumor (Vavra et al., 2004). Administration by two or three catheters (instead of one, as used for the first five doses of this trial) or increasing the infusion time might improve CpG ODN distribution, but it should be stressed that complete coverage of the tumor mass is not theoretically needed in a regimen that aims to trigger an immune response. Applying CpG ODN in newly diagnosed glioblastoma might also be a more appropriate setting than in recurrent glioblastoma because the tumor burden would be lower and these patients are likely to have less immunosuppression.

In summary, this study demonstrated that local treatment with CpG ODNs in patients with recurrent glioblastoma is feasible and well tolerated at doses up to 20 mg. A phase 2 trial is currently ongoing.

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