A Phase II, Randomized, Study of Weekly APG101+Reirradiation versus Reirradiation in Progressive Glioblastoma

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doi: 10.1158/1078-0432.CCR-14-0951-T

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Abstract

Purpose: Preclinical data indicate anti-invasive activity of APG101, a CD95 ligand (CD95L)—binding fusion protein, in glioblastoma.

Experimental Design: Patients (N = 91) with glioblastoma at first or second progression were randomized 1:2 between second radiotherapy (rRT; 36 Gy; five times 2 Gy per week) or rRT+APG101 (400 mg weekly i.v.). Patient characteristics [N = 84 (26 patients rRT, 58 patients rRT+APG101)] were balanced.

Results: Progression-free survival at 6 months (PFS-6) rates were 3.8% [95% confidence interval (CI), 0.1–19.6] for rRT and 20.7% (95% CI, 11.2–33.4) for rRT+APG101 (P = 0.048). Median PFS was 2.9 (95% CI, 2.3–3.8) months and 4.5 (95% CI, 3.7–5.4) months with a hazard ratio (HR) of 0.49 (95% CI, 0.27–0.88; P = 0.0162) adjusted for tumor size. Cox regression analysis adjusted for tumor size revealed a HR of 0.60 (95% CI, 0.36–1.01; P = 0.0559) for rRT+APG101 for death of any cause. Lower methylation levels at CpG2 in the CD95L promoter in the tumor conferred a stronger risk reduction (HR, 0.19; 95% CI, 0.06–0.58) for treatment with APG101, suggesting a potential biomarker.

Conclusions: CD95 pathway inhibition in combination with rRT is an innovative concept with clinical efficacy. It warrants further clinical development. CD95L promoter methylation in the tumor may be developed as a biomarker. Clin Cancer Res; 20(24): 6304–13. ©2014 AACR.

Introduction

Treatment regimens used for progressive glioblastoma are of very limited efficacy (1–3). For years, alkylating chemotherapy has been the mainstay, although patients already had been exposed to temozolomide in conjunction with radiotherapy after diagnosis and surgery. Lomustine (CCNU) is commonly used at progression. Recent and ongoing trials tested anti-vascular endothelial growth factor (VEGF; receptor) strategies (4, 5), protein kinase C-β inhibition with enzastaurin (6)
or they embarked on various temozolomide regimens (7, 8).

With the recognition that tissue recovery in the brain after radiotherapy might be much better than initially thought, options for a second radiotherapy (rRT) were explored. Retrospective analyses or uncontrolled trials reported positive clinical outcomes (9, 10). There is no consensus on one particular radiation regimen, but different concepts of hypofractionation, target delineation, and dosing, for example, 18 × 2 Gy, 15 × 2.33 Gy, 6 × 5 Gy, exist.

CD95 (Fas, APO-1) is a pleiotrophic receptor that regulates tissue homeostasis. During cancer progression, CD95 is frequently downregulated or tumor cells are rendered apoptosis resistant. However, evidence exists that cancer cells, regardless of their CD95 apoptosis sensitivity, depend on constitutive activation of CD95 for optimal growth (11), stimulated by CD95 ligand (CD95L) produced in an autocrine or paracrine manner. A growth-promoting role of the CD95/CD95L system has recently been described for glioblastoma in vitro and in an orthotopic syngeneic mouse model (12, 13) where activation of CD95 by CD95L stimulates AKT kinase- and β-catenin–dependent genes (12). CD95 activation in glioblastoma leads to invasive growth and migration facilitated by increased expression of matrix metalloproteinases (MMP), which are key mediators of glioma invasiveness (13). In vitro, blocking of CD95 activation was demonstrated to impede increased invasiveness of irradiated glioblastoma cells as an adaptive evasive response to radiation (14, 15). This insidious effect of radiotherapy may be mediated by stimulation of PI3K/AKT-dependent MMP-2 and MMP-9 activity (16, 17) or alternative mechanisms (18). APG101 is a CD95L-binding protein consisting of the extracellular domain of human CD95 fused to the Fc region of human IgG1. It interferes with CD95-dependent signaling by binding to CD95L, thereby blocking subsequent CD95-dependent activation (19). The possibility exists that not only cell-bound CD95L, but soluble, potentially systemic (20) as well as endothelial cell-bound CD95L may also serve as a therapeutic target (21). Single ascending doses of APG101 up to 20 mg/kg body weight (bw) administered as infusion over 1 hour were considered as safe and well tolerated in healthy volunteers in a phase I study. No maximum tolerated dose (MTD) has been reached and no anti-drug antibodies (ADA) were detected. After the application of multiple doses of 400 mg in 2 patients with glioma under compassionate use conditions, steady state for APG101 seemed to be reached, supporting further clinical evaluation of APG101 at a dose of 400 mg per week in patients with glioblastoma (19).

From the above, the combination of APG101 and radiotherapy might be particularly attractive, because APG101 might enhance radiation efficacy and reduce unwanted radiation-induced infiltrative growth, and radiotherapy might facilitate the delivery of APG101 to the tumor stroma by opening the blood–brain barrier (BBB). The primary objective of this phase II study was to evaluate the efficacy as determined by progression-free survival at 6 months (PFS-6) for patients with first or second progression of a glioblastoma. Secondary objectives included safety and tolerability of APG101, response rate (RR), overall survival (OS), PFS, and health-related quality of life. There were also correlative studies planned to find a tissue-based biomarker by immunohistochemistry.

Patients and Methods

Patients

Adult patients with first or second progression of a histologically confirmed glioblastoma either not being eligible for tumor resection or having macroscopic residual tumor after tumor resection, documented by contrast-enhanced magnetic resonance imaging (MRI) with the largest diameter measuring 1 to 4 cm and a Karnofsky performance score (KPS) ≥60 were eligible. No more than two prior therapy regimens including one or two resections, one or two chemotherapies of which one must have been temozolomide-containing, and one radiotherapy (completed ≥8 months before enrollment) were allowed. All patients were required to give signed informed consent before enrollment.

Trial design and conduct

The APG101_CD_002 study (NCT01071837) followed a Simon two-stage design. A randomized control arm with rRT alone was added to avoid under- or overestimation of a signal from APG101. The study was approved by the ethics committees (EC) of all 25 participating sites. The study started recruitment in December 2009, and the last patient was randomized on Sep 21, 2011. Patients were centrally randomized 1:2 to receive rRT (36 Gy) or rRT (36 Gy) + APG101 400 mg weekly until progression (Fig. 1). Treatment following disease progression was recorded.

In this open-label, multinational trial, the first 9 patients constituted a predefined run-in phase to evaluate the safety of rRT + APG101. An independent Data and Safety Monitoring Board (DSMB) reviewed all relevant patient data after completion of the rRT and endorsed further accrual to the trial. The second meeting was held after 25 patients completed the reirradiation (rRT) and the third took place at the end of stage 1 of the Simon two-stage design (after 28 patients reached the primary endpoint) combined with a safety evaluation after the first 49 patients completed rRT.

At the submission of this article, the collection of OS data is still ongoing. The study adhered to the Declaration
Radiotherapy

rRT at 36 Gy in 2-Gy single fractions was required to be performed as highly precise treatment, either as a stereotactic radiotherapy or as an image-guided radiotherapy. To assess the quality of each participating center, a dedicated dummy run evaluating technical equipment, quality assurance as well as treatment planning was performed. Only centers fulfilling all requirements were eligible to recruit patients into the trial. To ensure protocol-conform treatment planning, 2 test patients were distributed to each center and target volume definition as well as treatment planning and dose distributions carried out by the site were evaluated as a dummy run (Medical Centre Heidelberg, Heidelberg, Germany). In detail, as the basic inclusion criterion was an indication for rRT, any recurrence in- or outside the radiation field that occurred ≥6 months out of the initial RT was principally eligible. However, because >90% of the lesions occur inside the radiation volume, all except 1 patient, who had a recurrence at the 20% isodose, had a pre-RT at a similar region. For treatment planning, CT and contrast-enhanced MRI were mandatory. Target volume delineation was defined to include the gross tumor volume (GTV) defined as the contrast-enhancing lesion on MRI, adding 1 cm safety margin for potential microscopic spread (clinical target volume, CTV). After treatment, treatment plans of study patients were reviewed centrally in Heidelberg and protocol adherence was confirmed for all patients.

**APG101**

APG101 was given at 400 mg weekly as a 30-minute i.v. infusion until progression or undue toxicity. It was started on the same day as the rRT. APG101 was applied before rRT due to potential compliance reasons, for example, patients might be tired after the RT and therefore refuse the APG101 infusion on this day, which should be avoided.

**Randomization and masking**

Participant allocation was done according to an electronically generated randomization list stratified for maximum tumor diameter (<2.5 vs. >2.5 cm), a risk factor established for reirradiation (9). The sequence was generated before the start of study at the Contract Research Organization (CRO), Premier Research (Darmstadt, Germany).

Allocation took place by fax transmission from the CRO to the study site for patients fulfilling the eligibility criteria. This was an unblinded trial. Biases were prevented by strict adherence to an analysis plan that was written by the statistician (K. Junge) before any analysis of the data.

**Evaluations**

Baseline examinations included physical examination, vital signs, MRI, full blood cell counts, blood chemistry, serology (HbsAG, anti-HCV, and anti-HIV), urine analysis, ECG, abdominal ultrasound, Mini-Mental State Examination (MMSE), structured neurologic examination, and quality-of-life questionnaire (QLQ) assessment with European Organisation for Research and Treatment of Cancer (EORTC) EORTC QLQ-C15 PAL questionnaire and the brain module EORTC QLQ-BN-20 (22).

Patients randomized to rRT only had to attend 6-weekly visits; patients in the rRT+APG101 arm had weekly visits with i.v. application of APG101.

Toxicity/AEs, safety laboratory (blood chemistry and hematology), vital signs, and Karnofsky index were assessed at every visit (weekly in APG101+ rRT patients, 6-weekly in rRT-only patients). A more comprehensive evaluation that included physical examination, MRI, MMSE, structured neurologic examination, and QLQ was carried out every 6 weeks for all patients. Urine analysis, ECG, and abdominal ultrasound were done every 12 weeks.

Sites had to complete an MRI dummy run before study start for quality reasons. Tumor response or progression was defined according to modified MacDonald criteria taking pseudoprogression into account (23) by the local investigator and centrally. In detail, an apparent increase in tumor size considering the largest cross-sectional area or contrast-enhancement in the radiation field of ≥25% in the first or second scan post-rRT was called pseudoprogression and not deemed a progression until further confirmation on follow-
up. As recommended in the RANO criteria (24), minimal clinical worsening or increase in steroids (up to 4 mg) was not considered inconsistent with the diagnosis of pseudo-progression. Further progression resulted in backdating to the scan of the initial suspicion of a progression and stable disease on follow-up in retrospective rating as stable. Stable or decreasing contrast enhancement resulted in a continuation of trial treatment and/or follow-up. Further specified analyses of all patients were done for all irradiation plans (S.E. Combs and J. Debus) and tissue quality before molecular examinations (C. Hartmann and A. von Deimling) in a blinded fashion (25).

**Neuropathologic methods**

Archived tumor tissue was available from 81 patients. This tumor tissue was used to examine IDH1, O6-methylguanine-DNA methyl-transferase (MGMT), CD95, and CD95L as well as genome-wide methylation levels in a discovery set of 20 tumor samples. Only solid tumor tissue was evaluated. Areas that showed an infiltration zone or non-neoplastic brain parenchyma were excluded from the evaluation process. Expression of mutated IDH1 R132H protein was determined by immunohistochemistry (26). The MGMT promoter methylation status was analyzed after bisulfite treatment by methylation-specific PCR (27). Expression of CD95 and CD95L was determined by immunohistochemistry.

All CD95- and CD95L-stained slides were evaluated slide-by-slide in a single session by a board-certified neuropathologist (C. Hartmann). Vital tumor tissue of each slide was evaluated regarding the CD95 and CD95L staining intensities "high," "moderate," "low," and "absent." CD95 and CD95L calibration figures were used to standardize the evaluation. Because most tumors showed different staining intensities in separate areas, the percentage of these summed areas were counted. Each tumor was assigned to a specific value in percentage representing the area showing "high," "moderate," "low," and "absent" CD95 and CD95L staining intensities.

CD95L promoter methylation by MassARRAY at probes cg10161121 (CpG2) and cg06983746 (CpG1) was then examined in a validation set of 40 patients. For clinical data evaluations, a median-based cutoff at 0.85 was used (see Supplementary Methods and Supplementary Tables S3–S8).

**Biomarker identification**

To identify epigenetic differences distinguishing responders from non-responders, we performed genome-wide assessment of DNA methylation using the HumanMethylation450 BeadChip (Illumina) of 20 patients who received APG101 plus radiotherapy (10 patients with a PFS > 5 months and 10 patients with a PFS < 2 months, discovery cohort) at the Genomics and Proteomics Core Facility of the German Cancer Research Center (Heidelberg, Germany). Data normalization was performed following the manufacturer’s recommendations. Unsupervised hierarchical clustering was performed after removing probes (i) targeting the X and Y chromosomes, (ii) containing a single-nucleotide polymorphism within 5 base pairs and including the CpG site, and (iii) not mapping uniquely to the human reference genome (hg19), allowing for one mismatch. The Student t test assuming unequal variances was used to detect probes with significantly different mean methylation between the two groups.

**Biomarker validation**

Two CpGs upstream of the CD95L were screened in an independent validation cohort comprising all patients for whom sufficient DNA was available and who were not part of the discovery cohort (n = 40 patients) using the MassARRAY technique (Sequenom). This technology relies on detection of mass shifts, which are introduced through sequence changes following bisulfite treatment. In short, 500 ng genomic DNA was bisulfite-converted using the Epitect Bisulfite Kit (Qiagen). For PCR amplification, the following primers were used:

aggagaagtATTTTTTGGTTTTGAGAGGAG (forward)

cgataattctactataggagagctACTAACCCTCTTACAAAATCC (reverse)

Next, DNA methylation analysis was performed on a Sequenom mass spectrometer and the results were analyzed by the Epityper software (Version 1.05; Sequenom). For statistical analysis, both CpGs were dichotomized using their median methylation level, and Cox regression analysis using a model including tumor size (the main prognostic factor in the analysis of the trial data) and treatment (APG101 + radiotherapy vs. radiotherapy alone) was performed.

**Statistical analysis**

The primary endpoint was the proportion of patients free of progression based on the central assessment and alive at 6 months (PFS-6), calculated in days from randomization.

The sample size of the study was planned according to the optimal two-stage design of Simon (28) for the rRT+APG101 arm with a PFS-6 target rate of 30%, a nonintereste rate of 15%, first-type error rate of 0.05, and a power of 80%. A control arm of patients treated with rRT alone was added to the Simon design to calibrate the PFS-6 rate. The sample size of the control arm was defined as 50% of the investigational treatment arm. The Simon design required the recruitment of 55 patients for the rRT+APG101 arm (19 patients in stage I and 36 patients in stage II). With the addition of 28 patients in the control arm, the study was hence planned with a total sample size of 83 patients.

The study was not powered for comparisons between treatment arms. According to the Simon design, the study was considered positive if 13 PFS-6 responses were observed among the 55 patients treated with rRT+APG101, based on the specified target and noninteresting rate.
Secondary efficacy endpoints were: objective RRs, OS, PFS, quality-of-life as determined by EORTC PAL QLQ-C15 and -BN20, and cognitive function determined by MMSE. Safety and tolerability of APG101 were assessed by AEs with intensity mild/moderate/severe. PFS was defined as time from randomization to next progression for patients with progression or, respectively, as time to death of any cause for patients without progression described with Kaplan–Meier estimates. Patients without progression or death were censored at the day of the last assessment of tumor response. The significance level for remarkable findings was set to 0.05 for all tests in this study.

Within the framework of prospectively planned descriptive analyses:

- exact 95% confidence intervals (CI) according to Clopper–Pearson were calculated for the rates within treatment groups and asymptotic 95% CI were presented for the difference of rates between treatment groups. Descriptive treatment comparisons of rates were done by use of a Fisher test.
- Kaplan–Meier estimates (3) were used to describe PFS and OS and derive median survival times together with 95% CI. Cox regression models including prognostic factors tumor size (29) and proliferation rate, Karnofsky performance status (60–80 vs. 90–100), CD95 status (proportion of moderate + high), and CD95L status (proportion of moderate + high) as covariates were fitted to PFS and OS data to obtain estimates of treatment hazard ratios (HR) and corresponding 95% CI.

All analyses of the primary and secondary efficacy endpoints were based on the intention-to-treat (ITT) population, which included all randomized patients except patients who did not receive any dose of trial medication or rRT after randomization. The per-protocol analyses were limited to patients without major protocol violations (Fig. 1).

The safety analyses were done on the entire documentation of AEs (details in Supplementary Methods). Changes in Quality of Life scores with respect to baseline were classified as improved, unchanged, and worsened. Asymptotic 95% CI were calculated for the differences in improvement rates between treatment groups by visit.

Analyses were performed with SAS 9.1.3 (SAS Institute). During the study, the data were documented into the Oracle Clinical data management system of Premier Research (Darmstadt, Germany). Premier Research monitored the data quality. This trial is registered with ClinicalTrials.gov (NCT01071837).

Results

Patients

The trial enrolled and randomized 91 patients. The ITT population included 84 patients who were randomized and received at least one dose of APG101 or rRT. The per-protocol population consisted of 72 patients (Fig. 1). As of the data cutoff date (June 7, 2013), median follow-up was 11.4 months in both treatment arms. Baseline patient and disease characteristics were well balanced (Table 1).

Tolerability and toxicity

Most patients tolerated both treatments well. Toxicities are listed in Table 2. There were three patients in the rRT arm who discontinued rRT due to disease progression and one patient receiving 20 fractions. All other patients (22 of 26; 84.6%) received the planned 18 × 2 Gy. In the rRT + APG101 arm, all patients except of 1 received the planned RT (57 of 58; 98.3%). The median duration of APG101 treatment was 3.6 months (range, 0.1–24 months). Discontinuations from the study occurred because of disease progression (67 of 84; 79.8%), withdrawal of consent (2 of 84; 2.4%), investigator judgment (7 of 84; 8.3%), withdrawal from treatment (4 of 84; 4.8%), and other reasons (4 of 84; 4.8%).

Efficacy outcomes

At a minimal follow-up of 6 months [median 11.4 months (range, 2–36+ months)] after the last patient had
<table>
<thead>
<tr>
<th>AE by MedDRA preferred term</th>
<th>APG101 ^a (n = 58)</th>
<th>APG101 (n = 26)</th>
<th>Total (n = 84)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mild</td>
<td>Mod.</td>
<td>Severe</td>
</tr>
<tr>
<td>Any AE</td>
<td>9 (15.5)</td>
<td>27 (46.6)</td>
<td>22 (37.9)</td>
</tr>
<tr>
<td>Nervous system disorders</td>
<td>12 (20.7)</td>
<td>31 (53.4)</td>
<td>11 (19.0)</td>
</tr>
<tr>
<td>Aphasia</td>
<td>2 (3.4)</td>
<td>2 (3.4)</td>
<td>3 (5.2)</td>
</tr>
<tr>
<td>Brain edema</td>
<td>2 (3.4)</td>
<td>7 (12.1)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Cognitive disorder</td>
<td>2 (3.4)</td>
<td>5 (8.6)</td>
<td>2 (3.4)</td>
</tr>
<tr>
<td>Convulsion</td>
<td>2 (3.4)</td>
<td>5 (8.6)</td>
<td>3 (5.2)</td>
</tr>
<tr>
<td>Coordination abnormal</td>
<td>3 (5.2)</td>
<td>6 (10.3)</td>
<td>2 (3.4)</td>
</tr>
<tr>
<td>Headache</td>
<td>13 (22.4)</td>
<td>13 (22.4)</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td>Hemiparesis</td>
<td>3 (5.2)</td>
<td>4 (6.9)</td>
<td>3 (5.2)</td>
</tr>
<tr>
<td>Hypoaesthesia</td>
<td>8 (13.8)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Motor dysfunction</td>
<td>2 (3.4)</td>
<td>5 (8.6)</td>
<td>2 (3.4)</td>
</tr>
<tr>
<td>Neurologic decomposition</td>
<td>0 (0.0)</td>
<td>4 (6.9)</td>
<td>2 (3.4)</td>
</tr>
<tr>
<td>Partial seizures</td>
<td>5 (8.6)</td>
<td>6 (10.3)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>General disorders and administration site conditions</td>
<td>12 (20.7)</td>
<td>14 (24.1)</td>
<td>7 (12.1)</td>
</tr>
<tr>
<td>Disease progression</td>
<td>1 (1.7)</td>
<td>1 (1.7)</td>
<td>4 (6.9)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>4 (6.9)</td>
<td>11 (19.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>General physical health deterioration</td>
<td>0 (0.0)</td>
<td>4 (6.9)</td>
<td>2 (3.4)</td>
</tr>
<tr>
<td>Gastrointestinal disorders</td>
<td>20 (34.5)</td>
<td>7 (12.1)</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td>Nausea</td>
<td>7 (12.1)</td>
<td>4 (6.9)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>7 (12.1)</td>
<td>2 (3.4)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Musculoskeletal and connective tissue disorders</td>
<td>11 (19.0)</td>
<td>9 (15.5)</td>
<td>1 (1.7)</td>
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<tr>
<td>Pain in extremity</td>
<td>4 (6.9)</td>
<td>3 (5.2)</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td>Investigations</td>
<td>10 (17.2)</td>
<td>6 (10.3)</td>
<td>2 (3.4)</td>
</tr>
<tr>
<td>Karnofsky scale worsened</td>
<td>5 (8.6)</td>
<td>2 (3.4)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Psychiatric disorders</td>
<td>9 (15.5)</td>
<td>6 (10.3)</td>
<td>2 (3.4)</td>
</tr>
<tr>
<td>Depression</td>
<td>3 (5.2)</td>
<td>2 (3.4)</td>
<td>1 (1.7)</td>
</tr>
</tbody>
</table>

Abbreviations: N, number of patients; Mod, moderate.

^aPatients in the rRT + APG101 arm have been seen weekly during the post-rRT phase until progression, whereas patients in the rRT arm were seen 6-weekly.
been randomized, 84 patients were evaluable for the primary endpoint. In the control arm, rRT resulted in a PFS-6 rate of 3.8% (95% CI, 0.1–19.6), that is, one patient was free of progression, whereas PFS-6 in the rRT+APG101 arm was 20.7% (95% CI, 11.2–33.4), that is, 12 patients, one less than prespecified, were free of progression. The difference in PFS-6 rates was 16.9% (95% CI, 4.1–29.6; \( P = 0.0485, \chi^2 \) test). These data were confirmed in the central review.

Efficacy of rRT+APG101 was also suggested by the analysis of the per-protocol population (Fig. 1) for PFS-6 with 4.8% (95% CI, 0.1–23.8) and 21.6% (95% CI, 11.3–35.3; \( P = 0.1606 \)).

Median PFS was 2.5 months (95% CI, 2.3–3.8) and 4.5 months (95% CI, 3.7–5.4, \( P = 0.0162 \); Fig. 2A).

In the univariate analysis, median OS was 11.5 (95% CI, 6.5–15.4) months in the rRT+APG101 and 11.5 (95% CI, 8.8–16.2) months in the rRT arm (Fig. 2B). After correcting for tumor size, the HR for the secondary endpoint OS was 0.60 (95% CI, 0.36–1.01; \( P = 0.0559 \); Table 3).

In both arms, all patients experienced progression in the observation interval of the study. Pseudoprogression (21) was reported in 19 of 26 (73.1%) patients in the rRT and in 43 of 58 (74.1%) patients in the rRT+APG101 arm. It was confirmed in 8 of 19 and 22 of 43 patients, respectively (see Supplementary Table S1). Patients in both arms had similar types and frequencies of salvage therapies. These postprogression treatments are listed in the Supplementary Table S2.

Prognostic and predictive factors
Tumor size \( \leq 2.5 \) cm at rRT was a prognostic factor for OS with HR = 0.45 (95% CI, 0.27–0.75; \( P = 0.0022 \)) and for PFS with a HR = 0.61 (95% CI, 0.35–1.05; \( P = 0.0744 \); Table 3), but not proliferation rate or KPS. Tumor tissue was analyzed for IDH1 R132H mutation (7 of 84; 8.4%; Supplementary Table S3), MGMT promoter methylation (57 of 84; 67.9%; Supplementary Table S4), expression of CD95 (Supplementary Table S6) and the APG101 target CD95L (Supplementary Table S7), as well as CpG methylation analysis upstream of CD95L (Supplementary Fig. S1). Lower methylation at CpG2 in the CD95L promoter was a positive prognostic factor for PFS and OS in the rRT+APG101 arm (Supplementary Table S8 and Supplementary Fig. S2).

Quality-of-life assessments
Health-related quality of life (22) data were available from 92% of all patients. No clinically meaningful or statistically remarkable differences between the two groups over time in any of the scales or cohorts were observed. Baseline values and the last values available are depicted in Supplementary Table S9.

Discussion
The present trial evaluated a novel therapeutic approach for recurrent glioblastoma aiming to silence the proinvasive CD95/CD95L system. The fusion protein APG101 hereby acts as a CD95L scavenger. The addition of APG101 to rRT produces a promising number of patients with first or second progression of a glioblastoma eligible for rRT without progression at 6 months after randomization. Within the borders of the randomized non-comparative design of our trial, there is a beneficial PFS signal and also promising OS data after correction for tumor size. Patients with low methylation at CpG2 upstream of CD95L seemed to have a greater benefit from the addition of APG101 to rRT (Supplementary Fig. S2). Thus, CD95L promoter methylation may be developed as a selection marker. Expression of CD95L (Supplementary Fig. S3) seems to be associated with impaired prognosis in other malignancies as well (30).
The main reason for a poor outcome in glioblastoma is therapy resistance, the highly invasive behavior as well as the local immunosuppression. Although for patients with newly diagnosed glioblastoma the current standard of care is radiochemotherapy with temozolomide, no such standard exists for progressive disease. Therapeutic options at recurrence depend on the individual disease situation and include reoperation, rRT, alkylating chemotherapy with temozolomide or nitrosoureas, bevaxizumab, and experimental agents within clinical trials. Approaches targeting the pathologic tumor vasculature are numerous and bevaxizumab was approved in the United States and in countries outside the European Union. All other compounds addressing this concept recently failed in phase II/III evaluations, including the VEGF receptor 2 inhibitor cediranib (4), the protein kinase C inhibitor enzastaurin (6), the VEGF trap aflibercept (31), and the integrin inhibitor cilengitide (32) as well as a series of trials analyzing the efficacy of epidermal growth factor receptor inhibition (33). By targeting the invasive growth, APG101 addresses a pathologic hallmark of glioblastoma different from all previous approaches.

In uncontrolled series of rRT with fractionated stereotactic rRT (9, 10) or stereotactic radiosurgery (34), rRT appeared as a relatively safe and effective approach in well-selected patient groups as one option for salvage therapy. Because exposure of glioblastoma cells to RT induces upregulation of CD95L, the combination of rRT with APG101 should improve the efficacy of RT, as was demonstrated in preclinical experiments (35).

In the current trial, the PFS-6 rate (12 of 56 patients) of rRT+APG101 was remarkable given the prespecified target (13 of 56 patients) corrected for the performance of the rRT arm that was lower than assumed, but well within the range for recurrent therapy trials. Patients received similar post-progression treatments in both study arms (Supplementary Table S2). The positive PFS signal (Fig. 2A) also translates into a meaningful OS benefit in the rRT+APG101 arm (Fig. 2B). The macroscopic diameter of the tumor, as determined by MRI, is strongly prognostic. Hence, a neurosurgical reduction of the tumor size may be an adequate measure enabling patients to achieve a greater benefit from the combined treatment with APG101+rRT.

RRT+APG101 was well tolerated (Table 2). There were no serious AEs causally related to APG101, and APG101 did not impair tolerability of rRT resulting in a favorable risk/benefit assessment. Radiotherapy increases the permeability of the BBB, which may lead to edema and potentially worsening of neurologic symptoms. An increased BBB permeability may facilitate APG101 influx into the tumor stroma and the invasive tumor front.

The present phase II proof-of-concept study was designed to identify a therapeutic effect of APG101 when combined with rRT in the treatment of progressive glioblastoma. Thus, the study population was selected for smaller tumors and a long interval from first or second progression as needed for a second RT. The latter is documented by the high number of patients with MGMT promoter methylation (Table 1). There were insignificant imbalances in favor of the rRT+APG101 arm with more IDH-mutant tumors and in favor of the rRT arm with smaller tumors (Table 1). However, only 2 of the patients who reached PFS-6 had an isocitrate dehydrogenase (IDH)-mutant tumor. Every effort was made to ensure high quality and comparability of therapeutic and diagnostic measures applied during the study. MRI and RT dummy runs had to be completed by all sites and all sites had to obtain central approval before participation in the study. MRIs were assessed centrally in a blinded fashion and a strict algorithm to identify pseudo-progression was used. All measures ensured the accuracy of the observed therapeutic effects; however, the effect of rRT on PFS is at the lower end of what was reported in scientific publications.

The examination of tumor tissue for the expression of CD95 and CD95L was specified in the study protocol and complemented by the methylation analyses. Both tests were carried out on identical archival tumor samples obtained during surgery at the time of diagnosis and provided quantitative data with an easy to reproduce PCR-based assay. Patients with low methylation levels at CpG2 upstream of IDH showed the best response to treatment with APG101. These data demonstrate first signs of efficacy of APG101 in combination with rRT. Given the limited options at progression of glioblastoma, rRT+APG101 may represent a therapeutic chance for the subset of patients with the option for second RT. Clinical studies administering...
APG101 in combination with radiochemotherapy in newly diagnosed glioblastoma patients will fully exploit the potential of CD95/CD95L inhibition in this disease. In these trials, CD95L promoter methylation will need validation as a potential selection biomarker.

Disclosure of Potential Conflicts of Interest

W. Wick reports receiving a commercial research grant from Boehringer Ingelheim and Roche, speaker’s bureau honoraria from Prime Oncology; and is a consultant/advisory board member for Eli Lilly and Co. and Roche. B. Wiestler is a coinventor of a patent on Neutralization of CD95 activity blocks invasion of glioblastoma cells in vitro, which is owned by the German Cancer Research Center and licensed to Apogenix. J. Debus and C. Hartmann report receiving commercial research grants from Apogenix. C. Kunz and Harald Fricke are employees of Apogenix. No potential conflicts of interest were disclosed by the other authors.

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Acknowledgments

The authors are indebted to the patients and their families for agreeing to participate in this trial, as well as to the nurses and data managers for their collaboration. The authors gratefully acknowledge the expertise and time of the members of the study’s DSMB (Chair Prof. Roger Supp [Zurich, Switzerland]; members Prof. Minesh Mehta [University of Maryland Medical Center, Baltimore, MD] and Prof. Lee J. Hellman [National Cancer Institute, Bethesda, MD]); Premier Research (Darmstadt, Germany) served as CRO for monitoring and data collection. The authors also thank Matthias Schick and Roger Fischer from the DKFZ Genomics Core Facility for performing the microarray analyses to a very high standard.

Grant Support

This work was supported by Apogenix GmbH. The methylation analyses were supported by the Heidelberg Center for Personalized Oncology and Precision Oncology Program (DKFZ HIPO and NCT POP; PO9-024).

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Received April 17, 2014; revised August 19, 2014; accepted September 4, 2014. Published OnlineFirst October 22, 2014.
Syk kinase to trigger their recruitment to the inflammatory site. Immunity 2010;32:240–52.
Clinical Cancer Research

A Phase II, Randomized, Study of Weekly APG101+Reirradiation versus Reirradiation in Progressive Glioblastoma

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