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N^2M^2 (NOA-20) phase I/II trial of molecularly matched targeted therapies plus radiotherapy in patients with newly diagnosed non-MGMT hypermethylated glioblastoma

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Abstract

Background. Patients with glioblastoma without O^6-methylguanine-DNA methyltransferase (*MGMT*) promoter hypermethylation are unlikely to benefit from alkylating chemotherapy with temozolomide (TMZ). Trials aiming at replacing TMZ with targeted agents in unselected patient populations have failed to demonstrate any improvement of survival. Advances in molecular understanding and diagnostic precision enable identification of key genetic alterations in a timely manner and in principle allow treatments with targeted compounds based on molecular markers.

Methods. The NCT Neuro Master Match (N^2M^2) trial is an open-label, multicenter, phase I/IIa umbrella trial for patients with newly diagnosed isocitrate dehydrogenase (*IDH*) wildtype glioblastoma without *MGMT* promoter hypermethylation to show safety, feasibility, and preliminary efficacy of treatment with targeted compounds in addition to standard radiotherapy based on molecular characterization. N^2M^2 is formally divided into a Discovery and a Treatment part. Discovery includes broad molecular neuropathological diagnostics to detect predefined biomarkers for targeted treatments. Molecular diagnostics and bioinformatic evaluation are performed within 4

weeks, allowing a timely initiation of postoperative treatment. Stratification for Treatment takes place in 5 subtrials, including alectinib, idasanutlin, palbociclib, vismodegib, and temsirolimus as targeted therapies, according to the best matching molecular alteration. Patients without matching alterations are randomized between subtrials without strong biomarkers using atezolizumab and asinercept (APG101) and the standard of care, TMZ. For the phase I parts, a Bayesian criterion is used for continuous monitoring of toxicity. In the phase II trials, progression-free survival at 6 months is used as endpoint for efficacy.

Results. Molecular diagnostics and bioinformatic evaluation are performed within 4 weeks, allowing a timely initiation of postoperative treatment. Stratification for Treatment takes place in 5 subtrials, including alectinib, idasanutlin, palbociclib, vismodegib, and temsirolimus as targeted therapies, according to the best matching molecular alteration. Patients without matching alterations are randomized between subtrials without strong biomarkers using atezolizumab and asinercept (APG101) and the standard of care, TMZ. For the phase I parts, a Bayesian criterion is used for continuous monitoring of toxicity. In the phase II trials, progression-free survival at 6 months is used as endpoint for efficacy.

Discussion. Molecularly informed trials may provide the basis for the development of predictive biomarkers and help to understand and select patient subgroups who will benefit.

Keywords

CD95 ligand | MDM2 | MGMT | mTOR | radiotherapy

Importance of the study

It is conceivable that targeted precision treatments work in situations of a defined molecular background. The present study addresses this topic by focusing on newly diagnosed glioblastoma, in which the analyzed tumor material best reflects the molecular specifics; further, the trial uses multiple agents in an umbrella design with postsurgical standard radiotherapy as a backbone and deferral of TMZ outside a control arm by restriction to patients with glioblastoma harboring an unmethylated *MGMT* promoter. The study spearheads the concept in newly diagnosed glioblastoma and leaves room for future improvement by integrating novel compounds, combinations thereof, and molecular analyses better reflecting the heterogeneity of the disease. Each subtrial may evolve into a controlled phase II trial further strengthening the therapy and the attached molecular biomarker.

The understanding of glioblastoma at the molecular level has improved dramatically in recent years.[1–5] For the first time a limited defined set of molecular markers is implemented in the updated World Health Organization classification.[6] These and additional markers and some others are increasingly used to support clinical decisions.[7] Isocitrate dehydrogenase 1 and 2 (*IDH1/2*) mutations[8] and 1p/19q codeletion[7] are already routinely tested in glioma patients to guide diagnostics, and O^6-methylguanine DNA methyltransferase (*MGMT*) promoter methylation[9] is used to support treatment decisions.

Despite these advances, prognosis and treatment success in glioblastoma patients have only slowly been improving over the past decades, with an increase in median survival reflecting improved supportive measures and patient selection.[10–12] The current standard therapy for glioblastoma patients consists of maximal safe resection followed by radiochemotherapy with temozolomide (TMZ) and 6 maintenance TMZ cycles.[10] *MGMT* methylation status was shown to be a predictive biomarker with methylation indicating a response to alkylating chemotherapy such as TMZ or lomustine. The European Organisation for Research and Treatment of Cancer (EORTC) 26981/22981 National Cancer Institute of Canada (NCIC) CE.3 trial led to the practical use of *MGMT* testing in daily clinical routine[13]—and after final confirmation by the NOA-08[14] and NORDIC[15] trials, it was integrated as a predictive biomarker into the current European guidelines for diagnosis and treatment of glioblastoma at least in elderly patients.[7,9] Therefore, in clinical routine, treatment decisions are mainly *MGMT* based in elderly patients, if combined radiochemotherapy is not applicable due to age or comorbidities.[7] Most other patients are treated with combined radiochemotherapy despite the unlikely benefit from an alkylating chemotherapy with a non-hypermethylated *MGMT* promoter. *MGMT* promoter methylation status does not define a molecularly distinct glioblastoma subpopulation,[16] which means that other molecular lesions occur with the same frequency and there is no reason to believe that MGMT unmethylated tumors harbor further distinct molecular resistance features. However, clinical trials replacing TMZ by, for instance, temsirolimus, bevacizumab, or enzastaurin have failed to improve survival so far in molecular-wise unselected glioblastoma patients with unmethylated *MGMT* status.[17–19]

Recent developments of new targeted therapies increasingly allow subset-specific treatment for patients with expression of respective molecular markers. *IDH* mutations represent prognostic biomarkers and additionally are targetable by IDH inhibitors[20,21] or an immunotherapeutic approach with vaccination targeting the *IDH1 R132H* mutation.[22] Other examples for targetable alterations include variant III of epidermal growth factor receptor (EGFRvIII) mutation,[23] BRAF mutations[24] (although present in rare cases of adult gliomas), and CD95L.[25] Lower levels of methylation of carboxypeptidase G2 (CpG2) in the promoter of cluster of differentiation ligand (CD95L) may be predictive of an improved overall survival (OS) with the CD95 inhibitory treatment with asinercept (APG101) in glioblastoma patients.[25] In addition, the proneural subtype of glioblastoma according to expression analysis[26] might be predictive for response to bevacizumab treatment[27] and mismatch-repair deficiency or polymerase epsilon gene (*POLE*) mutations resulting in a hypermutator phenotype may predict response to checkpoint inhibition.[28] Furthermore, improved molecular diagnostics increasingly enable individual treatments based on molecular alterations in representative tissue,[29,30] building the basis for clinical trials.[31]

Growing evidence proposes a relevant genetic heterogeneity within one and the same disease manifestation, particularly in spatially or temporally separated tumors (ie, multifocal tumors or tumor recurrences). Whereas data from a recent study do not support uniformity within spatially heterogeneous tumors, they support the present concept of using new tissue information for informed decisions.[32,33] Since the NCT Neuro Master Match (N^2M^2) trial relies on tissue from the surgery immediately prior to trial inclusion, the restrictions may be less relevant. In a series of dry runs, we have demonstrated feasibility of the timely molecular analysis and application of an algorithm for decision making.[31]

The N^2M^2 trial intends to translate complex molecular diagnostics in glioblastoma into clinical decision making by prospectively allocating patients with molecular profiles that match with the mode of action of a targeted therapy and might thereby indicate a higher likelihood for a response to this treatment. Glioblastoma patients harboring an unmethylated *MGMT* promoter status most likely benefit from alternative treatment approaches to TMZ and therefore are chosen as the study population in this trial.

Study Design

This study is designed as an open-label, parallel group, nonrandomized phase I/IIa multicenter trial of molecularly matched targeted therapies plus radiotherapy in patients with newly diagnosed glioblastoma without *MGMT* promoter methylation. A "match" is defined as detection of one of the predefined biomarkers of the available targeted drugs. The study is formally divided into a Discovery and a Treatment part. Discovery consists of complex molecular diagnostics including whole exome, low coverage whole-genome and transcriptome sequencing, methylome analysis using methylation arrays, and gene expression arrays to identify defined biomarkers as well as new targets and to get a more comprehensive view of affected pathways. Importantly, data from Discovery are to be confirmed with established immunohistochemical and (Sanger) sequencing techniques. The detection of predefined biomarkers for the different arms, which are considered to indicate a response to a specific available targeted therapy, forms the basis for a "match"/"no match" decision in the Treatment part of this study. Matching patients receive the respective targeted therapy in combination with radiotherapy as first-line treatment in different subtrials which are subdivided in a phase I part for determination of safety and appropriate dose by dose-escalation and a phase IIa part evaluating preliminary efficacy. The warehouse of targeted therapies in this trial consists of asinercept, alectinib, idasanutlin, atezolizumab, vismodegib, palbociclib, and temsirolimus. For asinercept and atezolizumab, biomarkers have not been considered strong enough at the present, and the "non-matching" patients will be equally allocated to receive asinercept, atezolizumab, or the current standard of care: radiotherapy with TMZ. In the latter, patients will serve as a nonrandomized but contemporary control group to the molecular informed subtrials and a randomized control for the no-match subtrials.

Objectives and Endpoints

The main objective of the N^2M^2 study is to demonstrate the improvement of OS of glioblastoma patients with an unmethylated *MGMT* promoter based on molecular characterization and use of targeted compounds in a modern trial design. Further aims are the assessment of safety and feasibility of treatment with these targeted compounds in addition to radiotherapy. Subtrials that satisfy the safety and efficacy criteria will be considered as candidates for further investigation in randomized phase II/III trials independent of the current protocol.

The phase I part evaluates safety and tolerability of the systemic molecularly defined therapy and the proof of the proposed optimal monocompound dose in conjunction with radiotherapy. The primary safety endpoint is dose-limiting toxicity (DLT), defined as all adverse events (AEs) of grade ≥3 according to Common Terminology Criteria for Adverse Events (CTCAE) v4.03 that are related to the administration of the investigational agents. The secondary objective of the phase I part is the evaluation of efficacy by determination of progression-free survival (PFS) at 6 months (PFS-6), which also defines the primary objective of the phase IIa part. Secondary objectives of the phase IIa part consist of (i) safety and tolerability of experimental therapies, (ii) PFS, (iii) OS, and (iv) biomarker development.

Trial Population

The trial population is molecularly defined by glioblastoma patients harboring an unmethylated *MGMT* promoter status and an *IDH* wildtype status.

The inclusion criteria include: (i) written informed consent; (ii) open biopsy or resection to obtain enough

tumor material; (iii) availability of fresh-frozen tissue, formalin-fixed paraffin embedded tissue, and blood; (iv) histologically confirmed, newly diagnosed *IDH*-wildtype glioblastoma with unmethylated *MGMT* promoter determined by one of the accepted methods (quantitative PCR, pyrosequencing, methylation array);[32,33] (v) standard MRI ≤48 (+ 6) hours postsurgery according to the present guidelines; (vi) Karnofsky performance score (KPS) ≥70%; (vii) life expectancy >6 months; (viii) age ≥18 years; (ix) no stable or decreasing steroid levels below 4 mg/day of dexamethasone during the last 3 days prior to enrollment (for a complete list, refer to the Supplementary Material). After inclusion, unmethylated *MGMT* status needs to be reconfirmed prior to initiation of specific treatment, otherwise the patient will be excluded from this study.

The exclusion criteria include: (i) abnormal (grade ≥2 CTCAE v4.03) laboratory values for hematology, liver, and renal function; (ii) HIV, active hepatitis B or C infection, or active infection requiring antibiotics; (iii) immunosuppression; (iv) history of other malignancies within the last 5 years; (v) prior therapy for glioma (except surgery and steroids); (vi) insufficient tumor material for molecular diagnostics; (vii) pregnancy or breastfeeding; (viii) history of hypersensitivity to the investigational medicinal product; (ix) any clinically significant condition that could interfere with the conduct of the study or absorption of oral medication or that would pose an unacceptable risk to the patient (for a complete list, refer to the Supplementary Material).

Enrollment

Patients will be enrolled in 13 Neuro-oncology Working Group trial sites of the German Cancer Society (NOA) in Germany. Based on molecular findings ("match"/"no match"), patients will be allocated in 7 different subtrials or the control group.

For the "match"/"no match" decision, fresh tumor tissue and blood from glioblastoma patients with an unmethylated *MGMT* promoter will be widely examined by neuropathological analysis. Results will be available within a maximum of 3 weeks postoperatively allowing a dedicated bioinformatics evaluation which forms the basis for the final treatment decision by the molecular tumor board and afterward a timely initiation (≤4–6 wk) of postoperative treatments. The workflow and timelines of molecular diagnostics and treatment decisions are summarized in Fig. 1.

Discovery, Sequencing, and Data Processing

Molecular analysis consists of an epigenome-wide array, panel sequencing, whole exome, low-coverage whole genome, and transcriptome sequencing as well as expression array detecting somatic single nucleotide variants, small inserts/deletions, copy number variants, focal amplifications, or overexpression of affected genes and pathways.

The detected somatic mutations are assigned information from databases such as known cancer genes and the catalogue of somatic mutations in cancer, as well as custom lists of cancer-associated genes, drug targets and biomarkers (with special respect to the warehouse drugs), resistance mechanism, indirect druggability, and contraindications. The lists will continuously be updated and expanded during the project by external data and feedback from the study arms.

For cases with detection of several targetable mutations, a previously described ranking algorithm will be used.[31] A schematic study overview, including the ranking algorithm, is depicted in Fig. 2. If more than one mutation obtains the highest rank, the match will be randomly allocated to specific subtrials or assigned for the best-performing subtrial, if already known. This process does not introduce a bias into the final evaluation, but allows for more rapid detection of a positive subtrial. All experimental test results will be confirmed by an accepted genetic test (eg, Sanger sequencing) or immunohistochemistry.

Treatment Decision

The final decision about specific treatments is made by the molecular tumor board (MTB), consisting of members of the steering committee in Heidelberg, members of the participating site with patients under discussion and optionally all other participating sites invited via video conference. The aims of the MTB are to ensure reliable

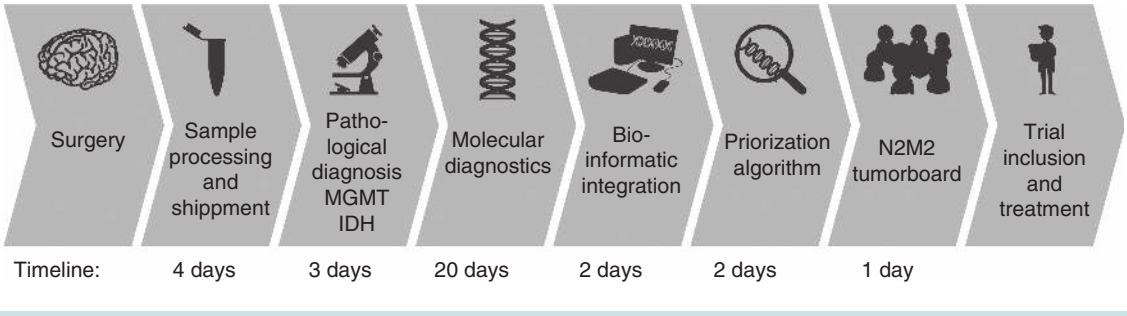

Fig. 1 Workflow and timelines of molecular diagnostics and treatment decision. Of note, patient tissue analyses in Heidelberg and with a start in an outside study center follow the same time intervals.

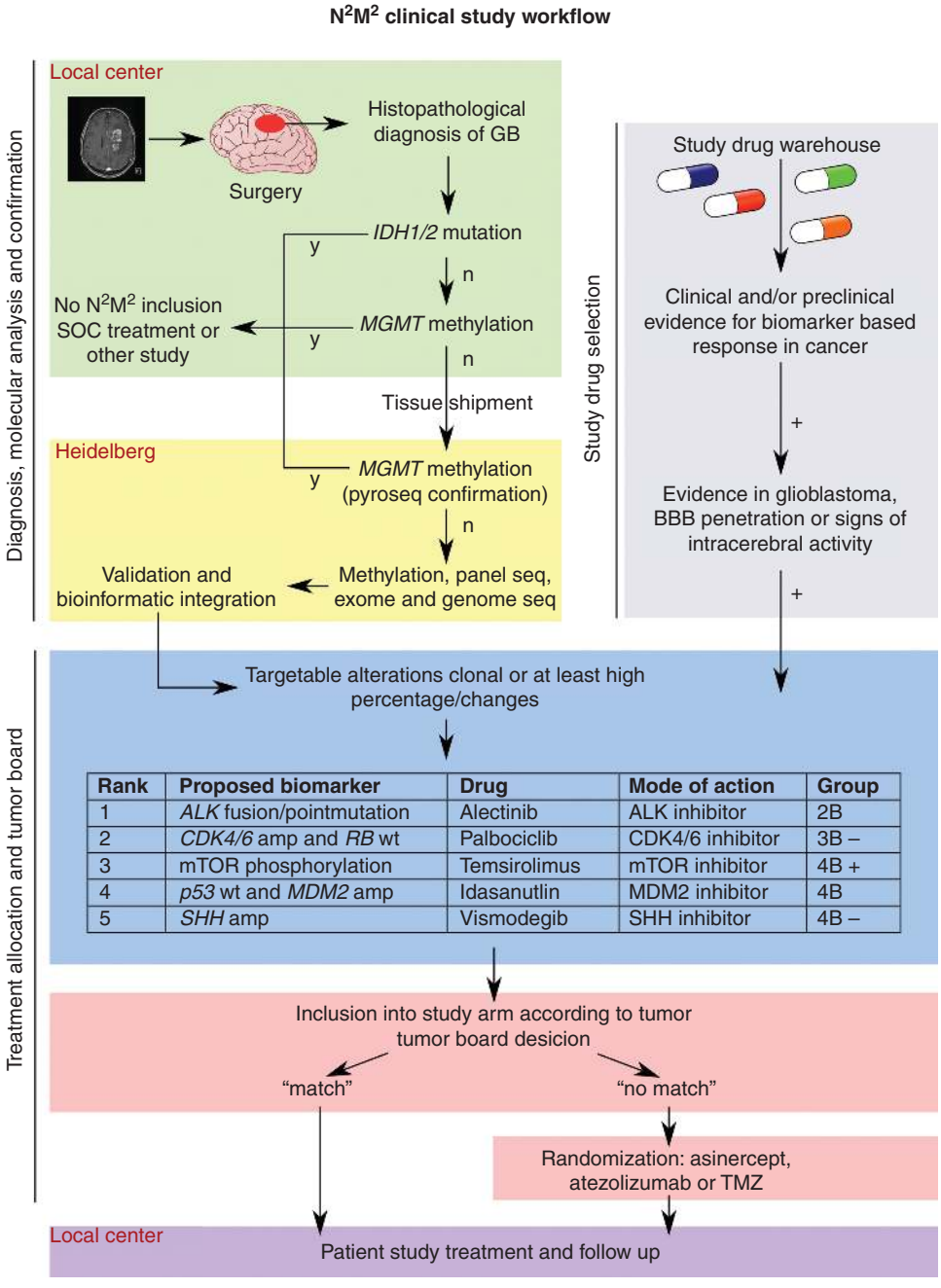

Fig. 2 Schematic study overview. Figure shows the workflow of the N²M² study with emphasis on molecular analysis and treatment allocation process. GB: glioblastoma; SOC: standard of care; seq: sequencing, BBB: blood–brain barrier.

and consistent decisions and to provide final recommendations regarding the enrollment of patients in specific subtrials. The molecular basis for the decisions will be based on the accepted tests, not on the experimental procedures. The algorithm for decisions about patient allocation is demonstrated in Fig. 2. At complete availability of molecular information and open slots in each subtrial, the data are pre-assessed by the study chair, a molecular neuropathologist, the study coordinator, and a bioinformatician to allow suggestion of a potential match or a non-match resulting in randomization. At the MTB, the patient's case plus the raw molecular information as well as the recommendation are intensively discussed and a decision on the allocation is rendered by consensus.

Trial Oversight Committees

An external Data Safety Monitoring Committee (DSMC) will consist of clinical and biostatistical experts to

- ☐ meet periodically (quarterly in the phase I part of the subtrials and twice per year in the phase II parts as well as via written approval on a mailing at the end of each phase I subtrial, prior to moving to phase II) to review summarized and individual patients' data related to safety, data integrity, and overall conduct of the trial
- ☐ re-review specific interim analyses for safety and/or efficacy, as appropriate
- ☐ provide recommendations to continue as originally planned, change or terminate the trial depending on these analyses
- ☐ communicate other recommendations or concerns as appropriate

The management of the complexity and innovation of N²M² will be facilitated by the formal implementation of a Steering Committee in addition to the DSMC. The Steering Committee will comprise representatives from all involved subspecialties to ensure input and counseling for the formal study leadership. Of note, decisions on the patient-relevant changes are made by the DSMC and the Coordinating Investigator (W.W.). The Steering Committee has advisory function; a formal role in the decision process would complicate, not improve, the study management.

Treatment, Intervention

Based on the decision of the MTB, patients will be enrolled in 5 different subtrials ("match") or randomized between asinercept, atezolizumab, and the control subtrial ("no match") (Fig. 3). A complete randomized allocation of patients to the subtrials is not feasible due to the fact that the subtrials differ in molecular targets. As radiotherapy is considered standard of care, it is not a study procedure and builds the backbone for each subtrial with radiotherapy at 60 Gy in 2-Gy fractions in working-daily radiotherapy sessions over a period of 6 weeks.[7] Experimental treatments start with the initiation of radiotherapy at the maximum tolerated dose (MTD), which is predefined or determined in phase I parts of the subtrials, and continue until progression, undue toxicity, death, or patient's decision, whichever comes first. As a control intervention, patients without any of the defined molecular alterations receive concomitant TMZ chemotherapy (75 mg/m² body surface area) plus radiotherapy followed by 6 cycles of TMZ maintenance therapy (150/200 mg/m² body surface) according to the standard of care. Safety endpoints of phase I parts will be determined until the end of combined modality treatment, and efficacy data will be collected until end of study or death, whichever comes first.

Subtrials, Targeted Therapies

The warehouse of targeted therapies for the different subtrials consists of alectinib, idasanutlin, vismodegib,

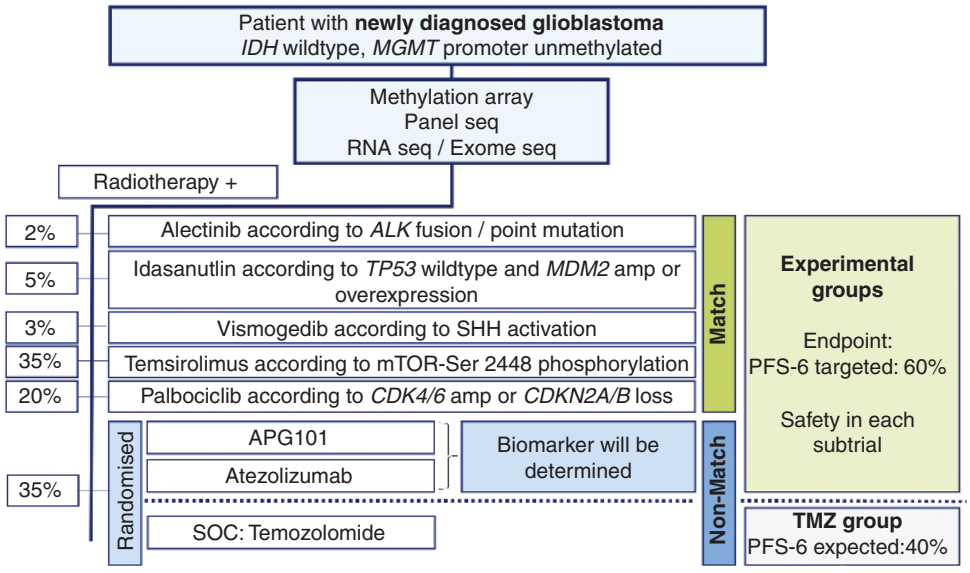

Fig. 3 Patient allocation and targeted therapies according to matching biomarkers. Percentages indicate proposed proportions of patients allocated to each subtrial. As for asinercept and atezolizumab, no specific biomarker is available so far; it will be assessed exploratively during the trial and patients will be equally allocated to each "non-match" subtrial including asinercept, atezolizumab, standard of care (SOC). RT: radiotherapy.

Table 1 Prioritization algorithm for biomarker-based targeted treatment

Group	Criterion
1	Biomarker with approved biomarker specific treatment in glioblastoma + with strong survival benefit – with moderate survival benefit or inconsistent
2A	Biomarker with approved biomarker specific treatment in another cancer indication with compelling clinical evidence in glioblastoma
2B	Biomarker with approved biomarker specific treatment in another cancer indication not tested in glioblastoma in a clinical setting
3A	Clinical evidence in glioblastoma, but not approved in glioblastoma or any other cancer indication + mutation – amp/expression
3B	Clinical evidence in another cancer indication, makes biological sense in glioblastoma, but no clinical evidence in glioblastoma + mutation – amp/expression

palbociclib, and temsirolimus for the match subtrials as well as asinercept, atezolizumab, and temozolomide for the non-match randomized subtrials. Targets and biomarkers of therapies, methods for their molecular detection, as well as the prevalence of the alterations in glioblastoma patients are summarized in Table 1. Of note, no prognostic value is so far attributed to these markers.[31]

Alectinib is a second-generation inhibitor of anaplastic lymphoma kinase (ALK), which showed clinical efficacy in ALK positive non–small cell lung cancer administered orally at 600 mg twice daily.[34] ALK fusions and mutations represent proven biomarkers for alectinib treatment.

The mouse double minute 2 homolog (MDM2) inhibitor idasanutlin activates the p53 pathway by blocking the inhibitory MDM2-p53 interaction in p53 wildtype tumors.[3,35] Preclinical studies demonstrated a higher sensitivity toward the drug for p53 wildtype tumors with MDM2 amplification and a primary resistance of tumors harboring p53 mutations.[36] Idasanutlin was effective and well tolerated in first-in-human studies in patients with acute myeloid leukemia and solid tumors. It is administered orally on 5 consecutive days of a 28-day cycle. Optimal dose will be determined in the phase I part by dose escalation from 100 mg daily until MTD.

Vismodegib, a small-molecule inhibitor of the sonic hedgehog (SHH) signaling pathway, has been approved for therapy of basal cell carcinoma in doses of 150 mg daily. Activation of the SHH pathway leads to cell proliferation, upregulation, of anti-apoptotic proteins, production of vascular endothelial growth factor, and angiopoietins[37] and is considered a biomarker for a response to vismodegib treatment.

Palbociclib, an oral inhibitor of cyclin-dependent kinases (CDKs) 4 and 6, has been approved for treatment of estrogen receptor–positive, human epidermal growth factor receptor 2–negative breast cancer in combination with aromatase inhibitors or fulvestrant.[38] Amplification of CDK4 and CDK6 results in dysregulation of the retinoblastoma pathway, a major regulator of cell cycle progression and proliferation. CDKN2A is an inhibitor of CDKs such as CDK4 and CDK6, and CDKN2B interacts with CDK4. Therefore, activation of CDK4 or CDK6 or CDKN2A/B codeletion serves as biomarkers for palbociclib treatment. Palbociclib will be administered initially at 75 mg with dose escalation steps to 100 and 125 mg during combination with radiotherapy and at 125 mg in adjuvant monotherapy on 21 consecutive days of a 28-day cycle.

Activation of the mechanistic target of rapamycin (mTOR) pathway is associated with reduced survival in glioma patients[39,40] and leads to increased cell growth.[40] Temsirolimus represents an inhibitor of the mTOR pathway, which is administered intravenously at 25 mg/week, and was evaluated as a first-line treatment in glioblastoma patients in the EORTC-26082 trial. Although primary endpoints were not reached in an unselected patient population, phosphorylation of mTOR-serine2448 (p-mTOR^{Ser2448}) was retrospectively found to be predictive for response to temsirolimus.[17] This association is worth prospective confirmation, which is attempted in the present subtrial. As the EORTC 26082 trial showed feasibility and safety of temsirolimus in the exact same patient population and treatment schedule, a formal phase I trial is not foreseen for this subtrial.

Asinercept (APG101), a CD95-fusion protein, has been shown to be effective and well tolerated in combination with second radiotherapy in progressive glioblastoma.[25] It blocks the interaction of CD95 and its ligand CD95L and thereby inhibits the CD95 pathway, resulting in reduced proliferation and invasion of glioblastoma cells.[41] Retrospective analysis suggested low methylation levels in CpG2 of the CD95L promoter as predictive for response to asinercept treatment.[25] Determination of the safe combination dose of asinercept i.v. started with 600 mg/week with 3 de-/escalation steps of 200 mg (ie, D0 = 400 mg, D1 = 600 mg, D2 = 800 mg) in conjunction with radiotherapy. Atezolizumab is a monoclonal antibody targeting programmed death ligand 1 (PD-L1). PD-L1 is an inhibitory cell surface molecule which is expressed on immune and tumor cells, suppresses T-cell migration, proliferation, and secretion of cytotoxic mediators, and restricts tumor cell killing by binding the inhibitory programmed death 1 (PD-1) receptor on T cells. Predictive biomarkers for atezolizumab are currently not yet defined, but high expression of PD-L1[42,43] or high numbers of nonsynonymous mutations driven by mismatch-repair deficiency[44] are potential candidates. Atezolizumab will be administered intravenously at 1200 mg every 3 weeks. Recent studies in colon cancer revealed that patients with mismatch repair deficiency respond better to anti-programmed death (PD)-1 therapy.[44,45] Additional studies indicate that other solid tumors

with mismatch repair deficiency, including glioblastoma, are sensitive to anti–PD-1 therapy.[46]

Temozolomide is an alkylating chemotherapy used as standard of care for patients with glioblastoma irrespective of *MGMT* status.[7]

Withdrawal of Patients

Patients must be withdrawn from trial at any time at their own request, in case of serious adverse events caused by the investigational medicinal product except for manageable abnormal laboratory values or other general safety issues by the investigator. All ongoing AEs and serious AEs of withdrawn patients will be followed up until stabilization or resolution.

Outcome Measures

An overview about diagnostic and therapeutic measures, timing of disease assessment, and study visits of participating patients is displayed in Supplementary Table 2. AEs, DLTs, concomitant medication, and safety hematological laboratory values will be recorded weekly during combined radiotherapy and medical treatment. Clinical chemistry laboratory values and physical examination will be performed every 4 weeks. MRIs are carried out twice-monthly starting 4 weeks after completion of radiotherapy. Six months after start of therapy, PFS-6 is assessed. After end of study (EOS)—that is, 6 months after start of study for the individual patient—patients will be routinely followed up until death every 3 months by phone. After EOS, patients will be routinely followed up and will be treated regarding standard of care according to the discretion of the treating physician. Patients who would still benefit from the experimental intervention after EOS might continue as part of an individual treatment or as an off-label use after consulting the coordinating physician, if medication is still available then.

Assessment of Endpoints and Statistical Analysis

Assessment of Safety

All AEs that occur during the trial after the first experimental treatment are recorded, graded according to the CTCAE v4.03 at every study visit, and followed up until resolution or stabilization. Safety endpoints will be assessed by frequency of AEs and the number of laboratory values that fall outside of predetermined ranges. AEs will be described by event, duration, seriousness, intensity, and relationship to the investigational medicinal product, actions taken, and clinical outcome and reported as tables of frequencies at Preferred Term (PT) and MedDRA System Organ Class.

Assessment of Efficacy

For the primary efficacy endpoint, PFS-6 (defined as proportion of patients with PFS 6 months after treatment start) is determined and presented in summary tables, along with Pearson–Clopper 95% CIs. Radiographic progression will be evaluated according to Response Assessment in Neuro-Oncology (RANO)[47] or immunotherapy RANO for atezolizumab[48] by the central neuroradiology and clinical progression by deterioration of KPS. Most importantly, the protocol contains detailed instructions to avoid too early cessation of study drug in case of presumed pseudoprogression and mandates a confirmatory scan whenever clinically possible.

For secondary efficacy endpoints, PFS and OS (defined as the time from treatment start until progression or death) will be determined and analyzed using the Kaplan–Meier method for survival curves and Greenwood's formula for estimating the standard error of event rates. Given the low number of patients in each subtrial and the multiplicity of the analyses, all statistical tests are of strictly exploratory nature.

Efficacy will be evaluated in each subtrial separately, based on a one-sided binomial test of the null hypothesis set as PFS-6 at 40%, the rate observed in a retrospective analysis of available data in patients undergoing standard treatment,[10,13] and an alternative hypothesis of 60% at the final analysis. No formal statistical comparisons between the subtrials are planned. However, results obtained for the control group and different subtrials may be used for considerations of changes regarding efficacy or recommendation for further phase II/III trials.

Interim Analysis and Stopping Rules

Two interim analyses per subtrial will be carried out once the PFS-6 endpoint has been determined for 15 and 25 patients, respectively. Tests for futility based on predictive power[49] and for decisions regarding acceptance of the DLT rate of experimental treatment for a phase IIa trial are performed. For that, the posterior distribution of the DLT rate is calculated with a binomial-beta model with a non-informative prior, and a Bayesian criterion is used for continuous monitoring of toxicity.[50] Recruitment will be suspended if the predictive power is lower than 10% or if the posteriori probability that the true toxicity rate (at the given dose level of dose-escalation in the phase I part of indicated subtrials) is 30% or higher exceeds 95%. In both scenarios, the DSMC will advise the coordinating investigator if patient accrual should be stopped.

Sample Size Estimation

In each of the 7 experimental subtrials, between 2 and 18 patients will be enrolled for phase I parts, depending on observed toxicities. In phase IIa parts, a maximum of 40 patients in each subtrial will be accrued, wherein 9 patients of the corresponding dose of the phase I part will be included. The exact number depends on early stopping for toxicity or futility. The "non-matching" group is anticipated to include approximately 35% of all screened study patients. Therefore, 12% of all screened patients are expected to be enrolled in the control group receiving TMZ.

Accordingly, about 450 patients with newly diagnosed glioblastoma harboring an unmethylated *MGMT* promoter will need to be screened, requiring approximately 5 years for recruitment and 84 months for overall duration of the trial with expected wide variability in the subtrials depending on frequency of the molecular alteration providing a match.

Ethical and Legal Aspects

The trial is conducted in accordance with the standards of Good Clinical Practice, the applicable version of the Declaration of Helsinki and local legal and regulatory requirements. The study protocol has been approved by the independent Ethics Committee (AFmu-207/2017) and the competent federal authority (Vorlagen Nummer 3051/01, Paul-Ehrlich-Institute in Langen, Germany).

For this trial, the EudraCT number 2015-002752-27 has been obtained. Monitoring and pharmacovigilance is performed by the Coordination Center for Clinical Trials (KKS) Heidelberg.

Patients are enrolled in a two-step consenting process. Oral and written explanation of the molecular testing, including interpretation and conduct of the MTB, is provided after surgery, and any trial-specific measure is started only after written informed consent. Consenting for the treatment step in the respective subtrial is done after the MTB decision prior to any subtrial-specific process.

The Discovery phase of the trial is funded by DKFZ/NCT Heidelberg. Study drugs will be provided free of charge by Hoffmann-La Roche Ltd., Apogenix AG, and Pfizer Pharma GmbH. The clinical phase is supported by funding of the German Cancer Aid (DKH, funding number 70111980) and by structural support via the German Ministry of Education and Research (BMBF) funded German Cancer Consortium (DKTK) as well as the Heidelberg Center for Personalized Oncology (HIPO 2-K25 and 2-K32R).

Discussion

The aim of this study is the development of a complex molecular and bioinformatics workup to prospectively identify patient subgroups with a potential higher likelihood for a response to a specific treatment based on predefined molecular profiles with the final objective of an improvement of OS for these patients.

At present, molecular markers are increasingly used to allocate patients for individual treatments. Some of these markers already represent a prerequisite for specific treatments, such as the *IDH* mutation for IDH inhibitors[20,21] or vaccinations[22] and the detection of EGFRvIII[23] or BRAF V600E[24] for respective inhibitor treatments. At least conceptually comprehensive diagnostics enable precision treatment concepts for patients based on molecular alterations and drugs with published mode of action if available.[29–31] Until now these treatments have not been approved for patients with glioblastoma, and molecular markers are not validated in this patient cohort. As studies evaluating the diagnostic pipeline and consecutive prospective patient allocation are lacking, its investigation is one of the aims in the N²M² trial. In order to translate molecular diagnostics into treatment decisions, diagnostic workup has to be performed within a maximum of 3–4 weeks to allow a timely initiation of postoperative treatment. For N²M², dry runs already demonstrated the feasibility of this timely diagnostic process.[31]

Heterogeneity is observed in the mutational profile changes during the natural course of the disease, among different patients, within the tumor and through selection pressure resulting from treatment.[32,33] Decisions for salvage treatments that are based on biomarker information from tissue acquired prior to any other treatment may therefore underestimate the molecular variability and result in incorrect conclusions.[29,30,32,33] For that reason, patients will receive treatment with respective targeted therapies as first-line therapy in N²M², which further enables the investigation and comprehensive molecular understanding of causes for treatment failure whenever tissue can be obtained at recurrence.

MGMT promoter status predicts the response to alkylating chemotherapies but does not define a fundamentally different subgroup of glioblastoma.[16] Prior studies replacing TMZ for patients with unmethylated *MGMT* status failed to demonstrate a survival benefit in unselected patient populations,[17–19] but retrospective analysis revealed potential predictive biomarkers for treatment response.[17,19] Therefore, prospective patient allocation to respective treatments based on molecular biomarkers represents a promising approach to improve OS and establish rational alternatives to TMZ for glioblastoma patients with unmethylated *MGMT* promoter status unlikely benefiting from TMZ treatment.

N²M² would be deemed successful if at least one arm made it to a full controlled phase II/III trial and if we considerably deepened our understanding of the disease and accepted molecular decisions to be integrated into primary patient care.

Supplementary Material

Supplementary data are available at *Neuro-Oncology* online.

Funding

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