A phase II, randomised, study of weekly APG101 + reirradiation versus reirradiation in progressive glioblastoma

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CONFLICT OF INTEREST

W.W. reports on having received consulting and lecture fees from MSD and Roche.

W.W. has received research support from Apogenix, Boehringer Ingelheim, Eli Lilly,
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MSD, and Roche. He serves on the Steering Committee of the AVAglio trial involving bevacizumab in glioblastoma.

H.F. (COO and CMO Apogenix) and C.K. are employees of the company developing the study drug and sponsoring the trial.


**Manuscript statistics:** 4,189 words; 190 words abstract; 35 references; 3 Tables and 2 Figures plus *Supplementary Information*

**Statement of Translational Relevance**

Inhibiting rather than inducing CD95 activity is a break-of-paradigm treatment approach for malignant gliomas. APG101 in combination with radiotherapy is a concept with good tolerability and clinical efficacy in patients with progressive glioblastoma. *CD95L* promoter methylation in the tumour tissue may be developed as a biomarker.
ABSTRACT

Purpose: Preclinical data indicate antiinvasive 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 randomised 1:2 between second radiotherapy (rRT) (36 Gy; 5 times 2 Gy per week; rRT) or rRT+APG101 (400 mg weekly i.v.). Patient characteristics [N=84 (26 patients rRT, 58 patients rRT + APG101)] were balanced.

Results: PFS-6 rates were 3.8% (95%-CI: 0.1 - 19.6) rRT and 20.7% (95%-CI: 11.2 - 33.4) for rRT+APG101 (p=0.048). Median PFS was 2.5 (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 tumour size. Cox regression analysis adjusted for tumour size revealed a HR 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 tumour 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 tumour may be developed as a biomarker.

Keywords: brain tumour, progression, glioblastoma, reirradiation, CD95 ligand

ClinicalTrials.gov identifier: NCT01071837

Other Study ID Numbers: APG101_CD_002, EudraCT No. 2009-013421-42r

Sponsor: Apogenix GmbH, Heidelberg, Germany
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-beta 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, e.g. 18 x 2 Gy, 15 x 2.33, 6 x 5 Gy, exist.

CD95 (Fas, APO-1) is a pleiotrophic receptor that regulates tissue homeostasis. During cancer progression, CD95 is frequently down-regulated or tumour 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 signalling 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 a therapeutic target (21). Single ascending doses of APG101 up to 20 mg/kg body weight (bw) administered as infusion over 1 h were considered as safe and well tolerated in healthy volunteers in a phase I study. No MTD has been reached and no anti-drug-antibodies (ADA) were detected. After the application of multiple doses of 400 mg in two glioma patients 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 glioblastoma patients (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.
METHODS

Patients

Adult patients with first or second progression of a histologically confirmed glioblastoma either not being eligible for tumour resection or having macroscopic residual tumour after tumour 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 prior to enrolment) were allowed. All patients were required to give signed informed consent prior to enrolment.

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 randomised on Sep 21, 2011. Patients were centrally randomised 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 manuscript, the collection of OS data is still ongoing. The study adhered to the Declaration of Helsinki and the Guideline for Good Clinical Practice (ICH-GCP). Intensity of adverse events was assessed as mild/moderate/severe.

**Radiation Therapy**

rRT at 36 Gy in 2 Gy single fractions was required to be performed as highly precise treatment, either as stereotactic radiotherapy, or as image-guided radiotherapy. To assess the quality of each participating centre, a dedicated dummy run evaluating technical equipment, quality assurance as well as treatment planning was performed. Only centres fulfilling all requirements were eligible to recruit patients into the trial. To ensure protocol-conform treatment planning, two test patients were distributed to each centre 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). In detail, as the basic inclusion criterion was an indication for rRT, any recurrence in- or outside the radiation field that occurred ≥8 months out of the initial RT was principally eligible. However, since >90% of the lesions occur inside the radiation volume, all except one patient, who had a recurrence at the 20% isodose had a pre-RT at a similar region. For treatment planning, CT as well as contrast-enhanced MRI were mandatory. Target volume delineation was defined to include the gross tumour volume (GTV) defined as the contrast-enhancing lesion on MRI, adding 1 cm
safety margin for potential microscopic spread (clinical target volume, CTV). The recommended total dose was 36 Gy in 2 Gy single fractions. After treatment, treatment plans of study patients were reviewed centrally in Heidelberg and protocol adherence confirmed for all patients.

**APG101**

APG 101 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 prior to rRT due to potential compliance reasons, e.g. patient might be tired after the RT and therefore refuse the APG101 infusion at this day, which should be avoided.

**Randomisation and Masking**

Participant allocation was done according to an electronically generated randomisation list stratified for maximum tumour diameter ($\leq 2.5$ cm *versus* $> 2.5$ cm), a risk factor established for reirradiation (9). The sequence was generated prior to study start at the Contract Research Organisation (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.J.) prior to any analysis of the data.

**Evaluations**

Baseline examinations included physical examination, vital signs, MRI, full blood cell counts, blood chemistry, serology, (HbsAG, anti-HCV, anti-HIV), urine
analysis, ECG, abdominal ultrasound, Mini-Mental State Examination (MMSE), structured neurological 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/adverse events, safety lab (blood chemistry, 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 which included physical examination, MRI, MMSE, structured neurological 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. Tumour 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 tumour 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 pseudoprogression. 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
prespecified analyses of all patients were done for all irradiation plans (S.E.C. and J.D.) and tissue quality prior to molecular examinations (C.H. and A.v.D.) in a blinded fashion (25).

**Neuropathological Methods**

Archived tumour tissue was available from 81 patients. This tumour tissue was used to examine *IDH1*, *MGMT*, CD95 and CD95L as well as genome-wide methylation levels in a discovery set of 20 tumour 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 analysed 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.H.). Vital tumour 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 tumour was assigned to a specific value in percent 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 cut-off at 0.85 was used (see Supplementary Methods and Tables S3-8).

**Biomarker identification**

To identify epigenetic differences distinguishing responders from non-responders, we performed genome-wide assessment of DNA methylation using the HumanMethylation450 BeadChip (Illumina, San Diego, CA, USA) 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 of and including the CpG site and (iii) not mapping uniquely to the human reference genome (hg19), allowing for one mismatch. Student’s 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 CD95 ligand were screened in an independent validation cohort comprising all patients for whom sufficient DNA was available and which were not part of the discovery cohort (n = 40 patients) using the MassARRAY technique (Sequenom, San Diego, CA, USA). 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, Hilden, Germany). For PCR amplification, the following primers were used:

aggaagagagTTATTTTGTAGTTGAAGTTGAGAAG (forward)
cagtaatacgactcactatagggagaaggctACTAACCTACTCTACAAAATCCC (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, San Diego, CA, USA). 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 six months (PFS-6), calculated in days from randomisation.

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 non-interesting 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 1 and 36 patients in Stage 2). 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 non-interesting rate.

Secondary efficacy endpoints were: objective response rates, OS, PFS, quality-of-life as determined by EORTC PAL QLQ-C15 and BN-20, and cognitive function determined by MMSE. Safety and tolerability of APG101 were assessed by adverse events with intensity mild/moderate/severe. PFS was defined as time from randomisation 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 tumour 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 versus 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 and corresponding 95%-CI.
All analyses of the primary and secondary efficacy endpoints were based on the intention-to-treat population, which included all randomised patients except patients who did not receive any dose of trial medication or rRT after randomisation. The *per-protocol* analyses were limited to patients without major protocol violations (Fig. 1).

The safety analyses were done on the entire documentation of adverse events (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, Cary, NC). 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 randomised 91 patients. The ITT population included 84 patients who were randomised and received at least one dose of APG101 or rRT. The per-protocol population consisted of 72 patients (Fig. 1). As of the data cut-off 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 (22/26, 84.6%) received the planned 18 x 2 Gy. In the rRT+APG101 arm, all patients except of one received the planned RT (57/58, 98.3%). The median duration of APG101 treatment was 3.6 months [range: 0.1-24 months]. Discontinuations from the study occurred due to disease progression (67/84, 79.8%), withdrawal of consent (2/84, 2.4%), investigator judgment (7/84, 8.3%), withdrawal from treatment (4/84, 4.8%) and other reasons (4/84, 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 been randomised, 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), i.e. one patient was free of progression, whereas PFS-6 in the rRT+APG101 arm was 20.7% (95%-CI: 11.2 - 33.4), i.e. 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-Square test). These data were confirmed in the central review.

Efficacy of rRT+APG101 was also suggested by the analysis of the per-protocol population (Figure 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 overall survival (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 tumour 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. Pseudoprogession (21) was reported in 19/26 (73.1%) patients in the rRT and in 43/58 (74.1%) patients in the rRT+APG101 arm. It was confirmed in 8/19 and 22/43 patients, respectively (see Supplementary Table S1). Patients in both arms had similar type and frequency of salvage therapies. These post-progression treatments are listed in the Supplementary Table S2.

**Prognostic and Predictive Factors**

Tumour size ≤ 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. Tumour tissue was analysed for IDH1 R132H mutation (7/84, 8.4%, Supplementary Table S3), MGMT promoter
methylation (57/84, 67.9%, Supplementary Table S4), expression of the APG101 targets CD95 (Supplementary Table S6) and CD95L (Supplementary Table S7), as well as CpG methylation analysis upstream of CD95L (Supplementary Figure 1). 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 Figure 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 pro-invasive 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 six months after randomisation. Within the borders of the randomised non-comparative design of our trial, there is a beneficial PFS signal and also promising OS data after correction for tumour size. Patients with low methylation at CpG2 upstream of \(CD95L\) seemed to have a greater benefit from the addition of APG101 to rRT (Supplementary Figure 2). Thus, \(CD95L\) promoter methylation may be developed as a selection marker. Expression of \(CD95L\) (Supplementary Figure 3) 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 behaviour as well as the local immunosuppression. While 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, bevacizumab and experimental agents within clinical trials. Approaches targeting the pathological tumour vasculature are numerous and bevacizumab was approved in the US and in countries outside the EU. 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 analysing the
efficacy of epidermal growth factor receptor inhibition (33). By targeting the invasive growth, APG101 addresses a pathological 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. Since 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 pre-clinical experiments (35).

In the current trial, the PFS-6 rate (12/56 patients) of rRT+APG101 is remarkable given the prespecified target (13/56 patients) corrected for the performance of the rRT arm which 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 tumour as determined by MRI is strongly prognostic. Hence, a neurosurgical reduction of the tumour 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 favourable risk/benefit assessment. Radiotherapy increases the permeability of the BBB, which may lead to oedema and potentially worsening of neurological symptoms. An increased BBB permeability may facilitate APG101 influx into the tumour stroma and the invasive tumour 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 tumours 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 favour of the rRT+APG101 arm with more IHD mutant tumours and in favour of the rRT arm with smaller tumours (Table 1). However, only two of the patients who reached PFS-6 had an 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 prior to participation in the study. MRIs were assessed centrally in a blinded fashion and a strict algorithm to identify pseudoprogression 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 tumour 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 archived tumour 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 CD95L 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.
FUNDING

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

We 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.

We gratefully acknowledge the expertise and time of the members of the study’s DSMB [Chair Prof. Roger Stupp, MD (Zurich, Switzerland); members Prof. Minesh Mehta, MD (University of Maryland Medical Center, Baltimore, USA) & Prof. Lee J. Hellman, MD (National Cancer Institute, Bethesda, USA)].

Premier Research (Darmstadt, Germany) served as CRO for monitoring and data collection.

We thank Matthias Schick and Roger Fischer from the DKFZ Genomics Core Facility for performing the microarray analyses to a very high standard.


CONTRIBUTORS
The concept of the trial was developed by H.F. and W.W. The protocol was drafted by C.K., H.F., K.J. and W.W.
All data were collected by Premier Research and reviewed in the parts respective to the individual responsibility by H.F., C.K., M.B., S.C., J.D. and W.W.
The statistical analyses were performed by K.J., a statistician at Premier Research.
Histological specimens were reviewed centrally at the Department of Neuropathology at the University of Heidelberg Medical Centre by C.H. and A.v. D. They also carried out the IDH1 analysis, CD95L staining and MGMT promoter analysis.
Genome-wide methylation analysis and CD95L promoter MassARRAY were performed by B.W. and M.G.S.
REFERENCES


**Table 1. Baseline patient and disease characteristics**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>rRT (n=26)</th>
<th>rRT + APG101 (n=58)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age, y (range)</td>
<td>59 (25-79)</td>
<td>57 (20-73)</td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>12 (46.2)</td>
<td>39 (67.2)</td>
</tr>
<tr>
<td>Karnofsky performance status, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60-80</td>
<td>8 (30.8)</td>
<td>17 (29.3)</td>
</tr>
<tr>
<td>90-100</td>
<td>18 (69.2)</td>
<td>41 (70.7)</td>
</tr>
<tr>
<td>MGMT status, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methylated</td>
<td>15 (57.7)</td>
<td>41 (70.6)</td>
</tr>
<tr>
<td>Non-methylated</td>
<td>8 (30.8)</td>
<td>14 (24.1)</td>
</tr>
<tr>
<td>Missing</td>
<td>3 (11.5)</td>
<td>3 (5.2)</td>
</tr>
<tr>
<td>IDH status, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mutated</td>
<td>0</td>
<td>6 (10.3)</td>
</tr>
<tr>
<td>Wild type</td>
<td>25 (96.2)</td>
<td>49 (84.5)</td>
</tr>
<tr>
<td>Missing</td>
<td>1 (3.8)</td>
<td>3 (5.2)</td>
</tr>
<tr>
<td>Recurrence status, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First</td>
<td>19 (73.1)</td>
<td>41 (70.7)</td>
</tr>
<tr>
<td>Second</td>
<td>6 (23.1)</td>
<td>15 (25.9)</td>
</tr>
<tr>
<td>Third</td>
<td>1 (3.8)</td>
<td>2 (3.4)</td>
</tr>
<tr>
<td>Mean time since first diagnosis, months (standard deviation)</td>
<td>20.3 (11.7)</td>
<td>23.9 (14.8)</td>
</tr>
<tr>
<td>Tumour diameter, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 2.5 cm</td>
<td>20 (76.9)</td>
<td>29 (50)</td>
</tr>
<tr>
<td>&gt; 2.5 cm</td>
<td>6 (23.1)</td>
<td>29 (50)</td>
</tr>
<tr>
<td>CD95L status, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>16 (61.5)</td>
<td>39 (67.2)</td>
</tr>
<tr>
<td>Negative</td>
<td>8 (30.8)</td>
<td>16 (27.6)</td>
</tr>
<tr>
<td>Missing</td>
<td>2 (7.7)</td>
<td>3 (5.2)</td>
</tr>
</tbody>
</table>

CD95L, CD95 ligand; IDH, isocitrate dehydrogenase; MGMT, O6-methylguanine-DNA methyltransferase
Table 2. Number of Patients with Adverse Events by Maximal Severity and MedDRA Preferred Term (Frequency of AEs ≥ 10.0% of the Total Patients by Preferred Term)

<table>
<thead>
<tr>
<th>Number of Patients with AE by MedDRA Preferred Term</th>
<th>APG101 + RT (N=58) N (%)</th>
<th>RT (N=26) N (%)</th>
<th>Total (N=84) N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mild</td>
<td>Mod.</td>
<td>Severe</td>
</tr>
<tr>
<td>Any Adverse Event</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>9 (15.5)</td>
<td>27 (46.6)</td>
<td>22 (37.9)</td>
</tr>
<tr>
<td>Nervous System Disorders</td>
<td></td>
<td></td>
<td></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 Oedema</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>- Hypoesthesia</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>0 (0.0)</td>
</tr>
<tr>
<td>- Neurological decompensation</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></td>
<td></td>
<td></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></td>
<td></td>
<td></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>
</tbody>
</table>

continued
Wick et al. APG101 + rRT

<table>
<thead>
<tr>
<th>Number of Patients with AE by MedDRA Preferred Term</th>
<th>APG101 + RT (N=58) N (%)</th>
<th>RT* (N=26) N (%)</th>
<th>Total (N=84) N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mild</td>
<td>Mod.</td>
<td>Severe</td>
</tr>
<tr>
<td>Musculoskeletal and Connective Tissue Disorders</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>11 (19.0)</td>
<td>9 (15.5)</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td>Mod.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain in extremity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>4 (6.9)</td>
<td>3 (5.2)</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td>Mod.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Investigations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>10 (17.2)</td>
<td>6 (10.3)</td>
<td>2 (3.4)</td>
</tr>
<tr>
<td>Mod.</td>
<td>5 (8.6)</td>
<td>2 (3.4)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Severe</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Karnofsky scale worsened</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>9 (15.5)</td>
<td>6 (10.3)</td>
<td>2 (3.4)</td>
</tr>
<tr>
<td>Mod.</td>
<td>3 (5.2)</td>
<td>2 (3.4)</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td>Severe</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N = number of patients; Mod = moderate

*: Patients in the rRT+APG101 arm have been seen weekly during the post-RT phase until progression whereas patients in the rRT arm were seen six-weekly.
Table 3. Efficacy Outcomes (ITT)*

<table>
<thead>
<tr>
<th></th>
<th>rRT n = 26</th>
<th>rRT + APG101 n = 58</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFS-6 rate (95%-CI)</td>
<td>3.8% (0.1 - 19.6)</td>
<td>20.7% (11.2 - 33.4)</td>
</tr>
<tr>
<td>Median PFS, months, (95%-CI)</td>
<td>2.5 (2.3 - 3.8)</td>
<td>4.5 (3.7 - 5.4)</td>
</tr>
<tr>
<td>Median PFS, HR, (95%-CI)</td>
<td></td>
<td>0.49 (0.27 - 0.88)</td>
</tr>
<tr>
<td>(p=0.0162 after correction for tumour size)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median OS, months, (95%-CI)</td>
<td>11.5 (6.5 - 15.4)</td>
<td>11.5 (8.8 - 16.2)</td>
</tr>
<tr>
<td>Median OS, HR, (95%-CI)</td>
<td></td>
<td>0.60 (0.36 - 1.01)</td>
</tr>
<tr>
<td>(p=0.0559 after correction for tumour size)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*exploratory analyses due to the non-comparative design of the trial
FIGURE LEGENDS

**Figure 1. Trial design and CONSORT flow chart.** Patients were randomized 1:2 to receive rRT or rRT+APG101 (Abbreviations: intention-to-treat population, ITT; reirradiation, rRT).

**Figure 2. Kaplan-Meier survival estimates.** Data of PFS (panel a) or OS (panel b) were analyzed by treatment arm.
Figure 1

Screened
histology, recurrence, KPS
n = 107

Randomized
n = 91

rRT
n = 30

rRT+APG101
n = 61

Not exposed to treatment [n=4]

Not exposed to treatment [n=3]

ITT
n = 26

ITT
n = 58

<8 eight months since last prior radiotherapy (n=1)
>4 four days of interruption of irradiation (n=2)
>2 two pre-treatment regimens (n=1)
Prior radiotherapy dose > 60 Gy (n=1)

<8 months since prior radiotherapy (n=1)
<3 three doses of APG101 (n=1)
>2 pre-treatment regimens (n=2)
Only one MRI after end of radiotherapy and not progressive (n=1)
Prior radiotherapy dose > 60 Gy (n=1)
No candidate for re-irradiation (n=1)

Per protocol population
n = 21

Per protocol population
n = 51

Not randomized [n=16]
• Treatment preference
• Not meeting inclusion criteria
• Declined to participate

Screened
histology, recurrence, KPS
n = 107

Randomized
n = 91

rRT
n = 30

rRT+APG101
n = 61

Not exposed to treatment [n=4]

Not exposed to treatment [n=3]

ITT
n = 26

ITT
n = 58

<8 eight months since last prior radiotherapy (n=1)
>4 four days of interruption of irradiation (n=2)
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Prior radiotherapy dose > 60 Gy (n=1)

<8 months since prior radiotherapy (n=1)
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Per protocol population
n = 51

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• Treatment preference
• Not meeting inclusion criteria
• Declined to participate

Screened
histology, recurrence, KPS
n = 107

Randomized
n = 91

rRT
n = 30

rRT+APG101
n = 61

Not exposed to treatment [n=4]

Not exposed to treatment [n=3]

ITT
n = 26

ITT
n = 58

<8 eight months since last prior radiotherapy (n=1)
>4 four days of interruption of irradiation (n=2)
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Prior radiotherapy dose > 60 Gy (n=1)

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Per protocol population
n = 21

Per protocol population
n = 51

Not randomized [n=16]
• Treatment preference
• Not meeting inclusion criteria
• Declined to participate
Figure 2

(a) Progression-free survival

(b) Overall survival
Clinical Cancer Research

A phase II, randomised, study of weekly APG101 + reirradiation versus reirradiation in progressive glioblastoma

Wolfgang Wick, Harald Fricke, Klaus Junge, et al.

Clin Cancer Res  Published OnlineFirst October 22, 2014.

Updated version  Access the most recent version of this article at: doi:10.1158/1078-0432.CCR-14-0951-T

Supplementary Material  Access the most recent supplemental material at: http://clincancerres.aacrjournals.org/content/suppl/2014/10/23/1078-0432.CCR-14-0951-T.DC1

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