

# A Pilot Clinical Study of CUSP9v3: Nine Repurposed Drugs Combined With Temozolomide for the Treatment of Recurrent Glioblastoma

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Anticancer Fund

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**Research Article**

**Keywords:** CUSP9v3, glioblastoma, polypharmacy, repurposing.

**Posted Date:** February 9th, 2021

**DOI:** <https://doi.org/10.21203/rs.3.rs-162394/v1>

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# Abstract

**Purpose:** The dismal prognosis of glioblastoma (GBM) may be related to the ability of GBM cells to develop mechanisms of treatment resistance. We designed a protocol called Coordinated Undermining of Survival Paths combining 9 repurposed non-oncological drugs with metronomic temozolomide - version 3 - (CUSP9v3) to address this issue. The aim of this proof-of-concept clinical trial was to assess the safety of CUSP9v3.

**Study Design:** Ten adults with histologically confirmed GBM and recurrent or progressive disease were included. Treatment consisted of aprepitant, auranofin, celecoxib, captopril, disulfiram, itraconazole, minocycline, ritonavir and sertraline added to metronomic low-dose temozolomide. Treatment was continued until toxicity or progression. Primary endpoint was dose-limiting toxicity defined as either any unmanageable grade 3-4 toxicity or inability to receive at least 7 of the 10 drugs at  $\geq 50\%$  of the per-protocol doses at the end of the second treatment cycle.

**Results:** One patient was not evaluable for the primary endpoint (safety). All 9 evaluable patients met the primary endpoint. Ritonavir, temozolomide, captopril and itraconazole were the drugs most frequently requiring dose modification or pausing. The most common adverse events were nausea, headache, fatigue, diarrhea and ataxia. Progression-free survival after 12 months was 50%.

**Conclusions:** CUSP9v3 can be safely administered in patients with recurrent GBM under careful monitoring. A randomized phase II trial is in preparation to assess the efficacy of the CUSP9v3 regimen in GBM.

## Key Points

- Glioblastoma escapes pharmacological treatment as a result of cellular heterogeneity and resistance mechanisms
- A treatment regimen with 9 different drugs (CUSP9v3) in addition to low-dose metronomic temozolomide was devised to tackle this issue
- CUSP9v3 is safe in patients with recurrent GBM and led to 50% of patients being progression-free 1 year after trial inclusion

## Introduction

As of winter 2020, current standard treatment of glioblastoma (GBM) with neurologically safe maximal resection, irradiation and temozolomide leads to median progression-free survival (PFS) of 8 months. Median overall survival (OS) remains under two years [1, 2].

Recurrence usually takes place within a year after initial treatment. There is no commonly accepted standard of care for recurrent GBM. No regimen has proven to be safe and markedly effective for this condition.

In an attempt to address this unmet need many groups have embarked on an exhaustive systematic search for already-marketed non-oncological drugs that might be able to set the stage for temozolomide to be more effective [3-8]. Our group and others have specifically focused on this potential of repurposed drugs for GBM [9-14].

A complex winnowing process led us to the final selection of the 9 drugs of CUSP9v3 on which we report here the first clinical experiences. Details of that selection process can be found in the three background papers [11-13]. In vitro testing of the drugs selected for CUSP9v3 showed greater activity than did the mainstay chemotherapeutic drug in GBM, temozolomide [10, 12].

Important criteria for drug selection were 1) robustness of preclinical data on GBM growth inhibition, 2) low side effect burden, 3) our own personal clinical familiarity with the drug in its general medicine (non-oncology) role, 4) a broad published, peer-reviewed research database on any candidate drug, 5) availability as a generic, non-proprietary drug and finally 6) lack of predictable serious pharmacological interactions. Regarding this latter point, detailed pharmacodynamic evaluation of the original CUSP9 regimen was carried out initially and updated with each iteration [10-13]. Candidate drugs were discarded if severe drug-drug interactions had been predicted.

The drugs of CUSP9v3 met all these selection criteria. They are listed with their basic pharmacological attributes in Table 1. The Appendix gives a more detailed overview of the clinical and pre-clinical database leading to the respective drug's inclusion in CUSP9v3.

CUSP9v3 uses the anti-nausea drug aprepitant; the anti-rheumatoid arthritis drug auranofin; the anti-hypertensive captopril; the analgesic celecoxib; the alcohol deterrent disulfiram; the antifungal itraconazole; the antibiotic minocycline; the anti-retroviral ritonavir; and the antidepressant sertraline. Remarkably enough, as outlined in the background papers, these drugs all had what we judged to be a strong preclinical database of robust anti-glioma or temozolomide-augmenting effects as well as meeting the inclusion criteria 1 to 6 above. All CUSP9v3 drugs were tested against GBM cell lines with clinically achievable plasma concentrations [10, 12].

CUSP9v3 also comprises low-dose, continuous temozolomide at 20 mg/m<sup>2</sup> body surface area (BSA) p.o. twice daily, without interruption. This choice was based on past trials of various temozolomide schedules. After evaluating the 15 trials reviewed by Chen et al. [15], and comparing these to data of Omuro et al., Clarke et al. and Reynés et al. [16-17] who used temozolomide at 50 mg/m<sup>2</sup> BSA/day without interruption, that of Stockhammer et al. who used 20 mg/m<sup>2</sup> BSA/day [18] and that of Zustovich et al. who used 40 mg/m<sup>2</sup> BSA/day [19], we concluded that any potential advantage of higher dosing was small and offset by a strongly reduced side effect burden associated with a regimen of 50 mg/m<sup>2</sup> BSA/day or less. Kong et al. reported that temozolomide at the dose of 40 mg/m<sup>2</sup> BSA/day was well-tolerated even in patients with Karnofsky Performance Status (KPS) < 70% [20]. We therefore chose 20 mg/m<sup>2</sup> BSA given twice daily, the dose used by Zustovich et al. and Kong et al. [19, 20].

Although the safety profile of each drug of the CUSP9v3 protocol is well-known, safety concerns may arise due to the risk of drug-drug interactions at the pharmacodynamic (e.g., in form of additive toxicity) or pharmacokinetic level (with effects on metabolism or elimination, requiring dose adjustments or drug pausing). PubMed and DrugBank database searches prior to study initiation showed that clinically relevant interactions between CUSP9v3 drugs are expected to occur mainly due to CYP3A inhibition by itraconazole (strong), ritonavir (strong) and aprepitant (moderate) [11, <https://go.drugbank.com/drugs/DB00995>].

The unusual risks of using 10 daily drugs over a protracted period was partially offset by the good safety profile when used as a single drug and the intensity of our monitoring of patients. Risks and the unusual nature of this trial were carefully explained and informed consent was obtained. This study was approved by the institutional review board of Ulm University Hospital (approval number 112/16) and the German competent authority Bundesinstitut für Arzneimittel und Medizinprodukte (BfArM; reference number 4041326) and registered at [clinicaltrials.gov](https://clinicaltrials.gov) (NCT02770378). All patients gave written consent after thorough discussion of risks and alternative treatments. The study fully complied with the 1975 Helsinki declaration, as revised in 2000.

We report here acceptable tolerability and a possible signal of potential benefit from the CUSP9v3 regimen for recurrent or progressive GBM.

## Patients And Methods

### Study Design

This is a pilot trial examining the safety of the CUSP9v3 regimen combined with temozolomide in patients with recurrent or progressive GBM. The primary endpoint was dose-limiting toxicity (DLT), and secondary endpoints were best tumor response, PFS and OS. Dose limiting toxicity was defined as either any unmanageable grade 3-4 toxicity at the end of the second treatment cycle or inability to receive at least 7 of the 10 drugs, all of them being given at  $\geq 50\%$  of the target doses, at the end of the second treatment cycle. Best tumor response was defined as the best therapeutic effect recorded from the start of the treatment until the last follow-up according to Response Assessment in Neuro-Oncology (RANO) criteria [21].

Overall survival was defined as the time in months between the CUSP9v3 induction cycle start date and the date of last follow-up or death of any cause, whichever came first. Patients alive at the time of last follow-up were censored.

Progression-free survival was defined as the time between the CUSP9v3 induction cycle start date and the date of last follow-up, progression according to RANO criteria, or death of any cause, whichever came first. Patients with no progression and alive at the time of last follow-up were censored.

### Sample Size

A sample size of 10 patients was selected to assess the primary endpoint. In this population, we expected a true rate of DLT of 40%. Sequential boundaries were used to monitor the DLT rate with accrual to be halted if excessive numbers of DLTs were seen. A Pocock-type stopping boundary yielded a probability of crossing the boundaries of maximally 10% when the actual rate of DLT was equal to the expected rate of 40% [22]. The boundaries are described in Supplement 1.

## **Patients**

Eligible patients were adults with histologically confirmed GBM and recurrent or progressive disease according to RANO criteria. In 3 cases, study inclusion was allowed based on early recurrence that had not yet met minimal RANO requirements (i.e., 10 mm x 10 mm diameters) but was judged as recurrence by external radiologists and confirmed by the trial's neuroradiologist (Be.S.). Additional key eligibility criteria were: no more than 3 prior episodes of tumor progression, KPS of at least 70%, stable steroid dose for at least 1 week prior to start of study treatment, sufficient interval since last treatment (at least 4 weeks for systemic treatment or surgery, at least 12 weeks for radiotherapy) and no known contraindication to any of the CUSP9v3 drugs.

## **Treatment Regimen**

Treatment initiation encompassed the addition of the 9 drugs to uninterrupted temozolomide as depicted in Supplement 2 and comprised an induction cycle with 2 phases: a low-dose drug-by-drug addition phase followed by an up-dosing phase. Patients were hospitalized during the drug-by-drug addition phase, which lasted 18 days, to monitor tolerability and drug-drug interactions.

In summary, as schematically depicted in Supplement 2, the treatment started with temozolomide (20 mg/m<sup>2</sup> BSA b.i.d.) and aprepitant (80 mg q.d.) on day 1, followed by the addition of 1 drug every 2 days (day 3, day 5 etc.) at the low-dose level. The last drug (auranofin) was added on day 17. On day 19, the up-dosing phase started with the dose of only one drug being increased every 2 days. The doses of temozolomide and aprepitant remained unchanged, 7 drugs were up-dosed only once and 1 drug (ritonavir) was up-dosed twice.

After reaching target doses of all drugs, the regimen remained unchanged until side effects mandated dose modifications and/or drug pausing or until tumor progression occurred. While the study was locked after the last recruited patient had completed 12 months of treatment, patients without tumor progression continued to receive the CUSP9v3 regimen beyond that point.

## **Safety and Dose Modifications**

In addition to the potential drug-drug interactions to be monitored, we assessed the cumulative toxicity of the regimen. Employing the summary of product characteristics of each of the 10 drugs, we were able to identify the side effects most likely to occur during treatment. By developing a simple algorithm based on the frequency of each side effect (from very common [occurs in  $\geq 1/10$  patients] to very rare [occurs in <

1/10,000 patients]), we elaborated a strategy for dose modifications, dose re-escalations, and on-hold rules.

During the induction cycle and the first 2 treatment cycles, adjustments (dose reductions and drug pausing) were allowed to accommodate the patients' individual tolerability of the regimen. These modifications were discussed by a team comprising a neurosurgeon (M-EH), an oncologist (RM-S), a pharmacologist (OZ) and a psychiatrist (REK). For each patient, the regimen tolerated at the time of completion of the second treatment cycle (around day 90) was used to assess the primary endpoint.

## **Response Assessment**

Response to study treatment was determined by neurological examination and contrast-enhanced magnetic resonance imaging (MRI) using the RANO criteria. Assessment was done at week 6, week 10 and then every 8 weeks. We used the best overall response, i.e., the best response recorded from the start of the treatment, as secondary endpoint. Pre-inclusion radiologic lesion expansion rate was measured as the difference of the maximum perpendicular axial diameter product of the largest contrast-enhancing lesion on MRI at study entry and on the directly preceding earlier MRI divided by the days in between.

## **Statistical Analysis**

Study data were initially analyzed by means of descriptive methods using frequencies (absolute and relative values) for categorical data as well as median and range for metric data. The Kaplan-Meier method was used to calculate PFS and OS. The median PFS and OS, respectively, are presented along with their corresponding 95% confidence intervals (CI). The most frequent adverse events (AE), frequency  $\geq 4$  were analyzed and displayed by means of a contingency table and dot chart. The latter diagram is provided as Supplement 3. Specifically, the interactive dot chart shows the association of each of the 10 drugs with the frequency of each AE. Spearman correlation was used to assess the possible association between the number of days of reduced dosing or drug pausing and PFS or OS. All analyses were performed using SAS (version 9.4, [www.sas.com](http://www.sas.com)) and R (version 3.5.2, [www.r-project.org](http://www.r-project.org)).

# **Results**

## **Patients Characteristics**

Ten patients were included between August 2016 and April 2018. A total of 12 patients were screened. One patient could not be included because of high serum transaminases and one because of acute deep vein thrombosis. Demographic characteristics of the 10 included patients are presented in Table 2.

## **Safety**

The defined Pocock-type safety boundaries for stopping the trial were not crossed at any time. Nine patients completed at least 2 treatment cycles. At the end of the second treatment cycle, no patient had experienced any unmanageable grade 3-4 toxicity, and all patients had received at least 7 of the 10 drugs,

given at  $\geq 50\%$  of the target doses. The primary endpoint was therefore met. Most frequently paused were ritonavir (for ataxia and fatigue), temozolomide (for diarrhea, nausea and laboratory abnormalities), captopril (for diarrhea and nausea) and itraconazole (for diarrhea and laboratory abnormalities) while ritonavir (for gait disturbance) and captopril (for fatigue) were most frequently dose-reduced.

Without consideration of Common Terminology Criteria of Adverse Events (CTCAE) grades, the total number of AEs ( $n = 320$ ) was evenly distributed among patients alive or deceased at the time of data lock (162 and 164 events of all grades, respectively). Grade 3 and 4 AEs occurred less frequently in patients alive after 12 treatment cycles (13 vs. 35 events,  $p < 0.001$ ).

All but a single one of the AEs attributed to CUSP9v3 drugs ceased upon pre-specified, targeted dose reduction or drug pausing within a range of 0 to 4 weeks (median 2 weeks; median number of drug modifications necessary to revert an AE: 1.5). None of the AEs that had ceased upon dose reduction or drug pausing recurred after the suspected drugs were reinstated.

In descending order, the 23 most frequently observed (i.e.,  $\geq 4$  times during the entire trial period) AEs and their possible or true relationship to CUSP9v3 study drugs as judged by trial investigators are listed in Supplement 4. Leading by far in terms of frequency were nausea, headache and fatigue with predominantly minor intensities that only very rarely reached grades 3 or 4. In total, the 23 most frequent AEs occurred 211 times with apportionment to CTCAE grades 1 and 2 vs. 3 and 4 of 178 vs. 28 times (86% vs. 14%;  $p < 0.001$ ). Only two types of AEs (i.e., lymphocytopenia and alanine aminotransferase increase) occurred more frequently in grades 3 and 4 than in 1 and 2.

For all except one (i.e., nausea) of the most frequently observed types of AEs, there was at least one CUSP9v3 drug that had no suspected relationship to the respective type of event. On the other end, for only 6 of the 23 most frequent events was a single drug always suspected; in 4 of these AEs, 1-2 other drugs were additionally deemed related but with a considerably lesser frequency. The 23 most frequent AEs were found related to a median of 1 (range: 0-10) drug, highlighting the need for a hierarchical drug order list in terms of dose reduction and drug pausing, a feature which had been successfully pre-specified in the study protocol. As a consequence, it became necessary to halt the entire CUSP9v3 regimen in only 2% of total scheduled treatment days.

The median number of AEs per cycle decreased during progression of the trial, resulting in a roughly 7.5-fold reduction of the median number of AEs in the 12th treatment cycle compared to the induction cycle (Fig. 1). An intuitive graphical depiction of the extent by which each of the CUSP9v3 drugs was associated with AEs of CTCAE grade 1-4 is presented in Supplement 5.

## **Efficacy**

Best overall response was stable disease (SD) in 6 patients and progressive disease (PD) in 4 patients. Median duration of response was 8 (range 1-11) months in responders at the time of data lock. For 5 patients, SD was ongoing at this time point. Three patients developed no detectable tumor on MRI during

study treatment but would not be assigned “complete response” according to RANO because their tumor was “non-measurable” on MRI (i.e., had maximal diameters of < 10 mm x 10 mm) at study entry. However, early recurrence had been initially diagnosed by external radiologists and was confirmed by the trial’s neuroradiologist (Be.S.) on the basis of a > 25% increase of “non-measurable” disease according to RANO.

Progression-free survival and OS are given in Fig. 2 and Fig. 3, respectively. Median PFS was 3.0 months (95% CI 2.2 - NA), and median OS was 7 months (95% CI 3.7 – non-applicable [NA]). Due to the small sample size, an upper CI bound could not be estimated. Progression-free survival at 12 months was 50% (95% CI 27% - 93%), the same applies for OS at 12 months. Table 3 shows each patient’s individual PFS on CUSP9v3.

Spearman’s rank order analysis revealed strong correlations between the applied cumulative CUSP9v3 total dose (in percent of the scheduled dose) and PFS or OS ( $\rho = 0.76$  and  $0.77$ , respectively; Supplement 6). On analysis of each individual drug’s applied cumulative total dose, those of minocycline, sertraline and auranofin each exhibited the highest respective correlations with PFS or OS (data not shown).

Collectively, we observed a dichotomous OS with CUSP9v3 as seen in Table 3. This dichotomy was also seen when comparing the pre-treatment radiologic lesion expansion rate (see *Methods* section for definition; Fig. 4) upon study entry of the deceased (mean =  $24 \text{ mm}^2/\text{d}$ , range  $8.1 - 44.2 \text{ mm}^2/\text{d}$ ) versus the living (mean =  $0.6 \text{ mm}^2/\text{d}$ , range  $0,1-1.5 \text{ mm}^2/\text{d}$ ) patients. Here, Wilcoxon ranked-sum test showed a significant difference between the pre-study lesion expansion rate of the living and deceased patients at the time of data lock ( $p = 0.016$ ).

## Discussion

In studying the last 120 clinical trials in GBM it became apparent that all had robust preclinical data showing benefit of the given drug in xenograft GBM models yet none translated into clinical benefit in phase 3 trials.

We designed CUSP9v3 to address the following attributes of GBM that we believe are the cause of failure of the last 120 clinical trials reported on PubMed.org:

1. Spatial heterogeneity of growth drive dependency;
2. Temporal heterogeneity of these;
3. Existence of mutually supporting cell communities;
4. Compensatory tumor responses to irradiation, chemotherapy by A. vertical clonal selection; B. horizontal sharing of resistance mechanisms via exosomes, etc.;
5. Existence of multiple cross-covering growth driving signaling pathways;

6. Metabolic flexibility in that if one energy source becomes inhibited, reliance is shifted to another;
7. Glioblastoma's enlistment of normal physiologic somatic systems that are normally functioning but pathologically engaged.
8. Entrance into a dormant state.

Our conclusion is that a polypharmaceutical approach is warranted until and unless a "silver bullet" is found.

CUSP9v3 is well-enough tolerated to be started in outpatients and to be fully introduced over a shorter time period than the 35 days that we used. In this pilot trial in recurrent or progressive GBM, we found that 9 carefully selected non-oncological repurposed drugs together with twice daily 20 mg/m<sup>2</sup> BSA temozolomide was safe and generally well-tolerated if individual dose adjustments were performed.

Other trials in pediatric and adult high-grade glioma had reported the safety of various multi-agent regimens combining chemotherapy with repurposed drugs, using a range of 4-7 agents [23-25]. Here we show that it is possible to combine 9 repurposed drugs given careful evaluation of potential drug-drug interactions and cumulative toxicity. Knowing that many non-oncological drugs target pathways relevant to GBM, precision oncology approaches could expand their armamentarium by evaluating non-cancer drugs and combining them with classical cancer drugs [26, 27]. Such was recently reported in a trial in diffuse intrinsic pontine glioma [28].

Strategies targeting cell membrane marker-defined GBM cells may be limited in that growth drive dependency is an "ever shifting target" in GBM [29, 30]. CUSP9v3 is consciously intended as a biomarker-independent approach.

In the small group of participants studied and reported here, longer-than-expected PFS and OS were observed also in patients whose tumors had unfavorable molecular profiles (i.e., absence of *isocitrate dehydrogenase* [*IDH*] mutation and/or of *O<sup>6</sup>-methylguanine-DNA methyltransferase* [*MGMT*] promoter hypermethylation).

While in our trial the cumulative doses of minocycline, sertraline and auranofin each strongly correlated with PFS and OS, the most effective antiproliferative drugs against GBM cells in vitro were ritonavir, disulfiram and auranofin when used in clinically relevant concentrations (Halatsch et al., unpublished data), with auranofin notably listed for both settings.

During the protocol development for this trial, a hierarchical drug list had been developed (based on AE information contained in each drug's summary of product characteristics) that correlated AEs to ranked sequences of drugs to be halved in dose or paused until pre-specified lower CTCAE grades were reached. If that was not the case within 3 days, the next drug on the hierarchical list was halved in dose or paused etc. This strategy proved successful in managing AEs.

Noteworthy, the three AEs most frequently observed in this trial were nausea, headache and fatigue. While a causal relationship between the CUSP9v3 drugs and these cardinal AEs cannot be excluded, these symptoms may also be caused by the underlying disease itself and/or its primary treatment, temozolomide.

In terms of the strong Spearman rank correlation between the applied scheduled cumulative CUSP9v3 total dose and PFS or OS, respectively (Supplement 6), there are several paths of putative causality. In most cases of pronounced CUSP9v3 cumulative dose reductions, tumor progression and the associated decrease of functional status impaired the ability of oral drug intake; in some patients with good functional status, however, specific drug side effects may have indirectly caused tumor progression via (albeit targeted) dose reductions and/or drug pausing. Those patients may benefit from an adaptive trial protocol with a pre-specified “shadow cocktail” designed to selectively replace individually intolerable drugs before or even upon tumor progression, with participants conditionally remaining on-study (in a future trial).

Lastly, a smaller fraction of patients – despite having a good functional status – may find simultaneous oral intake of a multiplicity of separate drugs genuinely challenging. For this latter group, the so-called polypill approach with possible integration of all CUSP9v3 drugs into one 3D-printed polypill (with flexible dosing options) may prove advantageous [31].

While the trial was not designed to assess efficacy of the CUSP9v3 regimen, we observed that 5 patients progressed quickly, dying within a range of 1.5-7 months. The 5 other patients did well on treatment, all 5 having a PFS of 12 or more months (range 12-29 months at the time of data lock).

Such pattern of failure, as seen in the Kaplan-Meier survival curve (Fig. 3) is distinctly unusual in GBM trials. In recurrent GBM, single-agent trials have generally reported PFS at 6 months of 20-30% [32, 33]. The rate of patients being alive and progression-free at 6 months has been suggested as an appropriate surrogate end point for predicting OS [34].

Regarding the correlation between cumulative CUSP9v3 total dose and PFS and OS, possible CUSP9v3 efficacy cannot be causally distinguished from longer survival simply enabling longer treatment duration.

Because of the small number of patients, the observed dichotomy of response is difficult to interpret. One hypothesis is that some or all of the 5 patients with a long PFS had pseudo-progression that erroneously led to inclusion in the study, although a 12-month period of stability would still not be common. The trial did not require histopathological confirmation of recurrence prior to study entry. Therefore, despite radiological judgement and reasonable time periods between the completion of radio- and chemotherapy on the one hand and the beginning of the study on the other hand (12 and 4 weeks, respectively), patients with a favorable course could have had pseudo-progression upon starting CUSP9v3. This issue of pseudo-progression is inherent to non-randomized trials in the recurrent setting.

Another hypothesis is that we are seeing a differential response according to pre-study lesion expansion rate, which would indicate that CUSP9v3 may be particularly effective in patients with slower proliferating tumors and/or lower tumor burden, suggesting that this regimen may have a role in a prophylactic maintenance setting after first-line treatment. The data presented in Fig. 4 would strongly support this notion. While a 12-month PFS of 50% intuitively appears discrepant with a median PFS of 3.0 months, this apparent discrepancy directly results from the pronounced dichotomy of response to CUSP9v3 that was observed in this trial.

A further note of caution in interpreting our results are the relatively favorable prognosis attributes of the patients with recurrent GBM recruited for this pilot study. Of the 5 study patients still alive at 12 months, 3 had tumors with *MGMT* promoter hypermethylation, one of whom was also *IDH*-mutated. The 2 other patients alive at 12 months had tumors without *MGMT* promoter hypermethylation but were 31 years of age or under upon study inclusion. These favorable prognostic factors could contribute to longer-term survival of these patients.

Drug repurposing represents a large source of therapeutic options in cancer [35]. In GBM in particular, notably 76 repurposed drugs were reported as potentially useful in treating GBM [26]. The selection of the 9 drugs to be included in CUSP9v3 was a long iterative process within a conceptual framework which considered the specific and relevant preclinical, pharmacological, and empirical features of each drug in addition to the 5 criteria listed in the Introduction. It should not be assumed that combining other repurposed non-cancer drugs will automatically yield similar results; other regimens may prove more or less toxic or more or less effective.

In the common aggressive cancers, and especially in GBM, phenotypic spatial and temporal heterogeneity, in both stem and non-stem subsets, is a dynamic process responding to treatment interventions and driven further and faster by hypoxia [30, 36-39]. GBM may be considered a collection of mutually interacting, mutually supporting cellular subpopulations demanding the use of a multi-drug combination to achieve prolonged treatment response. As in CUSP9v3 and in Palmer et al. we advocate “the 50-year old hypothesis that a curative cancer therapy can be constructed on the basis of independently effective drugs having non-overlapping mechanisms of resistance, without synergistic interaction, which has immediate significance for the design of new drug combinations” [40].

## Conclusions

We report here the first clinical trial of the CUSP9v3 regimen in recurrent or progressive GBM. The treatment regimen was safe under clinical, laboratory and ECG monitoring. Its results in terms of 12-month PFS and OS can be explained either by pseudo-progression upon study entry, the effect of low-dose metronomic temozolomide in a one-arm trial design, or by the efficacy of the combined study treatment. A randomized multicenter follow-on trial is in preparation to assess efficacy of the CUSP9v3 regimen in GBM.

This pilot study uncovered no unpredicted and no untoward side effects resulting from the CUSP9v3 combination of drugs. There has been a signal of potential positive effect of CUSP9v3 although multiple confounders that are typically present in a small one-arm study warrant confirmation in larger trials.

## Abbreviations

adverse event (AE); body surface area (BSA); confidence interval (CI); Common Terminology Criteria of Adverse Events (CTCAE); dose-limiting toxicity (DLT); glioblastoma (GBM); isocitrate dehydrogenase (IDH); Karnofsky Performance Status (KPS); non-applicable (NA); O<sup>6</sup>-methylguanine-DNA methyltransferase (MGMT); overall survival (OS); progression-free survival (PFS); Response Assessment in Neuro-Oncology (RANO); stable disease (SD).

## Declarations

### Acknowledgments:

None of the authors have any conflict of interest related to this work.

### Author Contributions:

Conceptualization: M-EH, REK, GB, KB;

Data curation: BiS, AH, BM, TH;

Formal analysis: M-EH, BM, BiS, AH, TH;

Funding: M-EH;

Investigation: M-EH, GK-M, OZ, BeS, AS, LM, LB, RM-S, CS, KZ, ZE, PP, AH, MDS, AD, M-AW, TH;

Methodology: M-EH, BM, BeS, GB, TH;

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Supervision: M-EH, REK;

Validation: GB, M-EH;

Writing: M-EH, REK, GB;

Writing revision: M-EH, REK, GB, TH.

### Funding:

Anticancer Fund grant number A37 and CuresWithinReach grant number 102015 (both to M.-E. H.)

### **Conflict of interest statement:**

None of the authors have any conflict of interest to declare.

### **Ethics approval:**

This study was approved by the institutional review board of Ulm University Hospital (approval number 112/16) and the German competent authority, Bundesinstitut für Arzneimittel und Medizinprodukte (BfArM; reference number 4041326) and registered at clinicaltrials.gov (NCT02770378). This study fully complied with the 1975 Helsinki declaration, as revised in 2000.

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## Tables

**Table 1.** Drugs included in CUSP9v3 with selected pharmacological and biological characteristics.

| Drug                     | p450 inhibition | Half-life | Core survival pathway or process inhibited or enhanced      |
|--------------------------|-----------------|-----------|---|
| Aprepitant               | 3A4, 2C9        | 10 h      | NK-1 receptors  |
| Auranofin                | None            | 10 d      | Thioredoxin, ROS, STAT3                                     |
| Captopril                | None            | 2 h       | ACE, AT1 receptors, MMPs                                    |
| Celecoxib                | 2C9, 3A4        | 12 h      | COX-1 and -2, CA-2 and -9                                   |
| Disulfiram               | 2E1             | < 2 h     | ALDH, ROS   |
| diethyl-dithio-carbamate | None prominent  | 6 h       | Same  |
| Itraconazole             | 3A4             | 19 h      | P-gp efflux transporters, BCRP, hedgehog, 5-lipoxygenase    |
| Minocycline              | None            | 10-20 h   | monocyte, macrophage and microglial contributions to growth |
| Ritonavir                | 3A4             | 4 h       | P-gp, proteasome, Akt, cyclin D3                            |
| Sertraline               | Weak            | 1 d       | Akt, mTOR, TCTP   |

See the Appendix for a fuller overview of the rationale for the use of each drug in its GBM-inhibiting role. ACE: angiotensin-converting enzyme; Akt: protein kinase B; ALDH: aldehyde dehydrogenase; AT1: angiotensin II receptor type 1; BCRP: breast cancer resistance protein; CA, carbonic anhydrase; COX: cyclooxygenase, MMPs: matrix metalloproteinases; mTOR: mammalian target of rapamycin, NK-1: neurokinin 1; P-gp: P-glycoprotein; ROS: reactive oxygen species; STAT3: signal transducer and activator of transcription 3; TCTP: translationally controlled tumor protein.

**Table 2.** Demographic characteristics of the 10 patients included in the CUSP9v3 trial.

| <b>Sex</b>  | <b>N (%)</b> |
|---|--------------|
| Male  | 6 (60)       |
| Female  | 4 (40)       |
| Median age at diagnosis in years (range)                        | 41(25-60)    |
| <b>Type of GBM</b>  |              |
| Primary   | 8 (80)       |
| Secondary   | 2 (20)       |
| <b>KPS at baseline</b>  |              |
| 100   | 4 (40)       |
| 90  | 1 (10)       |
| 80  | 2 (20)       |
| 70  | 3 (30)       |
| <b>Recurrence/progression at inclusion</b>                      |              |
| First   | 6 (60)       |
| Second  | 4 (40)       |
| Median time between first diagnosis and CUSP9v3 start in months | 16           |
| <b>Tumor location at time of study entry</b>                    |              |
| Frontal lobe  | 2 (20)       |
| Temporal lobe   | 2 (20)       |
| Parietal lobe   | 1 (10)       |
| Disseminated - basal ganglia                                    | 1 (10)       |
| Disseminated - midbrain and brainstem                           | 2 (20)       |
| Disseminated - callosal   | 2 (20)       |
| <b>Initial extent of resection</b>                              |              |
| Gross total   | 7 (70)       |
| Subtotal  | 3 (30)       |
| <b><i>MGMT</i> promoter status</b>                              |              |
| Hypermethylated   | 6 (60)       |
| Non-hypermethylated   | 4 (40)       |

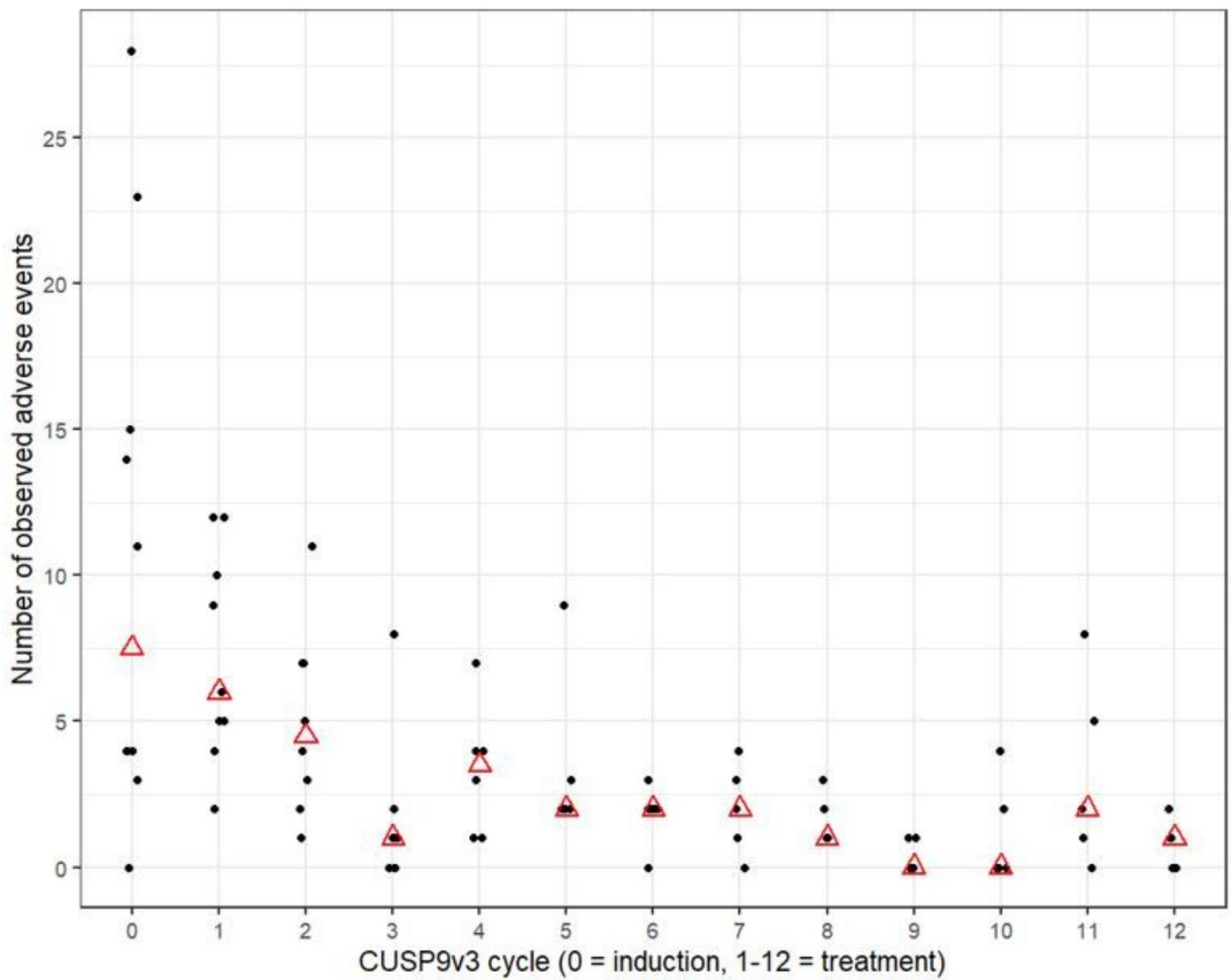
|                        |          |
|------------------------|----------|
| <i>IDH1/2</i> status   |          |
| Mutated                | 3 (30)   |
| Wild-type              | 7 (70)   |
| <b>Prior therapies</b> |          |
| Surgery                | 10 (100) |
| Radiotherapy           | 10 (100) |
| Temozolomide           | 10 (100) |
| Bevacizumab            | 1 (10)   |
| Tetrahydrocannabinol   | 1 (10)   |
| TTFields™              | 1 (10)   |

**Table 3.** Individual progression-free survival for all 10 patients with CUSP9v3 treatment.

| Patient ID | Age (years) | KPS (%) | prior second line Tx | <i>IDH1</i> mutation status | <i>MGMT</i> promoter status | Survival at data lock | PFS months at data lock |
|------------|-------------|---------|----------------------|-----------------------------|-----------------------------|-----------------------|-------------------------|
| 1          | 31          | 100     | No                   | Mutated                     | Methylated                  | Alive                 | 29                      |
| 4          | 53          | 100     | No                   | Wild                        | Methylated                  | Alive                 | 21                      |
| 5          | 41          | 100     | Yes                  | Wild                        | Methylated                  | Alive                 | 21                      |
| 7          | 30          | 90      | No                   | Wild                        | Non-methyl                  | Alive                 | 17                      |
| 10         | 27          | 100     | Yes                  | Wild                        | Non-methyl                  | Alive                 | 12                      |
| 9          | 25          | 90      | Yes                  | Mutated                     | Non-methyl                  | Dead                  | 3                       |
| 8          | 47          | 70      | Yes                  | Wild                        | Methylated                  | Dead                  | 2                       |
| 2          | 48          | 80      | Yes                  | Wild                        | Methylated                  | Dead                  | 2                       |
| 3          | 60          | 70      | Yes                  | Wild                        | Non-methyl                  | Dead                  | 0                       |
| 6          | 41          | 70      | Yes                  | Wild                        | Methylated                  | Dead                  | 0                       |

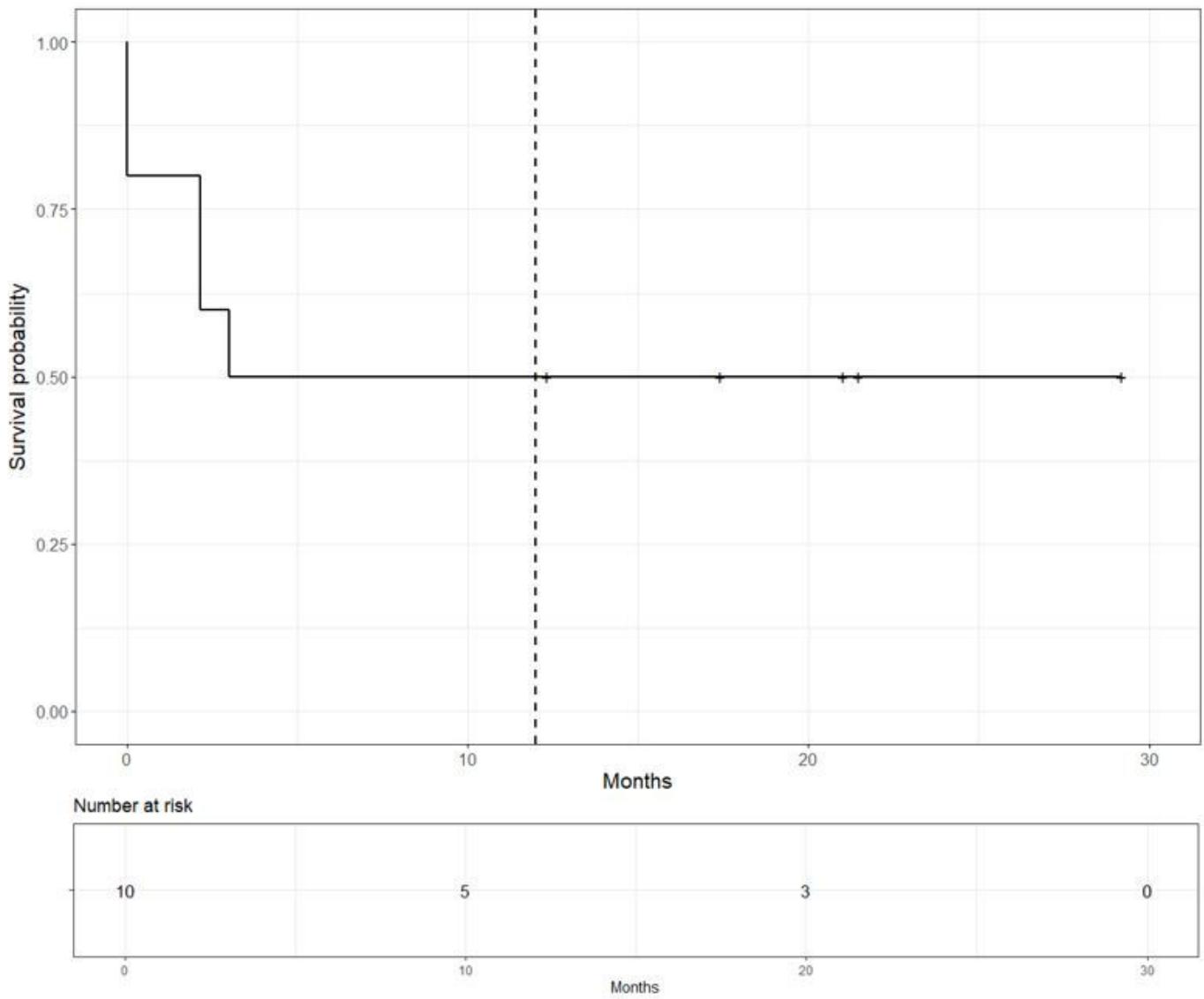
*IDH1/2*: isocitrate dehydrogenase 1 or 2 gene; *MGMT*: *O*<sup>6</sup>-methylguanine-DNA methyltransferase; non-methyl: non-hypermethylated; Tx, treatment.

## Figures



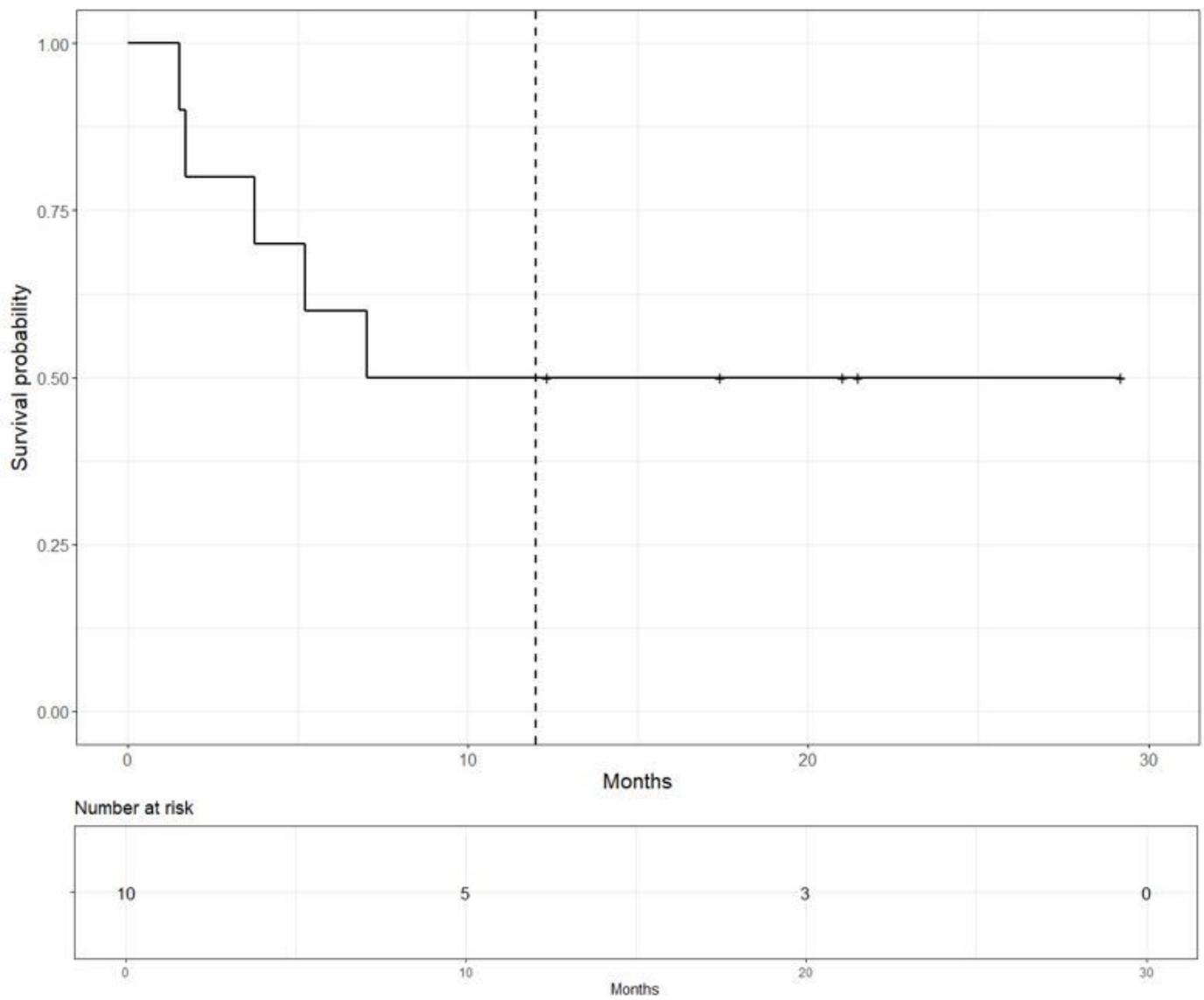
**Figure 1**

Dot plot of cumulative frequencies of AEs of all CTCAE grades in all patients over 13 cycles of CUSP9v3. The total number of AEs for every patient in every cycle is represented by a dot and the calculated mean across all patients with a triangle.



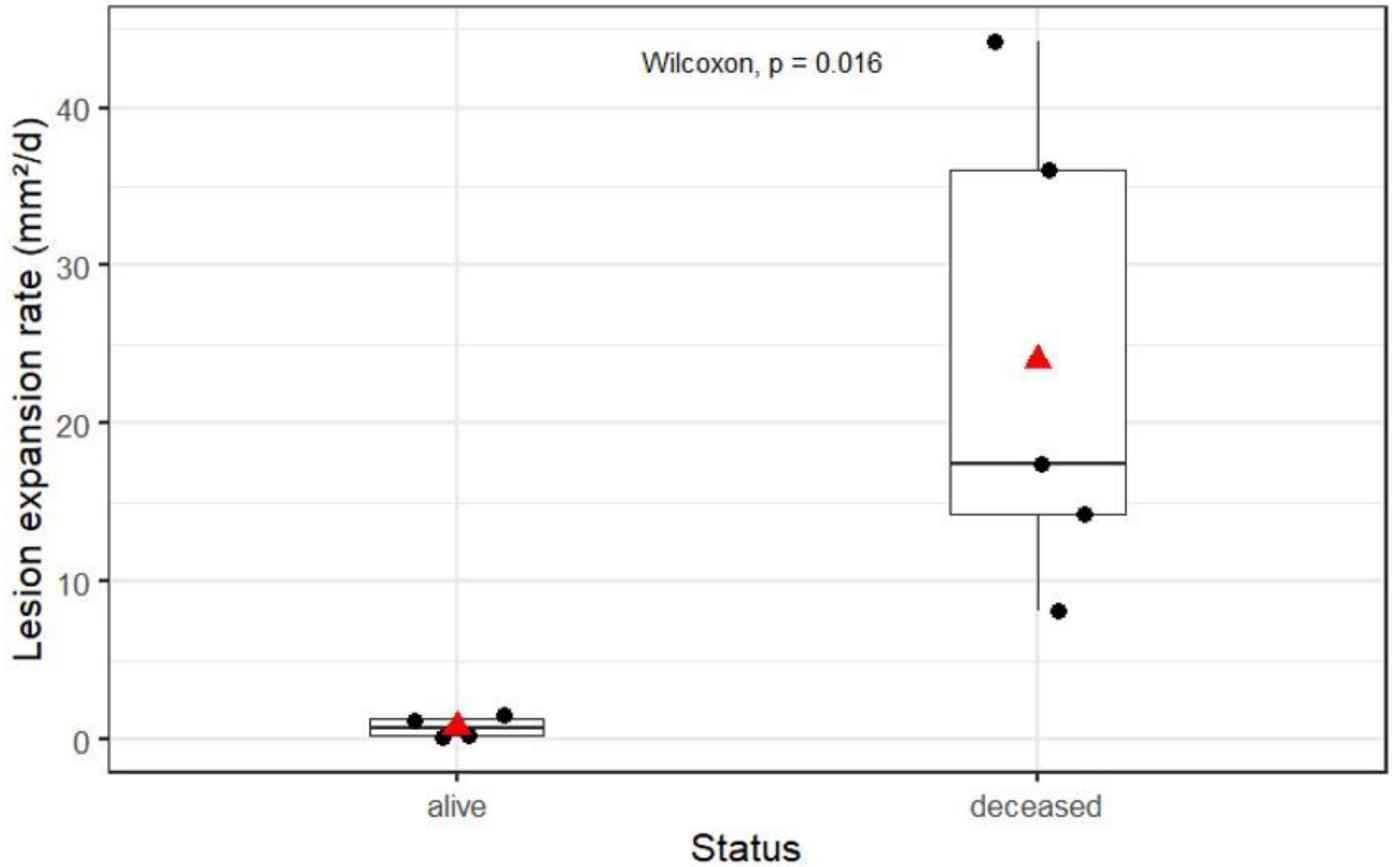
**Figure 2**

Progression-free survival since CUSP9v3 start. Kaplan-Meier plot with "+" indicating censored events at the time of data lock.



**Figure 3**

Overall survival since CUSP9v3 start. Kaplan-Meier plot with "+" indicating censored events at the time of data lock.



**Figure 4**

Lesion expansion rate at the time of study entry. In the „alive“ group, one patient was excluded from this analysis due to gross total recurrent tumor resection prior to study entry. Triangle: mean value; horizontal line within the box: median value.

## Supplementary Files

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