Final Results of the Prospective Biomarker Trial PETra: [11C]-MET-Accumulation in Postoperative PET/MRI Predicts Outcome after Radiochemotherapy in Glioblastoma

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Conflict of Interest:

In the past 5 years, Dr. Krause received funding for her research projects by IBA (2016), Merck KGaA (2014-2018 for preclinical study; 2018-2020 for clinical study), Medipan GmbH (2014-2018). Dr. Krause and Dr. Linge are involved in an ongoing publicly funded (German Federal Ministry of Education and Research) project with the companies Medipan, Attomol GmbH, GA Generic Assays GmbH, Gesellschaft für medizinische und wissenschaftliche genetische Analysen, Lipotype GmbH and PolyAn GmbH (2019-2021).

In the past 3 years, Dr. Beuthien-Baumann received remuneration for lecturing by BRACCO und Roche Pharma AG.

In the past 5 years, Dr. Michael Baumann attended an advisory board meeting of MERCK KGaA (Darmstadt), for which the University of Dresden received a travel grant. He further received funding for his research projects and for educational grants to the University of Dresden by Teutopharma GmbH (2011-2015), IBA (2016), Bayer AG (2016-2018), Merck KGaA (2014-open), Medipan GmbH (2014-2018). He is on the supervisory board of HI-STEM gGmbH (Heidelberg) for the German Cancer Research Center (DKFZ, Heidelberg) and also member of the supervisory body of the

Charité University Hospital, Berlin. As former chair of OncoRay (Dresden) and present CEO and Scientific Chair of the German Cancer Research Center (DKFZ, Heidelberg), he has been or is still responsible for collaborations with a multitude of companies and institutions, worldwide. In this capacity, he discussed potential projects with and has signed/signs contracts for his institute(s) and for the staff for research funding and/or collaborations with industry and academia, worldwide, including but not limited to pharmaceutical corporations like Bayer, Boehringer Ingelheim, Bosch, Roche and other corporations like Siemens, IBA, Varian, Elekta, Bruker and others. In this role, he was/is further responsible for commercial technology transfer activities of his institute(s), including the DKFZ-PSMA617 related patent portfolio [WO2015055318 (A1), ANTIGEN (PSMA)] and similar IP portfolios.

Dr. Krause, Dr. Linge, Dr. Beuthien-Baumann and Dr. Baumann confirm that to the best of their knowledge none of the above funding sources was involved in the preparation of this paper.

Relevance of the study for translational research

Patients with glioblastoma still have a poor prognosis despite intense multimodal treatment. However, local treatments, such as high-precision radiotherapy have the potential to further improve local control. Therefore biomarkers are required not only to predict the risk but particularly the location of a recurrence. Amino-acid-PET/MRI is not a wide available method for diagnosis or radiotherapy treatment planning in glioblastoma but it is a widely promising approach to guide therapy intensification. While prospective data is scarce, retrospective evaluations suggest a superior diagnostic impact versus standard magnetic resonance imaging and a co-localization of tracer uptake before standard RCT with later location of recurrence. This prospective trial confirms the impact of [^{11}C]-methionine-PET/MRI before postoperative radiochemotherapy of newly diagnosed glioblastoma in 89 patients. Tumor residues in positron emission tomography (PET) were included in gross tumor volumes for radiotherapy treatment planning. Exploratory analysis showed a spatial correlation of the

recurrence region with pre-radiochemotherapy PET tracer accumulation in the majority of patients, which could guide future dose-escalation trials.

Abstract

Background

This prospective trial investigates the association of time to recurrence (TTR) in glioblastoma with [^{11}C]methionine (MET) tracer uptake before postoperative radiochemotherapy (RCT) aiming to guide radiotherapy boost regions.

Methods

Between 2013 and 2016, 102 patients with glioblastoma were recruited. RCT was performed with concurrent and adjuvant temozolomide to a total dose of 60 Gy. Tumor residues in post-resection PET and MRI were together defined as gross tumor volumes for radiotherapy treatment planning. [^{11}C]methionine (MET)-PET/MRI was performed before RCT and at each follow-up.

Results

The primary hypothesis of a longer TTR for patients without increased tracer accumulation in postoperative MET-PET was confirmed in 89 patients. With 18.9 months (95% CI 9.3-28.5 months), median TTR was significantly ($p<0.001$) longer for patients without (n=29, 32.6%) as compared to 6.3 months (3.6-8.9) for patients with MET accumulation (n=60, 67.4%) in pre-RCT PET. Although MRI often did not detect all PET-positive regions, an unfavorable impact of residual tumor in post-surgical MRI (n=38, 42.7%) on TTR was observed (4.6 [4.2-5.1] versus 15.5 months [6.0-24.9], $p<0.001$). Significant multivariable predictors for TTR were MRI positivity, PET-positive volume and O^6-methylguanine DNA methyltransferase (MGMT) hypermethylation.

Conclusion

Post-surgical amino-acid-PET has prognostic value for TTR after RCT in glioblastoma. Due to the added value of the metabolic beyond the pure structural information, it should complement MRI in radiotherapy planning if available with reasonable effort, at least in the context of maximal therapy. Furthermore, the spatial correlation of regions of recurrence with PET-positive volumes could provide a bioimaging basis for further trials e.g. testing local radiation dose-escalation.

Introduction

Postoperative radiotherapy remains a mainstay clinical treatment for glioblastoma. The introduction of combined postoperative radiochemotherapy (RCT) more than 10 years ago [1] has for the first time led to a noticeable proportion of patients surviving five years after primary treatment. For prediction of the general prognosis of patients with glioblastoma, the Recursive Partitioning Analysis (RPA) classification in its original [2] or modified version [3] including patient´s age, performance status, extent of surgery, tumor grading and brain function, is widely used. Increasing evidence supports additional value of molecular markers and presence of IDH mutation and 1p/19q codeletion is now part of the WHO 2016 classification [4]. However, none of these markers has become standard for treatment stratifications in clinical routine for glioblastoma [5]. Several studies have tested escalated radiotherapy doses using recent techniques to improve outcome of radiotherapy in glioblastoma [6-14] and most of them showed higher local control rates and survival [7-11,13,15], sometimes at the price of higher toxicity. However, randomized data are missing, and some of these studies were retrospective and thus prone to selection bias. Still, precise definition of boost areas for dose escalation only in those patients with high risk of early locally confined recurrence might help to reduce severe late effects at improved local tumor control rates. For optimization of local treatments, such as high-precision radiotherapy, biomarkers are needed that do not only predict the risk but also the location of a recurrence. While retrospective evaluations suggest a superior diagnostic value of pre-RCT amino-acid positron emission tomography (PET) with [11C]methionine (MET) versus magnetic resonance imaging (MRI) [16] and a co-localization of MET tracer uptake before standard RCT with later location of recurrence [17,18], prospective data on this question are scarce. In a small prospective study on 34 patients, a spatial correlation of recurrences with the initially amino acid PET (O-(2-18F-fluoroethyl)-L-tyrosine (FET)) positive areas was shown with no additional recurrences within initially MRI-positive areas [19].

The prospective study presented here aims to investigate the association of high MET tracer uptake before postoperative RCT and concurrent temozolomide (TMZ) with time to recurrence (TTR) in patients with glioblastoma. To gain information towards a potential individualized radiation dose

escalation strategy, secondary aims were definition of prognostic groups for TTR and to evaluate the spatial co-localization of recurrences with the initially MET-PET-positive areas.

Methods

Trial design

The PETra trial is a prospective one-arm, single-center, non-randomised biomarker study. The study was conducted in accordance with the Declaration of Helsinki in the University Hospital/Faculty of Medicine Carl Gustav Carus of the Technische Universität Dresden in cooperation and with financial support of the German Cancer Consortium (DKTK)/German Cancer Research Center (DKFZ) Heidelberg and the Helmholtz-Zentrum Dresden-Rossendorf (HZDR). The protocol was reviewed and approved by the institutional review board (EK 41022013) and registered in a clinical trials database (clinicaltrials.gov; NCT01873469). All patients gave written informed consent.

Patients

Adult patients (age $\geq$ 18 years) with histologically confirmed newly diagnosed glioblastoma and clinical indication for standard radiochemotherapy with temozolomide were consecutively recruited if they were in adequate general conditions (Karnofsky Performance Score $\geq$ 60, ECOG $\leq$ 2). Radiochemotherapy had to start within 7 weeks after surgery (macroscopic total tumor resection or biopsy allowed) and adequate contraception was required. Participation in other clinical trials was allowed, if not competing with the PETra trial. Exclusion criteria were previous radiotherapy of the brain, previous chemotherapy with temozolomide, known other malignant disease likely to impact survival prognosis and/or require interventions interfering with study treatment, pregnancy or lactation, claustrophobia and non-MRI compatible material in the body (metal implants, pacemakers/defibrillators, insulin pumps, neurostimulators, cochlear implants).

Image acquisition and evaluation

MET-PET/MRI was performed before RCT and at each follow-up visit every three months until objective tumor recurrence. Details of image acquisition are given in supplement S1.

PET-data of the timeframe of 20-40 min post injection were evaluated qualitatively and quantitatively. For visual comparison of different scans, the images were displayed using an SUV window of [0-4]. Qualitative evaluation comprised the evaluation of abnormal distribution of MET suspicious for tumor (i.e. focal uptake in areas without physiologically enhanced uptake and not compatible with post-surgical alteration). Tumor 3D-volumes of interest (VOI), encompassing the suspicious tumor area, were delineated by a nuclear medicine expert (BBB), using the software package ROVER® (ABX, Radeberg, Germany). The VOIs were defined manually by applying an adaptive threshold for each suspicious lesion, since using a fixed threshold led to erroneous delineations. Tumor VOIs were exported to the commercial treatment planning software RayStation (Version 6.0, RaySearch, Stockholm, Sweden). MRI tumor volumes were re-contoured (AS) for this evaluation in the treatment planning system as T1 contrast enhancing regions in the first MRI 24 – 48 hours after surgery, considering nodular lesions as residual tumor and linear enhancement as post therapeutic changes. Residual tumor status was dichotomised using primarily this early post-surgical MRI under auxiliary consideration of operative reports and the second baseline MRI obtained contemporaneously with PET. As the second MRI was median 23 (minimum 9, maximum 45) days after post-surgical MRI it is prone to unspecific changes. Therefore residual tumor evaluation could change to MRI negative if the second MRI showed no residual tumor at all (false-positive post-surgical e.g. due to unspecific changes, blood etc.). On the other hand it could only change from negative to positive if there was conversion from missing residues in post-surgical scans to unambiguous macroscopic tumor in second MRI, i.e. in case of distinct progression between these two pre-RCT scans. Difficult cases were independently reviewed by an experienced radiologist (IP).

For evaluation of spatial correlations with recurrences, treatment plans including the treatment volumes (see below) were rigidly co-registered with the follow-up images and with the contoured initial PET/MRI images (see above) in RayStation. Classification of the location of recurrences was initially done according to the proportion of the recurrence volume covered by the 95% isodose (V95)

as follows: central recurrence if V95≥95%, in-field if 95%>V95≥80%, marginal if 80%>V95≥20% and distant if V95<20% [20,21]. As this classification did not prove satisfactory to the assessment with respect to a future dose escalation trial based on PET/MRI, it was adjusted for clinical meaningful judgement and, all cases were reviewed together with the principal investigator (MK) as follows: distant in case of no anatomical connection to the former primary tumor or resection cavity, local if within the region of former primary tumor or resection cavity and loco-regional if local plus spread outside the V95. Anatomical shifts over time were carefully taken into consideration and visual classification corrected if necessary.

Treatment

Concurrent and adjuvant TMZ was prescribed according to clinical standards [2]. Certified commercial treatment planning systems were used for treatment planning. Images of the baseline diagnostic PET/MRI were co-registered to the planning CT. For target volume definition and follow-up imaging of this trial, the radiation oncologists were supported by the diagnostic imaging team, i.e. specialists in radiology and in nuclear medicine. According to the clinical standard, the cavity plus contrast enhancing lesion in MRI was delineated as GTV. Specific for the PETra study, the PET-positive volume was also included into the GTV. Tumor residues in PET or MRI were included in the gross tumor volumes (GTV). The GTV was expanded by a 20 mm or 5 mm isotropic margin to create the 50 Gy (RBE) or 60 Gy (RBE) clinical target volume (CTV) under consideration of anatomical borders, respectively. If non-enhancing lesions or edema in T2/FLAIR MRI were not included in this 20 mm margin, the CTV was enlarged to include these regions in line with clinical standards. Radiotherapy was applied with photons (6/15 MV linear accelerators, three-dimensional conformal radiotherapy or intensity-modulated radiotherapy) or protons (100 MeV to 230 MeV, double scattering technique, field shaping by range compensators and lateral apertures). As important processes, such as clinical target volume creation, were similar between the two treatment modalities and dose was normalized to the commonly accepted factor of 1.1 for the relative biological effectiveness, the effect of proton and photon therapy on tumor control are expected to be equal [22].

Each treatment plan was approved by two radiation oncologists and two medical physicists. Constraint doses to organs at risk were used according to current clinical guidelines based on QUANTEC criteria [23-25].

Analysis of MGMT promoter methylation status

For the majority of patients (n=86) the MGMT promoter methylation status was obtained in routine diagnostics using the Therascreen MGMT Pyro Kit (Qiagen GmbH, Hilden, DE). Samples with <8% methylation were considered non-methylated [26,27].

For 3 patients with missing MGMT status, MGMT methylation was determined using methylation-specific PCR. After the tumour content of the respective formalin-fixed paraffin-embedded tissues was estimated from haematoxylin and eosin stained tissue sections by an experienced neuropathologist, tissues with <70% glioblastoma content were subjected to macrodissection. Extraction of genomic DNA was carried out from 5µm FFPE sections using the QIAamp® DNA FFPE Tissue Kit (Qiagen GmbH, Hilden, DE) following the instruction of the manufacturer. Bisulfite conversation and methylation-specific PCR has been carried out as described previously [28,29]. Quantifications of the optical signals of the methylated (M) and unmethylated (U) product were performed with ImageJ (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, https://imagej.nih.gov/ij/, 1997–2018), according to [30]. Samples with an M/U ratio=0 were considered non-methylated [28].

IDH1 immunohistochemistry analyses

FFPE material from 85 patients was available for mutant IDH1 (R132H) specific immunohistochemistry analyses. Following deparaffinisation and antigen retrieval in target retrieval solution (pH 6.1; Dako, Glostrup, DK) for 28 min at 630 W, immunohistochemical staining was performed. Endogenous peroxidase activity was blocked (Peroxidase Block, Dako). Sections were then incubated with the monoclonal mouse anti-IDH1 (R132H) antibody (dilution 1:40; clone H09; dianova GmbH, Hamburg, DE) in Dako REAL Antibody Diluent for 30 min. Negative control slides

were incubated with corresponding IgG antibody control (Dako). The staining was visualised by DAB immunostaining (Dako REAL EnVision Detection System, Peroxidase/DAB, Rabbit/Mouse). Blinded samples were evaluated semi-quantitatively. Tumours with strong cytoplasmic staining were considered positive for IDH1 (R132H) mutation [31].

Study endpoints, quality assurance and statistical considerations

The primary hypothesis was that the TTR is shorter in patients with MET tracer uptake before RCT as compared to patients without such uptake. Secondary objectives were to study the association of overall survival (OS) with MET tracer uptake and the comparison of localization of MET uptake at baseline with subsequent sites of recurrence. The primary endpoint TTR and the secondary endpoint OS were calculated from the first day of radiotherapy to the date of event or censoring. Definite judgement for recurrences was postponed until confirmation in further follow up scans in some inconclusive cases. Time of recurrence was then set to the first scan indicating progression.

Trial monitoring was performed ten times during the trial duration, capturing adherence to protocol, correct documentation and definition of recurrences for all patients at the first three monitorings and for at least 10% of all patients for all further monitorings. Before data evaluation, an additional final monitoring was performed to check correct documentation of the primary endpoints for all included patients.

For statistical planning and data analysis, a certified biostatistician (SL) was involved. The necessary sample size for the primary endpoint of this trial was estimated for Cox proportional hazards regression. A sample size of 79 patients is needed if the hazard ratio (HR) of TTR between patient groups stratified by a median-split of MET uptake is 2.0, the 90% confidence interval of this HR (α=0.1) does not contain the value 1.0 with a power of 90%, accrual rate is 5 patients per month and follow-up continues at least for six months after accrual of the last patient.

In secondary analyses, the impact of potential prognostic variables on the endpoints was evaluated using univariable Cox regression for continuous variables and the log-rank test for categorical variables. Significant parameters were included in a multivariable Cox regression model using

forward variable selection with $p<0.05$ for inclusion and $p>0.1$ for exclusion. Based on the 1st and 3rd quartiles of the corresponding linear predictors, patients were assigned to three risk groups. Differences between these risk groups in TTR and OS were evaluated by the log-rank test. Statistical analyses were performed using IBM SPSS Statistics 25 (IBM Corporation, Armonk, NY). Two-sided tests were performed and p-values<0.05 were considered statistically significant without multiple testing correction.

Results

Patients

A trial overview is given in supplementary figure SF1. A checklist referring to reporting recommendations for tumor marker prognostic studies (REMARK criteria) is shown in supplementary table ST4. From August 2013 to September 2016, 102 patients were included. Thirteen patients (12.7%) dropped out of the study early. Reasons were missing slots for baseline PET/MRI (n=5), repeated surgery without new baseline imaging (n=1), changes in the therapeutic concept due to massive progression before RCT (n=5), cancelling of participation (n=1) and the delayed start of RCT (n=1). Eighty-nine patients remained for the final analysis of the primary endpoint TTR. Median follow-up was 17.2 months (range 1.5-73.0 months) at the time of closure of the database for final data analysis (April 23th 2020). Baseline characteristics are shown in table 1.

All patients received radiotherapy per protocol. Slight changes in overall treatment time or combined treatments are described in supplement S2.

Imaging

Baseline PET imaging showed elevated MET uptake in 60 out of 89 patients (67.4%). In contrast, baseline MRI exhibited contrast enhancement indicating residual tumor in only 38 out of 89 patients (42.7%). While all except for one patient with residual tumors in MRI also showed MET-accumulation in PET imaging, only 37/60 (61.7%) of all patients with elevated MET uptake had a

visible tumor residuum in MRI imaging (table 2). MET-positive volumes ranged from 0.0 to 74 ml, the median was 1.3 ml. Median volume of residual tumors was 2.9 ml.

Primary endpoint time to recurrence

At closure of the database, 9 patients were still under follow-up without tumor recurrence (censored), whereas 77 patients developed recurrences. The remaining 3 patients were censored due to early death from pulmonary embolism (before first follow-up) and in two cases because a 2nd line chemotherapy was initiated by the treating oncologist without tumor recurrence (14 and 27 months follow-up).

The hazard ratio of MET accumulation for TTR determined in Cox regression was 2.46 (90% CI 1.59-3.80; p=0.001), confirming the primary hypothesis of this study. Median TTR in the whole patient group was 7.6 months (95% CI 6.9-8.2 months; figure 1a). A significantly increased median TTR was observed for patients without pathological MET accumulation (18.9 months [9.3-28.5]) compared to patients with pathological MET accumulation (6.3 months [3.6-8.9]) in the pre-radiochemotherapy PET (figure 1c). In addition, larger MET accumulation was associated with shorter TTR (p<0.001). Patients without MET accumulation were mostly male (p=0.002), showed more MGMT methylated tumors (p=0.043), lower age (p=0.049) and less residual tumor in MRI (p<0.001, table 1).

Although fewer patients showed residual tumor in MRI versus PET imaging (table 2), residual tumor detected by postoperative MRI was also significantly associated with time to recurrence (15.5 months [6.0-24.9] versus 4.6 months [4.2-5.1], p<0.001) (figure 1e). While patients with residual tumor in MRI and PET showed the lowest TTR, patients without residual tumor in both analyses performed best and PET positivity but MRI negativity resulted in intermediate TTR, see supplementary figure SF3. No correlation with outcome was detected for performance status, presumably due to narrow inclusion criteria of the trial. Effect sizes of the considered patient characteristics for TTR were similar for patients with and without residual tumor in MRI (supplementary table ST1).

Secondary endpoint overall survival

Thirteen patients were still alive whereas 76 had died at the time of closure of the database. Median overall survival (OS) in the whole patient group was 17.2 months (95% CI 13.6-20.7 months; figure 1b). OS differed between patients with MET-negative and MET-positive PET-scans (23.1 months [9.4-36.8] vs. 14.5 months [12.2-16.8], p=0.003) as well as between patients without and with contrast enhancing tumor residuum in MRI (22.6 months [15.0-30.1] versus 12.7 months [9.6-15.7], p=0.001) (figure 1d, f). Larger MET accumulation was associated with shorter OS (p=0.002). For progression-free survival (event: death or recurrence), similar results as for TTR were observed, shown in supplementary table ST2. Although there is no clear standard treatment in case of recurrent glioblastoma, salvage therapy can impact OS. If performance status allowed further treatment, patients were discussed in interdisciplinary tumor boards. Recurrence surgery was performed in 47 patients, in 10 of them more than one recurrence resection. Additionally, 11 patients underwent another series of radiotherapy for recurrent GBM. As salvage treatment is highly individual, it is not useful to account for this issue with statistical methods for this patient number.

Multivariable analysis

Parameters significantly related to TTR in univariable analysis were included in multivariable Cox regression with forward variable selection (table 3). While MGMT hypermethylation ($p<0.001$), MET-positive volume (p=0.004) and residual tumor in MRI ($p<0.001$) remained significantly associated with TTR, PET positivity (p=0.33) and IDH status (p=0.19) were not selected. For OS, age was additionally selected ($p<0.001$). Additional explorative statistical analyses, excluding the 7 patients who underwent only biopsy, the 4 patients with clinical or histopathological classification of secondary glioblastoma or the 9 patients with mutated or unknown IDH status, yielded similar results in comparison to the whole cohort (not shown).

A risk score (rs) was defined based on the linear predictor of the presented multivariable Cox model, rs = -1.598 x MGMT + 1.007 x MRI positivity + 0.0328 x MET-positive volume (ml). Using that risk score, three patient groups were exemplarily defined by the 1st and 3rd quartiles, -0.635 and 0.971. The three risk groups differed significantly in TTR (all pairwise p-values < 0.001, figure 2). Similarly,

for OS the risk score was defined by rs = -1.858 x MGMT + 0.625 x MRI positivity + 0.0413 x MET-positive volume (ml) + 0.0401 x age (years), and the quartiles were -0.972 and 0.929.

A freedom from recurrence rate of 59.3% and OS of 72.7% at 2 years, and a median TTR of 29.0 months (16.3-41.6) and OS of 48.0 months (15.7-80.4) was observed in the best prognosis group. In contrast, for the worst prognosis group a freedom from recurrence rate of 0% and OS of 0% at 2 years, a median time to recurrence of 4.3 months (2.8-5.8) and a median OS of 9.2 months (5.5-13.0) were observed.

To assess the impact of IDH-mutation on the presented results, we performed an additional sensitivity analysis excluding the 6 patients with IDH-mutated tumors. Effect sizes for TTR and OS were similar in comparison to the analysis of the whole cohort (supplementary table ST3 and figures SF2 and SF3). As expected, overall TTR and OS were slightly reduced due to the exclusion of the patients with IDH-mutated tumors with an inherent better prognosis.

Spatial correlation of recurrence regions with postoperative PET

At the time of closure of the database, 77 out of 89 evaluable patients (86.5%) showed disease progression. In 6 of these patients (7.8%) no follow-up imaging was available due to early clinical progression during or shortly after RCT. All of these 6 patients had both MET-positive lesions and contrast enhancing residual tumors in scans before therapy. Of the remaining 71 patients, 13 patients (18.3%) were imaged by MRI only at time of recurrence because of symptomatic progression and imaging performed outside the trial schedule, while 58 patients (81.7%) were imaged using PET/MRI at time of progression. These 71 patients (79.8% of the evaluated study population), were used for investigating the spatial correlation between the region of recurrence and baseline PET/MRI.

Relative to the high-dose irradiation volume, most patients developed local recurrences, but there were also some loco-regional or distant failures or combinations (table 4). Relative to the pre-RCT MET accumulation, of the 71 evaluable patients for this endpoint, the recurrence occurred within the previously PET-positive area in 43 patients (60.6%). In 28 patients (39.4%), there was no pre-RCT tracer accumulation in the later recurrence region, either because these patients were initially PET-negative (n=21) or because tracer accumulation before RCT and region of recurrence did not show

spatial overlap (n=7). Independent of the initial MET-positive region, in 57 patients (80.3%) tumor recurrence occurred at the resection margin. Of the 16 evaluable patients in the worst prognosis group (figure 2), all recurrences occurred in the previously PET-positive area. For illustration, imaging before RCT and at time of recurrence 6 months thereafter of a 60-year old patient after initial gross total resection is shown in figure 3.

Discussion

This prospective clinical trial confirmed our primary hypothesis that TTR is significantly shorter in patients with MET positivity in post-resection PET-imaging before RCT compared to MET negative patients. More patients showed PET-positive areas after surgery as compared to MRI-positive areas (table 2). Thus, PET negativity defined a patient group with longer TTR, while MRI positivity defined a group at particularly high risk of early recurrence (figure 1). Combining MRI positivity with MGMT status, age and MET-positive volume further improved its prognostic value. In line with retrospective data [32] and three prospective studies on 44 [33], 79 [34], and 16 patients [35], using FET-PET, our data underscore a high prognostic value of amino acid PET for patients with glioblastoma. FET and MET-PET have shown equivalence in a previous study [36].

In light of the importance of IDH-status in the WHO 2016 classification [4] we performed a sensitivity analysis with exclusion of the six patients with IDH-mutated tumors showing similar results for TTR and OS. Patients with IDH-mutation were not excluded for the main analyses, however, as IDH analysis was not clinical standard and also not part of glioblastoma classification at the time of patient diagnosis and treatment (2013-2016). Thus, it was also not part of the inclusion criteria. Nevertheless, results for the patients without IDH-mutation are shown as supplementary material, which allows for the comparison of the results with future publications according to the WHO 2016 classification [4].

PET positive areas before RCT were not always located along the resection cavity, and in a sizable fraction of patients (38.3%, table 2) they were not accompanied by contrast enhancement in MRI 24-72 hours after surgery. Thus, another important conclusion of this and also previous studies [14,19,37,38] is,

that amino acid PET has substantial value for precise target volume definition for radio(chemo)therapy.

To the best of our knowledge, we have for the first time prospectively shown an association of overall survival and freedom from recurrence with risk groups defined by MET-positive volume, MRI-positivity and MGMT hypermethylation status. Pending validation of this model is an important limitation, which should be addressed in future trials. Nevertheless, this prospectively obtained result is in line with retrospective data, suggesting a prognosis score of MGMT status, biological tumor volume defined by amino acid PET, patient age, and performance status [32].

For an individualized radiation dose prescription strategy, besides the determination of the prognostic value of MET-PET and the definition of prognosis groups, the co-localization of recurrences with regard to pre-RCT MET accumulation is important to assess. In our study, for tumors that were PET positive before RCT, consecutive tumor recurrences occurred in the initially PET-positive region in 86.0% of cases (43 of 50). Only 7 recurrences were found outside of the former PET positive region. However, 21 additional patients developed recurrences without MET accumulation before RCT. Radiotherapy dose escalation to the PET-positive area (if present) would thus apply higher radiation dose in the region of highest risk of recurrence in 60.6% of the patients with recurrence, i.e. would have a sensitivity of 60.6%. Of the 9 patients who are still under follow-up without recurrence, 3 showed pre-RCT MET accumulation (33.3%). Those three patients would potentially have been overtreated with a local radiotherapy boost, i.e. the specificity would be 66.7%. For another 8 patients with initial PET-positive volume, a co-localization with recurrence was not evaluable due to early recurrence without follow-up PET (6) or due to death without recurrence (2). Another approach of radiotherapy dose escalation would be to boost the resection cavity instead of a PET-positive post-surgical residual tumor volume. Although this would increase the sensitivity to 80.3%, it would lead to a dose escalation in many patients of the low risk group (PET-negative before RCT). Interestingly, in the worst prognosis group according to our multivariable model, all recurrences occurred in

initially PET-positive areas. This observation suggests that it may be promising to test a local boost concept in this very high risk group.

A dose escalation applying 72 Gy to amino acid PET positive areas (here FET) has been tested in a small single-arm prospective study on 13 patients with glioblastoma [39]. While recurrent tumor volumes in FET-PET overlapped only to one third with the boost target volume, potentially due to shrinking and shifting of the resection cavity, a simulated target volume of the initially FET positive area plus 7 mm margin covered a median of 100% (54-100) of the region of recurrences and was still smaller than a MRI-based definition of the boost region. This study supports our conclusion that initially PET-positive areas are at high risk for recurrences, however, it does not answer the question whether a local boost application to the PET-positive area would reduce the number of recurrences.

Conclusion

Post-surgical amino-acid-PET has prognostic value for TTR after RCT in glioblastoma. Due to the added value of the metabolic beyond the pure structural information, it should complement MRI in radiotherapy planning if available with reasonable effort, at least in the context of maximal therapy. Furthermore, the spatial correlation of regions of recurrence with PET-positive volumes could provide a bioimaging basis for further trials e.g. testing local radiation dose-escalation.

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Tables

Table 1: Patient characteristics for all patients and for the subgroups of MET negative and MET positive patients. The characteristics of the subgroups were compared by statistical tests.

	All patients (89)		**MET negative patients (29)**		**MET positive patients (60)**		
Characteristics	**Number**	**%**	**Number**	**%**	**Number**	**%**	**p-value**
Gender							
Male	53	59.6	24	82.8	29	48.3	
Female	36	40.4	5	17.2	31	51.7	**0.002**[#]
ECOG status							
0	47	52.8	20	69.0	27	45.0	
1	37	41.6	8	27.6	29	48.3	
2	5	5.6	1	3.4	4	6.7	0.11[#]
Resection							
Complete (in post-op. MRI)	51	57.3	28	96.6	23	38.3	
Partly (in post-op. MRI)	31	34.8	1	3.4	30	50.0	
Biopsy	7	7.9	0	0.0	7	11.7	**<0.001**[#]
MGMT							
Methylated	30	33.7	14	48.3	16	26.7	
Wildtype	59	66.3	15	51.7	44	73.3	**0.043**[#]
IDH							
Mutated	6	6.7	4	13.8	2	3.3	
Wildtype	79	88.8	25	86.2	54	90.0	
Unknown	4	4.5	0	0	4	6.7	0.081[#]
	Median (range)		**Median (range)**		**Median (range)**		**p-value**
Age (years)	58 (23 – 82)		53 (26 – 82)		60.5 (23 – 76)		**0.049**[*]
MET-positive volume (ml)	1.3 (0.0 – 74)		0 (0 – 0)		4.5 (0.1 – 74)		**<0.001**[*]
Post-surgical enhancing tumor volume in MRI (ml)	0.0 (0.0 – 41)		0 (0 – 8.9)		1.0 (0 – 41)		**<0.001**[*]

Abbreviations: ECOG, Eastern Co-operative Oncology Group; MET, methionine; MGMT, O^6-methylguanine DNA methyltransferase; IDH, isocitrate dehydrogenase; [#] chi-squared test; [*] Mann-Whitney-U test

Table 2: Comparison between accumulation of 11C-methionine (MET) and MRI contrast agent in pre-radiochemotherapy PET + MRI.

		MET tracer accumulation		
		No	Yes	Total
Contrast enhancement in MRI	No	28	23	51
	Yes	1	37	38
	Total	29	60	89

Abbreviations: MET, methionine; MRI, magnetic resonance imaging

Table 3: Univariable and multivariable Cox regression of time to recurrence and overall survival.

	Univariable analyses		Multivariable analyses	
Variable	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**
Time to recurrence				
Age (years)	1.01 (1.00-1.03)	0.089		
Gender (female vs male)	1.07 (0.67-1.69)	0.78		
ECOG status (0 vs >0)	1.17 (0.75-1.83)	0.50		
MGMT (methylated vs wildtype)	0.26 (0.16-0.44)	**<0.001**	0.20 (0.12-0.35)	**<0.001**
IDH (methylated vs wildtype)	0.26 (0.08-0.82)	**0.022**		
MET accumulation (yes vs no)	2.46 (1.47-4.13)	**0.001**		
MET-positive volume (ml)	1.04 (1.02-1.06)	**<0.001**	1.03 (1.01-1.06)	**0.004**
Post-surgical enhancing tumor volume in MRI (ml)	1.02 (0.99-1.04)	0.16		
Residuum in MRI (yes vs no)	2.52 (1.59-4.00)	**<0.001**	2.74 (1.63-4.61)	**<0.001**
Overall survival				
Age (years)	1.03 (1.01-1.04)	**0.002**	1.04 (1.02-1.06)	**<0.001**
Gender (female vs male)	0.95 (0.60-1.50)	0.81		
ECOG status (0 vs >0)	1.27 (0.80-2.01)	0.30		
MGMT (methylated vs wildtype)	0.26 (0.15-0.45)	**<0.001**	0.16 (0.08-0.29)	**<0.001**
IDH (methylated vs wildtype)	0.32 (0.10-1.02)	0.054		
MET accumulation (yes vs no)	2.15 (1.29-3.60)	**0.004**		
MET-positive volume (ml)	1.04 (1.01-1.06)	**0.002**	1.04 (1.02-1.07)	**0.002**
Post-surgical enhancing tumor volume in MRI (ml)	1.01 (0.98-1.04)	0.56		
Residuum in MRI (yes vs no)	2.12 (1.34-3.35)	**0.001**	1.87 (1.11-3.14)	**0.018**

Abbreviations: ECOG, Performance Status according to Eastern Cooperative Oncology Group; MGMT, O^6-methylguanine DNA methyltransferase; IDH, isocitrate dehydrogenase; MET, [^{11}C]methionine; MRI, Magnetic Resonance Imaging; HR, hazard ratio; 95% CI, 95 percent confidence interval.

Table 4: Location of recurrences relative to the high-dose area of radiotherapy.

Recurrence location			No. of patients	Percentage
solely local	-	-	41	57.7
local	+ locoregional	-	13	18.3
local	-	+distant	7	9.9
local	+ locoregional	+distant	3	4.2
-	solely locoregional	-	0	0
-	locoregional	+ distant	1	1.4
-	-	solely distant	6	8.5

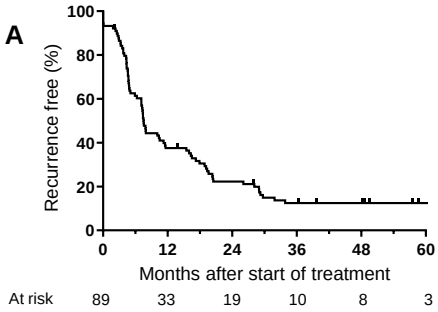
Figure legends

Figure 1: Kaplan-Meier curves for freedom from tumor recurrence (a) and overall survival (b) for the whole patient cohort, for patient groups without versus with MET accumulation in the pre-radiochemotherapy PET/MRI (c, d) and for patients with or without suspected tumor residuum in the 24h postoperative MRI (e, f).

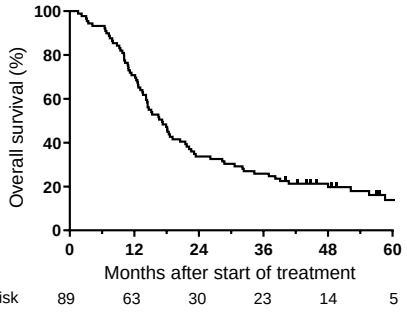
Figure 2: Kaplan-Meier curves for freedom from tumor recurrence (a) and overall survival (b) for three risk groups combining MGMT hypermethylation, MET-positive volume and residual tumor in MRI. The risk groups were defined by the 1st and 3rd quartiles, -0.635 and 0.971 of the risk score (rs) based on the linear predictor of the presented multivariable Cox model, rs = -1.598 x MGMT + 1.007 x MRI positivity + 0.0328 x MET-positive volume (ml). Similarly, for OS the risk score was defined by rs = -1.858 x MGMT + 0.625 x MRI positivity + 0.0413 x MET-positive volume (ml) + 0.0401 x age (years), and the quartiles were -0.972 and 0.929.

Figure 3: Imaging before radiochemotherapy (upper row) and at time of recurrence 6 months thereafter (lower row) of a 60-year old patient after initial gross total resection is shown. MRI (T1 with contrast enhancement) is displayed in the left column beneath the fusion of this MRI with MET-PET in axial, coronal and sagittal direction. PET-positive volume according to the nuclear medicine expert (orange) in comparison to the radiooncologist (yellow) visualizes interobserver-variability. The inner rose line surrounds the CTV prescribed with 60, the outer rose line the CTV prescribed with 50 Gy.

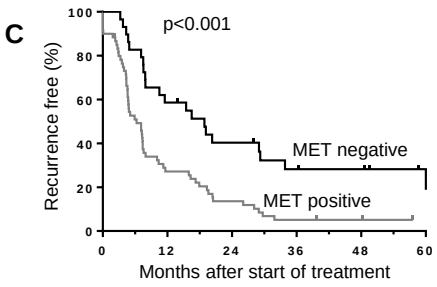
Figure 1



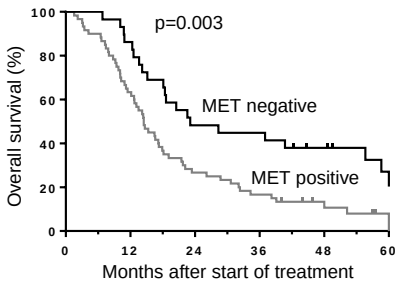
At risk	89	33	19	10	8	3



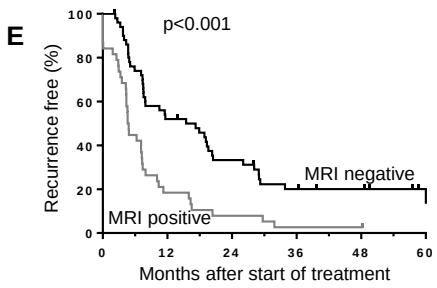
At risk	89	63	30	23	14	5



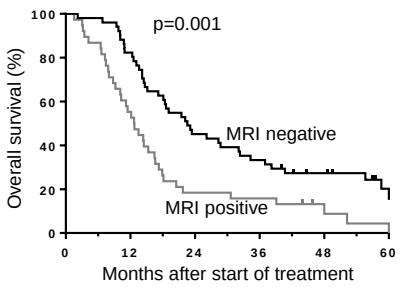
MET positive	60	16	8	3	2	0
MET negative	29	17	11	7	6	3



MET positive	60	38	16	10	5	1
MET negative	29	25	14	13	9	4

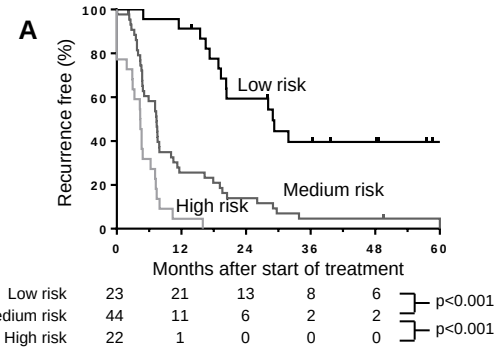


MRI positive	38	7	3	1	1	0
MRI negative	51	26	16	9	7	3

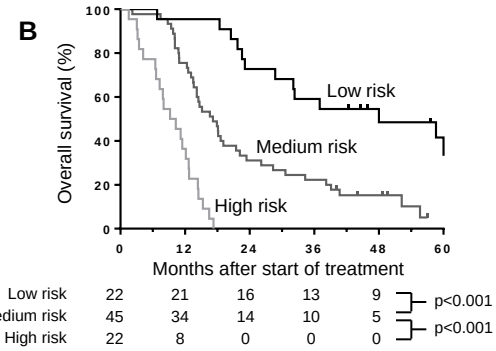


MRI positive	38	21	7	6	3	1
MRI negative	51	42	23	17	11	4

Figure 2



Low risk	23	21	13	8	6	p<0.001
Medium risk	44	11	6	2	2	p<0.001
High risk	22	1	0	0	0	



Low risk	22	21	16	13	9	p<0.001
Medium risk	45	34	14	10	5	p<0.001
High risk	22	8	0	0	0	

Figure 3