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Refined brain tumor diagnostics and stratified therapies: the requirement for a multidisciplinary approach

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Abstract

Individualized therapies are popular current concepts in oncology and first steps towards stratified medicine have now been taken in neurooncology through implementation of stratified therapeutic approaches. Knowledge about the molecular basis of brain tumors has expanded greatly in recent years and a few molecular alterations are studied routinely because of their clinical relevance. However, no single targeted agent has yet been fully approved for the treatment of glial brain tumors. In this review, we argue that multidisciplinary and integrated approaches are essential for translational research and the development of new treatments for patients with malignant gliomas, and we present a conceptual framework in which to place the components of such an interdisciplinary approach. We believe that this ambitious goal can be best realized through strong cooperation of brain tumor centers with local hospitals and physicians; such an approach enables close dialogue between expert subspecialty clinicians and local therapists to consider all aspects of this increasingly complex set of diseases.

Introduction

Advances in the understanding of tumor biology and the availability of more sophisticated technical tools for molecular genetic analyses in recent years has led to an exponential increase in the knowledge of genetic alterations linked to gliomagenesis [117, 118]. While these findings have widened our understanding of the underlying biology of the disease, they have also increased our awareness of its complexity. For example, intertumoral and intratumoral heterogeneity may influence outcome and treatment response; the vascular niche, the (hypoxic) stem cell niche or infiltrating glioma cells (to name but a few) all have their own molecular characteristics [76, 116]; and molecular changes occur on multiple regulatory levels (genomic, transcriptional, epigenetic) and are interconnected, such as miRNAs may control sophisticated signaling networks [83, 85, 122]. Taken together, these different facets of the disease have an enormous potential to influence diagnostic decisions and to stimulate the development of novel therapies.

At the same time, the molecular changes that have been translated into a clinically meaningful context are not many and no targeted agent has yet been fully approved for the teatment of gliomas. A few molecular alterations such as *MGMT* methylation, deletions on chromosome arms 1p/19q and *IDH1/2* mutations have been successfully linked to predictive and/or prognostic information [114, 115]. However, although frequently evaluated, their use for clinical decision-making is not yet widespread in the general community [56]. In addition, novel genetically defined subgroups within histologically homogenous tumor entities and global molecular signatures with prognostically relevant content have been identified [120]. Further, we are on the verge of understanding the molecular correlates of chemotherapy resistance. These are first steps emphasizing the transfer of basic research insights into clinically relevant applications. Finally, the interconnection of tissue-based information with data from imaging methods such as magnetic resonance techniques and positron emission tomography plays increasing roles in diagnostics and therapeutics [35]. Here, all types of information converge to establish an optimized treatment strategy.

With a multitude of molecular alterations now known through high-throughput profiling studies [22], one of the major future challenges will be the determination of how those changes might be exploited to our patients' benefit. While we are far from providing final answers to this important challenge, we here review approaches that connect molecular with

histopathological and clinical as well as imaging information. We further believe that such approaches can be best realized in an environment where clinicians and basic scientists work closely together to illuminate the many aspects of this increasingly complex disease and where they connect to local hospitals and physicians to bring optimal treatment to patients. As such we argue that current optimum neuro-oncology treatment and research must be multidisciplinary and interdisciplinary. In the following commentary, we provide a series of arguments and a conceptual framework in support of this statement (Fig. 1).

Argument #1: Diagnosis is necessarily complex and requires expert neuropathologists

#1a: Histology and molecular pathology are increasingly interconnected

Since its first edition in 1979, the WHO classification of tumors of the central nervous system has been developed periodically by correlating histopathological findings with clinical information, primarily survival data. To date, the WHO brain tumor classification (most recently updated in 2007) is still based on histological and immunohistochemical findings [78]. However, given the emerging knowledge of the molecular basis of brain tumors, molecular analyses will likely be increasingly incorporated in future revisions of the classification [77]. A few examples in this direction are as follows (also compare Table 1):

- *Molecular subtypes of glioblastomas*

  Histologically defined glioblastoma represents a molecularly diverse set of entities. For example primary (de novo) and secondary (derived from a lower-grade precursor lesion) glioblastoma have long been distinguished from one another [68, 93]. While histologically indistinguishable, these tumors exhibit divergent molecular profiles [74, 93, 114]. Data from large-scale profiling approaches (for details see below) identified even higher numbers of molecularly defined glioblastoma subgroups with clinical relevance [99, 140]. For molecular-driven therapies this additional information would have to be incorporated into the overall tissue diagnosis and passed from the neuropathologist to the clinician; to do so in many instances, particularly in the setting of complex diagnostic tests, requires subspecialized expertise.

- *Molecular differential diagnoses of astrocytic gliomas*

  Molecular genetics may be beneficial in stratifying astrocytomas with ambiguous histological features into groups that are more likely to behave similarly in response to treatment. One example in this respect would be the case of high-grade glial neoplasms that appear highly pleomorphic but in which histological criteria for the diagnosis of glioblastoma are not completely fulfilled and uncertainty remains whether this may be merely due to incomplete sampling. In such cases, the presence of a mutant *IDH* might raise caution for the diagnosis of a WHO grade IV lesion, while the *IDH* wildtype situation may support the possibility that the tumor is likely to behave in an unfavorable, glioblastoma-like fashion [4, 47, 59]. As *IDH* mutations are tumor-specific alterations that do not occur in non-neoplastic cell populations, they may also aid in the sometimes subtle differential diagnosis between reactive astrogliosis or the infiltrative rim of a low-grade glioma containing only isolated invading tumor cells [21, 121]. Other molecular alterations may support the sometimes difficult differential diagnosis between infiltrating astrocytomas and astrocytic tumors with a more circumscribed growth pattern [78, 119]. As such, *BRAF* gene alterations (and in particular a BRAF-KIAA1549 fusion gene) might help to differentiate pilocytic astrocytomas from diffusely infiltrating low-grade astrocytomas (the latter in turn show common *IDH* gene mutations) [5, 64, 65]. *BRAF V600E* mutations may also provide support for the diagnosis of an (anaplastic) pleomorphic xanthoastrocytoma over that of a glioblastoma [125, 143].

- *Molecular diagnostics of oligodendroglial neoplasms*

  Because oligodendroglial tumors follow a better clinical course and because recent data has shown that 1p/19q-codeleted oligodendroglial tumors should be treated with combined radiochemotherapy, their precise histological identification is of major clinical importance [20, 112, 138]. However, the histological features of oligodendroglial differentiation are prone to marked interobserver variability [71, 135]. In such cases, the integration of histological and molecular information becomes important. While it is still a matter of debate if the presence of 1p/19q deletions should define oligodendroglioma, these prognostically favorable molecular alterations are very common in oligodendroglial tumors (up to 80% of oligodendrogliomas and 30-50% of oligoastrocytomas) [18, 19, 20, 136, 138, 148]. Thus, in cases with questionable oligodendroglial histology, 1p/19q testing can help to identify those patients that follow

a better clinical course. In terms of therapies for oligoastrocytoma, this combination of histological and molecular information is also important because these tumors do not form a homogeneous group of neoplasms and histology alone does not sufficiently reveal their nature. Only molecular analysis can segregate lesions with the molecular characteristics of an oligodendroglioma (1p/19q codeletion) from those that more resemble a diffusely infiltrating astrocytic neoplasm (*TP53* mutation, 17p loss, chromosome 7 gain) [81, 88].

These three examples emphasize that a combination of histology, immunohistochemistry and molecular genetics is required for diagnostic approaches. The combination is more than the mere synopsis of the information obtained from the different investigative levels. Accurate brain tumor classification has to weigh histological and molecular information carefully in light of the therapeutic options available. In many instances, brain tumor diagnostics is not straightforward and requires a high degree of expertise and knowledge about the clinical consequences of a specific differential diagnosis.

The diagnosis of rare tumor entities also carries challenges. Tumors such as angiocentric glioma, papillary tumors of the pineal region, papillary glioneuronal tumor and rosette-forming glionueronal tumor of the fourth ventricle have only recently been added to the WHO classification [78]. Most of these tumors are rare and awareness of their existence and differential diagnosis is more likely in neuropathological institutions where such tumors are seen in greater numbers. Once identified, the lack of extensive published experience treating such tumors means that the clinical implications of such diagnoses have to be individualized. Thus, although the details of these rare entities is beyond the scope of this review, their rarity makes them as important as common entities in terms of this conceptual framework of interdisciplinary clinical approaches.

#1b: Proper molecular diagnostics requires dual molecular and histopathological expertise

Tissue expertise is a required starting point for state-of-the-art molecular diagnostics. The proper work-up of tissue specimens can be complex and requires the expertise of a neuropathologist. For example, because most molecular assays are tumor-lysate based approaches, histopathological characterization to identify representative tumor tissue is essential prior to homogenization. In addition, tumor cell content should be carefully

controlled for since some assays (such as *MGMT* methylation testing, which requires cut-off values and reports percentages) depend on a sufficient tumor cell content within the sample [115]. In this context, it is important to point out that nearly all molecular tests have not yet been validated independently, leading to discrepancies between laboratories.

For example, several methylation assays have been described for *MGMT* promoter methylation testing. In addition to the methylation-specific PCR assay [52], combined bisulfite restriction analysis, methylation specific sequencing and pyrosequencing as well restriction enzyme-based approaches (that do not require bisulfite conversion, e.g. methyl-QESD [10]) are in use. For the assays, primers are not standardized between laboratories so that different regions of the gene promotor and a different number of CG sites are being assessed. In this regard, it is unclear which methylation site corresponds best to clinical response [27]. Such pitfalls handicap the comparability of testing results between individual laboratories and make definition of clear cut-off levels questionable [115]. For these reasons, the establishment of a consensus testing method that enables interlaboratory testing would be desirable. New directions could involve combined assessment of gene methylation with protein expression [72, 146]. Such consensus would require inter-institutional agreements, perhaps through the involvement of national and international trial organizations.

Similar quality and comparability aspects have to be taken into consideration for assays of 1p and 19q deletions where PCR-based and FISH methods coexist and deletions of specific regions may bear an inverse prognostic meaning [41, 139]. All these natural limitations of the methods have to be kept in mind and, in combination with the histological findings, have to be adequately communicated to the clinician.

#1c: Correct diagnosis and tissue processing is the basis for interpretation of research results.

Another important aspect that requires the input of neuropathologists is brain tumor banking. Tumor banking stimulates basic, translational and clinical research aspects in many ways. The collection of prospective cohorts of clinically well-annotated tumor samples supplies a unique resource for basic researchers and clinicians by combining molecular and histopathological with clinical and prognostic information [54, 139]. As a consequence, tissue banks in brain tumor centers are a common resource usually goverened by a steering board that involves all disciplines (e.g., neurosurgery, neuropathology, neurology, medical oncology etc.) and decides together on the use of the tissue specimens. A critical step is the proper processing

and characterization (e.g., with respect to representativeness and tumor cell content) of surgical tissue specimens prior to banking, which requires expert neuropathological input, particularly from neuropathologists interested in research applications. If research-orientated neuro-oncologists and neurosurgeons then complement clinical information and other additional features (such as patient blood samples), tissue banks become a valuable resource. These resources become potentially yet more valuable if materials and information can be shared in inter-institutional ways, for example to support large trials or large genomic studies. Thus, tumor banks by linking clinical and basic research are an integral part of our conceptional framework and foster innovative treatment approaches (Fig. 1).

Argument #2: Clinical care is necessarily complex and needs expert neurooncologists

#2a: Molecular markers increasingly influence therapy decisions

In addition to aiding in diagnosis and classification, molecular markers are now also affecting therapeutic decisions. The prognostic or therapy-predictive role of these markers has been partly clarified within clinical trials. To date, a number of adequately powered phase III- or randomized phase II-clinical trials have been performed or are enrolling. These trials may change the standards for the treatment of high-grade gliomas on basis of molecular evaluations (Table 2). Therefore, molecular markers, particularly in high-grade tumors, will gain an increasing role for therapy stratification, make therapeutic decisions more individual and thus necessitate a close dialogue between clinicians and neuropathologists. This is not only true for medical neurooncologists, but also for neurosurgeons, radiotherapists and diagnostic disciplines as neuroradiology.

In the following we provide examples on how molecular information supports current therapeutic decisions in glioblastomas and anaplastic gliomas:

- *Clinical impact of molecular markers in glioblastomas*

  The EORTC 26981/22981 NCI-C3.0 trial demonstrated the relevance of *MGMT* promoter methylation in glioblastomas by comparing treatment with temozolomide radio-chemotherapy with radiotherapy only [52, 131]. In this trial, methylation was predictive for benefit from chemo- and radiotherapy, a result that was later verified in the phase III RTOG 0525 trial [1]. Results from the EORTC 26891 combined treatment

arm are often used as standard arm in trial design, and still, there has been no fully published trial that shows better results. RTOG 0525 failed to show that an intense regimen of temozolomide is more effective than the standard regimen either in patients with glioblastoma with a methylated or unmethylated *MGMT* promoter [1]. However, as there is no approved alternative to radio-chemotherapy with temozolomide at this time, the predictive value of the *MGMT* promoter methylation does not lead to a stratified treatment of patients with glioblastoma. This is different in elderly patients with glioblastoma above an age of 65: efficacy of temozolomide as a monotherapy in older patients was verified in the Nordic trial [82] and the german randomised phase 3 NOA-08 trial; as shown by NOA-08, dose-dense temozolomide and radiotherapy in a conventional fractionation scheme are generally equally effective [149]. However, patients with *MGMT* promoter methylation have an increased overall survival under temozolomide monotherapy. Therefore, these patients should be treated with chemotherapy, whereas patients without *MGMT* promoter methylation or with an unknown *MGMT* status should be treated with radiotherapy. Finally, data that bevacizumab increases PFS from around 6 to 10 months [26] and possible approval of bevacizumab may lead to a shift in the first-line treatment of glioblastomas, leading to bevacizumab treatment in patients with an unmethylated *MGMT* promoter (see #2b, below).

- *Clinical impact of molecular markers in anaplastic gliomas*

In the german NOA-04 trial for anaplastic astrocytomas and anaplastic oligodendroglial tumors, hypermethylation of the *MGMT* promoter, mutation of *IDH1* and oligodendroglial histology were verified as favorable prognostic markers [148]. However, *MGMT* promoter methylation was not predictive for a benefit from chemotherapy. Other publications substantiate the prognostic value of molecular markers in grade III gliomas [137]. In addition, data from two studies on WHO grade III oligodendroglial tumors [20, 138] recently showed that the overall survival of patients with a combined loss of 1p and 19q doubles from approximately 7 to about 14 years if combined treatment with radiotherapy and chemotherapy with procarbazine, CCNU and vincristine (PCV) is used. Even if these data were evaluated retrospectively, they appear so convincing that a paradigmatic change in the treatment of these patients may result; in contrast, patients without 1p/19q loss will most likely be treated with radiotherapy, temozolomide or PCV as a monotherapy.

Most of the classical molecular markers have not been prospectively verified in independent trials. In the EORTC 26981/22981 NCI-C3.0 trial, for example, *MGMT* promoter methylation was evaluated post hoc in a subset of 206 of 573 treated patients. The evaluation of fewer than half of the specimens, with the rest of specimens not being available or investigable, may have introduced a statistical bias. Even in later trials with a prospective evaluation of *MGMT* methylation, the rate of evaluated specimen has been in the range of 50-60% [1, 148]. Also, the situations discussed above do not offer a stratified therapeutic approach for all patients with high-grade gliomas, with the situation being even less clear in WHO grade II glioma patients.

In such instances, crosstalk between tissue and imaging diagnostic disciplines also comes into play and is essential to guide neurosurgery, radiotherapy and treatment response assessment. Therapeutic disciplines increasingly rely on functional and metabolic imaging to guide diagnostics and treatment [2]. For example, neurosurgeons use functional and biological imaging to increase the extent of resection or to guide biopsy of the anaplastic focus of diffuse tumors [40, 96, 145]. However, diagnosis and evaluation of response phenomena such as pseudoprogression and pseudoresponse cannot be solely based on imaging methods, such as MRI and PET [35, 86, 151], as the rate of false positive or negative imaging results is still considerably high. Radiotherapists increasingly plan treatment along the biological tumor volume that is evaluated by positron emission tomography [100, 144], and a value for magnetic resonance spectroscopy has been suggested [39]. In this situation, correlation of the biological tumor volume to histopathological and molecular features will be helpful to verify this apporach.

No single reliable tumor marker has yet been detected for gliomas and serum markers for glioma diagnosis are not available at the present time. Nonetheless, in regard to a potential serum markers for monitoring tumor load or tumor progression, small molecules such as miRNAs might bear promising diagnostic perspectives [57]. Thus, additional controlled studies involving proper histological and molecular work-up and development of novel imaging and serum markers are needed.

Regardless of the modalities involved, the number of different technologies and the nuances inherent in understanding the evidence levels offered by such approaches, it is clear that

treatment decisions for such patients are best discussed in multidisciplinary tumor boards that include experts well aware of the strengths and weaknesses of all of these technologies.

#2b: Targeted agents provide therapeutic options beyond standard alkylating chemotherapy

The current goal of many areas of oncology is the development of innovative targeted therapies. Achieving effective targeted approaches will require close interaction between basic scientists and clinicians, as well as a developmental pipeline from basic to translational to clinical research. Most of these approaches are still at an experimental stage and most are therefore introduced later in this review (see #4a below).

The currently most advanced example of a therapeutic strategy beyond standard alkylating agents is the selective blockade of pro-angiogenic pathways with the humanized monoclonal antibody bevacizumab [62, 101]. By binding the VEGF-A ligand, bevacizumab inhibits the receptor-ligand interaction [66, 129]. Bevacizumab has been used as a monotherapy or in combination with chemotherapeutic agents as irinotecan or temozolomide. In the first phase II trials in relapse of high-grade gliomas, high response rates of up to 63%, a significant increase of progression free survival (PFS) at 6 months (38%), and a small increase of overall survival have been shown in comparison to historical data [142] - observations that however could not be fully substantiated in subsequent trials. In most trials, response rates of about 30% have been reported. Bevacizumab has been preliminarily approved by the FDA for the treatment of relapsing or progressive high-grade gliomas [43], and several alternative regimens have been tested using bevacizumab as monotherapy or combined with other cytotoxic agents, i.e., irinotecan, temozolomide or nitrosourea. A trial of the EORTC focusing on the sequential therapy of patients with first relapse of glioblastoma (EORTC 26101) using bevacizumab and CCNU in several combinations is enrolling patients at this time and additional controlled studies involving proper histological, molecular and imaging work-up are urgently needed to identify those patients that are most likely to benefit.

In addition to the use of bevacizumab in relapse, two first-line phase III trials have been initiated that have shown early promising results of a significant increase of PFS in glioblastoma [26]. The difference in OS was not significant at the time of presentation, suggesting that bevacizumab may only prolong the clinically relevant first phase of the disease until first progression. As such, an improved understanding of the side-effects of

targeted agents appears necessary. Antiangiogenic therapy has been shown to increase the invasive properties of glioma cells. Early in vitro data [73] have recently been challenged by observations from human high-grade glioma trials using bevacizumab, where an increased FLAIR-enhancement suggesting increased invasion has been observed using magnetic resonance imaging (MRI) [3, 102]. However, other investigators could not reproduce these results [147]. These multidisciplinary results from both basic research as well as clinical studies raise the question if a combined use of anti-angiogenic and anti-invasive drugs may be advantageous and warrant careful evaluation of clinical and imaging response as well as effects on tumor progression and overall survival.

Argument #3: Basic research is needed to drive translational research

The development of novel anticancer therapies is a major goal in neurooncology, yet progress in this direction is still moderate. Since many substances fail in controlled clinical trials, the costs for the substances that reach market approval are tremendously high. Thus, less fragmented and instead more streamlined and cost-effective research approaches are needed. Translational research is a promising way to bridge basic and clinical research. There is a growing awareness of the fact that basic scientists on the one hand should provide input into the development of clinical trials and that pre-clinical research projects would benefit from the input of clinicians. Molecular aspects have to be better incorporated into clinical studies and clinical demands have to be considered for defining relevant basic research projects. A translational research pipeline that turns basic scientific discoveries into clinicial applications is a long and multistep process that requires a committed dialogue between experts from multiple disciplines. It will also be enriched by qualified individuals with medical and laboratory-based knowledge (so-called physician scientists). In our conceptional framework academically-based brain tumor centers that harbor both clinical and basic research disciplines are therefore ideally suited for addressing this task (Fig. 1).

#3a: Deeper insights into tumor biology are needed to identify novel promising targets of clinical use

Innovative treatment approaches necessarily require insights into the molecular basis of the disease. Over the past twenty years, tremendous progress has been made in unveiling the

different pathophysiological events contributing to gliomagenesis and glioma progression. Indeed, “glioma” is a heterogenous group of diseases, and the subsequent hope is that the different molecular events could be targeted by distinct treatment approaches. Processes may be targeted, for example angiogenesis or tumor cell proliferation. Experimental data from *in situ* analyses and microdissected glioma cell populations from different tumor regions suggest, for example, that infiltrating glioma cells contain unique molecular profiles [33, 84] and some of these differentially activated molecules and pathways could serve as targets for therapies aiming at reduction of the infiltrative nature of the disease: among others, the dysregulation of EGFR and integrin signaling pathways could affect the tumor infiltration zone [74, 95, 116].

Recent studies have raised the possibility that tumor-initiating cells such as cancer stem cells may be the most relevant targets for successful therapies [9, 12, 141]. The hypoxic niche is appreciated as a major relevant local factor for the growth and propagation of glioma stem cells [128]. In this regard, targeting the vascular components of the niche can lead to eradication of brain tumor initiating cells (BTICs) [42, 55]. Interestingly in this context, the expression of C/EBPbeta (one of the master regulators of the mesenchymal expression signature; see below) is closely associated with areas of necrosis, i.e., areas of intratumoral hypoxia [28]. Thus, targeting tumor cells with a hypoxia-tag may be particularly promising in eradicating those cell populations that sustain tumor growth and regrowth. Nevertheless, the definition and identification of tumor-initiating cell populations has to be refined to enable potential novel stem-cell directed therapeutic approaches.

While the above studies suggest the possibility of reasonable targets, this is only the first part in a long experimental pipeline for the development of successful clinical therapies. Experimental findings derived from cell culture and animal models have to be verified for their relevance to the human *in vivo* situation. Novel potential target molecules should be loaded with clinical prognostic and predictive information. As such, close interfaces between basic scientists and clinicians are essential to drive the development of these basic research insights into practical clinical applications and will require a multidisciplinary and translational research environment.

#3b: Molecular alterations have to be put into a meaningful context to extract those molecular changes that really matter

While many individual molecules could be targeted, approaches directed against single molecules have failed to date (see below, #4a). One reason for such failure may be that the tumor does not depend on the specific targeted molecular alteration, given the complexity of intracellular signaling relationships. Multiple alternative upstream alterations may lead to the dysregulation of identical downstream signaling intermediates in pathways [113, 116]. The situation becomes even more complex due to intratumoral heterogeneity and because multiple levels of molecular regulation affect one another (Fig. 2). In addition to genomic alterations (such as mutations or gene amplification), RNA and protein alterations contribute to a molecular make-up of the tumor. Epigenetic regulation superimposes an additional regulatory layer, with not only gene methylation and histone modifications but also miRNAs regulating complex networks of cancer genes. These signaling relationships have to be kept in mind when designing targeted therapeutic approaches and basic research can therefore best drive translational research by *putting genes into context*.

In 2008, The Cancer Genome Atlas (TCGA) reported an integrative analysis of DNA copy number, gene expression and DNA methylation profiling in a collection of 206 human glioblastomas [22]. This study confirmed the multitude of molecular aberrations that had been previously identified by individual researchers but also highlighted three signaling pathways of major importance in the broad majority of these tumors: CDK/cyclin/CDK inhibitor/pRB, p53, and RTK/RAS/PI3K (Fig. 3). A second approach substantiated these major molecular pathways defining glioblastomas [98]. These common pathways may serve to provide central targets to target the disease efficiently.

As mentioned above, global molecular approaches may also identify novel prognostic subclasses of high-grade astrocytomas: proneural, proliferative and mesenchymal expression patterns [11, 28, 75, 99, 140]. While tumors from the proneural subclass are highly enriched for neuronal lineage markers and exhibit better survival, proliferative and mesenchymal tumor subclasses are enriched for neuronal stem cell markers and display shorter survival. Upon recurrence, a frequent shift in expression patterns towards the mesenchymal subclass has been observed. Of note, two transcription factors (C/EBPbeta and STAT3) have been suggested as master regulators that could control the transition into a prognostically unfavorable gene expression profile [23]. Experimentally, the ectopic co-expression of C/EBPbeta and STAT3

reprogrammed neural stem cells along the aberrant mesenchymal lineage, while elimination of the two factors in glioma cells resulted in a loss of the mesenchymal signature and a reduction of tumour aggressiveness. Given that a hierarchy with a concerted regulation of multiple molecules exists and that a shift of whole expression patterns can be induced by single molecules, C/EBPbeta and STAT3 could be priority targets for therapeutic intervention.

In addition, meaningful molecular signatures may not only be restricted to the transcriptional level. For example, the TCGA highlighted a distinct subset of samples that had concerted hypermethylation at a large number of loci, indicating the existence of a glioma-CpG island methylator phenotype (G-CIMP) [92]. This hypermethylation signature overlapped with the proneuronal expression signature described above, and was more prevalent among lower-grade gliomas and associated with a significantly improved outcome. More recently, additional subgroups of glioblastoma with distinct global methylation patterns have been suggested defined by H3F3A mutations affecting two critical amino acids (K27 and G34) of histone H3.3 [132], and others have subgrouped glioblastomas according to miRNA expression profiles [67, 105] or histone modification patterns [80].

These findings indicate that biological knowledge of glioma biology is becoming increasingly complex and that advanced bioinformatic methods are needed to allow cross-platform correlations for extracting those molecular changes that are most meaningful in a clinical context. Basic and translational scientists as well as bioinformaticians need to be included in multidisciplinary research-orientated brain tumor teams. While not directly involved in clinical patient care, they can well contribute innovative impulses by identifying promising and relevant molecular targets for further clinical exploration.

Argument #4: Translational research is needed to drive clinical research

"From bench to bedside" is a goal occasionally envisaged in ambitiuous research grant proposals. As obvious from the examples provided above, basic tumor-biology orientated research approaches may be distant from clinical applications and benefit from the input of clinically orientated researchers in order to be catalyzed into practical patient care. On the

other hand, since basic research is necessarily more speculative and hypothesis-driven it may therefore - in the long run - lead to more fundamental breaktroughs and more radical paradigm shifts in practice than would clinical research alone. As such clinical research depends on basic research input to ask the most innovative questions, leave well-trodden trails and thereby accelerate therapeutic advancements.

#4a: Clinical studies are needed that are based on attractive molecular targets derived from basic research approaches

Translational research requires the transfer of basic scientific findings into clinical applications. Earlier in this review we introduced bevacizumab as the currently most advanced targeting agent that supplements therapeutic options in glioma care. In addition, other promising molecular targets have been or are currently being tested in early clinical trials. The most important of these are summarized in the following section.

- *Antiangiogenic agents other than bevacizumab*

  In addition to bevacizumab, several small molecule tyrosine kinase inhibitors as sunitinib [91, 97], sorafenib [34, 90], cediranib [7, 8], pazopanib [60], vatalanib [14, 107] and vandetanib [36, 70] have anti-angiogenic properties (Fig. 3). The molecular mechanism of these agents is similar in that each binds angiogenesis-relevant receptor tyrosine kinases or intracellular signaling molecules and therefore inhibits angiogenic signaling pathways. Inhibiting multiple targets at once may overcome the redundancy of intracellular signaling pathways. Suprisingly, none of the mentioned antiangiogenic agents except bevacizumab has been effective in clinical studies. Cediranib showed significant efficacy in an animal model and promising results in an early phase I/II clinical trial and therefore entered phase III in newly diagnosed glioblastoma, based on a strong drug development pathway of the respective company [6, 8, 61]. However, it then failed, leading to the assumption that it might be more promising to inhibit the ligand rather than the receptor or signaling cascade of antiangiogenic pathways.

- *Targeting the integrin cell adhesion receptor family*

  The alphaV-beta3 and alphaV-beta5 integrin receptors are expressed in glioma and tumor endothelial cells [24], contribute to tumor angiogenesis and migration, and may thus constitute promising targets for specific approaches. Cilengitide is a selective

inhibitor of integrins on endothelial cells with a predominant antiangiogenic effect, but has a bimodal biological effect since it also shows anti-invasive properties on tumor cells [124]. The inhibitor was investigated in several clinical protocols [87, 109, 110, 111, 130] and a promising median PFS of 8 months and 12- and 24-month overall survival rates of 68% and 35% have been reported in first-line therapy [130], especially for patients whose tumors have a methylated *MGMT* promoter. These clinical data led to the initiation of a large registration trial for patients in the primary therapy of glioblastoma with *MGMT* promoter metylation (CENTRIC; [133]) and a smaller phase II trial for patients whose tumors have non-methylated *MGMT*. Results were recently communicated in a press release by Merck; there was no benefit for the combination of radiochemotherapy with cilengitide in comparison to radiochemotherapy alone.

- *Tyrosine kinase receptor inhibition*

EGFR is amplified or overexpressed in its truncated form (EGFRvIII) in many glioblastomas, inducing excess kinase activity [115]. However, EGFR kinase inhibitors such as gefitinib, erlotinib and lapatinib have been unsuccessful in clinical trials [29, 103, 152]. Successful treatment has been claimed for some patients with coexpression of EGFRvIII and PTEN [89], but this has not been confirmed in subsequent studies. Recently, a investigational immunotherapeutic vaccine that targets the tumor-specific EGFRvIII has been developed and is currently being investigated in the international phase II and III ACT IV trial [31, 32].

PDGFR is another attractive target for the treatment of high-grade gliomas given the presence of *PDGFR* amplification/overexpression in many of these tumors [115]. The small molecule inhibitor imatinib mesylate was developed in the 1990s and is a ground breaking targeted agent that shows an over 90% response rate in chronic myeloid leukemia [37, 38]. Following the publication of first results from these trials, imatinib was investigated in a number of solid tumors. However, after promising early results [108], a phase III registration trial in high-grade gliomas was negative [106], illustrating that tumors that do not depend on a single driver oncogene will likely not respond to such therapeutic approaches.

- *Other targeted approaches*

Inhibitors of protein kinase Cß [15, 16, 69, 104, 150], PI3K/Akt/mTor inhibitors [25, 44, 123] and inhibitors of other receptor kinase or intracellular targets (e.g. notch, SHH,

histone deacetylase) have been investigated in phase II or early phase I trials and have failed, despite promising laboratory and animal data. In terms of immunmodulatory therapies, TGF-ß2 targeted antibodies, antisense oligonucleotides or small molecule inhibitors are the most advanced in clinical application [48, 49, 50, 126, 127], but their efficacy remains unclear at this time.

Most investigated targeted agents, therefore, have been unsuccessful to date in clinical phase II and III trials. This raises the question of why these biologically compelling molecules did not turn out to be clinically effective. The most ready explanation for such failure is that agents targeting a single molecule may not be sufficient to tackle the highly complex molecular oncogenic backbone of glioblastomas. Instead, multitargeted approaches may constitute an attractive and improved option and, as a result, first attempts in this direction have been made by using multi-target inhibitors as sorafenib [34, 90], cediranib [13] and sunitinib (against VEGFR1-3, PDGFR-a/b, FLT-3, c-KIT and RET) [91, 97] or combinations of cilengitide plus temozolomide [130]), EGFR-targeted vaccination and temozolomide [53], or bevacizumab plus irinotecan (Genentech trial; [43]).

#4b: Mechanisms of therapy failure and resistance have to be understood to improve therapeutic regimens

Most glioblastoma patients receive intensive neurooncological postsurgical care and are included into controlled clinical trials or are treated with standard radiochemotherapy using concomitant and adjuvant temozolomide [131]. Thus, the issue of chemotherapy resistence or failure becomes more important in terms of improving therapeutic regimens. Major preexisting tumor-intrinsic reasons for low efficacy of chemo- and targeted therapy against glioblastoma are poor blood-brain barrier penetration of cytostatic agents (especially in the therapeutically relevant periphery of the tumor) [46], expression of drug efflux pumps (multidrug resistance genes) [30, 63, 79], and the expression of resistance-associated proteins such as MGMT [51]. However, because of genetic instability and clonal selection, tumor cells may also develop molecular escape mechanisms under therapy that counteract the benefical effects of alkylating chemotherapeutic agents.

In a recent study by the German Glioma Network, pairs of primary and recurrent tumors from 64 glioblastoma patients treated with radiotherapy and TMZ were investigated, revealing significantly lower expression levels of the mismatch repair genes *MSH2*, *MSH6* and *PMS2* but no relevant changes in *MGMT* promoter hypermethylation [134]. Indeed, *MSH6* in this context appears to be a relevant player. A large-scale sequencing screen of the functional domains of 518 protein kinases identified inactivating somatic mutations of the mismatch repair gene *MSH6* in two gliomas that had recurred after treatment with alkylating agents [58] and sequencing of *MSH6* in 46 clinically well-characterized glioblastomas revealed that the frequency of *MSH6* mutations was significantly increased in recurrent glioblastomas [17]. These data suggest that *MSH6* deficiency (and maybe also the deficiency of other mismatch repair genes) may contribute to recurrences during maintenance treatment and that patients who initially responded to a frontline therapy may evolve treatment resistance by developing a hypermutator phenotype [153]. Further i*n vitro* data indicated that through exposure of an *MSH6* wild-type glioblastoma line to temozolomide resistant clones evolved with one of them harboring an *MSH6* mutation [22]. Also, knockdown of *MSH6* in the U251 glioblastoma cell line increased resistance to temozolomide cytotoxicity and its reconstitution restored cytotoxicity in *MSH6*-null glioma cells. It is hoped that better understanding of the biological mechanisms by which tumor cells escape the response to chemotherapy may be utilized to develop novel strategies to overcome or at least minimize chemotherapy resistance; for example, with respect to *MSH6*, a possible approach would be an upfront combination of alkylating agents with selective agents targeting mismatch-repair-deficient cells.

Further studies are needed to extend these observations, including to the epigenetic level. For example, in temozolomide resistant glioma cells, LINE-1 methylation, an indicator of global DNA-methylation and a positive prognostic factor in gliomas, is reduced [45, 94]. This could suggest that a lower global DNA methylation impairs DNA stability and activates novel chemotherapy resistance. Based on the knowledge about molecular markers for early response and resistance, adaptive clinical trials could be designed that would better overcome the therapeutic resistance of gliomas.

Conclusions

The approaches described above, within or outside clinical trials, can only be coordinated at dedicated brain tumor centers. A number of such centers have been founded within the last decades, starting from the US and now reaching Europe and Asia. Such centers typically include departments of neurosurgery, radiation ooncology, neurology and medical oncology, and specialized diagnostic units for neuropathology and neuroradiology. Our experiences working in such environments suggest that the individual patient case must be coordinated prospectively (including discussions on diagnostics and treatment planning) to meet the goals of quick decision making and structured, tailored treatment. We would argue further that a strong agenda combining translational development of new treatment approaches with the performance of clinical trials is best suited to serve patients both within clinical trials and also on an individual basis.

Equally important is a close connection of brain tumor centers with local institutions and community-based physicians. Such connectivity is essential to make local treatment possible, especially with regard to supportive treatments and management of complications. Local medical centers in this regard assume an important role in our conceptional framework of multidisciplinary and multiinstitutional brain tumor management (Fig. 1). Expertise can be brought to local centers by use of electronically connected consults and tumor boards. In such cooperative networks, local medical centers would be able to incorporate expertise from highly specialized disciplines, such as neuropathology and neuroradiology that might not be locally availaible. Bringing cutting edge diagnostics and treatment "close to home" is an optimal patient-oriented solution that can be reached when central and local strengths synergize.

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Tables and figure legends

Table 1. Molecular markers with diagnostic relevance.

***IDH1/2* mutation**	glioblastoma: genetically either primary (*IDH* wildtype) or secondary (*IDH* mutated) glioblastoma high-grade glioma with overtly anaplastic histology lacking necrosis and not fulfilling all histological criteria for the diagnosis of glioblastoma: lack of *IDH* mutations might point to a tumor that behaves like a glioblastoma gliosis versus glioma: detection of invading tumor cells by the presence of a mutation increases the sensitivity of glioma diagnostics
1p/19q deletion	tumors with borderline oligodendroglial features: detection of the 1p/19q codeletion may reflect a prognostically favorable oligodendroglioma diagnosis oligoastrocytoma: may guide consideration as oligodendroglial (1p/19q deleted) or astrocytic in nature (1p/19q retained, *TP53* mutated).
***BRAF* alterations**	*BRAF-KIAA1549 fusion*: presence conistent with pilocytic astrocytoma rather than a diffusely infiltrative astrocytoma (particularly when tested in tandem with *IDH1* and *IDH1* not mutated). *BRAF V600E mutation*: for differentiation of pleomorphic xanthoastrocytoma, gangliogliomas and extra-cerebellar pilocytic astrocytomas (frequent *BRAF V600E* mutation) from diffusely infiltrating astrocytic gliomas (rare *BRAF V600E* mutation).

Table 2. Ongoing or recently closed phase III clinical trials in adult patients with first line or relapsed glioblastoma, based on a search in ClinicalTrials.gov. All listed trials are recruiting or have not published results yet, except abstracts at scientific meetings. Only trials from major study groups and industry are listed. AvaGlio in abstract form has reported a PFS of 6.2 months for the standard arm and 10.6 months for the experimental arm (p<0,0001).

Treatment	Short name	Disease	No. of Pat.	Endpoint	Mol. selection	Primary study group / company	Trial identifier
Bevacizumab + TMZ/RT vs. TMZ/RT	RTOG 0825	GBM, first-line	942	PFS, OS	no	RTOG	NCT00884741
Bevacizumab + TMZ/RT vs. TMZ/RT	AvaGlio	GBM, first-line	920	PFS, OS	no	Roche	NCT00943826
Cilengitide + TMZ/RT vs. TMZ/RT	CENTRIC	GBM, first-line	504	OS	yes (MGMT)	EORTC / Merck	NCT00689221
NovoTTF-100A + TMZ/RT vs. TMZ/RT	-	GBM, first-line	700	PFS	no	NovoCure	NCT00916409
Rindopepimut + GM-CSF vs. TMZ/RT	ACT IV	GBM, first-line	440	OS	yes (EGFRvIII)	Celldex	NCT01480479
RT (3 weeks) vs. TMZ/RT (3 weeks)	-	GBM, first-line (> 65 a)	560	OS	no	NCIC	NCT00482677

Figure 1. Conceptional framework of an optimized multidisciplinary brain tumor management as proposed in this review article. Affiliation of brain tumor patients to academically-based brain tumor centers guarantees state-of-the art treatment and enables access to innovate research-driven clinical approaches. Collaborative clinical networks convey expertise from brain tumor to local medical centers and allow for supportive treatment and management of complications "close to home" in a socially embedded environment.

Figure 2. Illustration of the layers of histological and molecular information that coexist within a patient's diagnostic tumor sample. Typing and grading based on the histological classification represent the basis for the estimation of the tumors biological behavior. Histological diagnosis may be supplemented by molecular information on a multitude of genes, molecular markers or complex gene signatures. This molecular information is represented on different molecular levels (epigenetic, genomic, transcriptional) or in different areas of the tumor microstructure (e.g. tumor center vs. tumor border), but highly mutually interconnected: *IDH* mutations, e.g., might lead to global epigenetic changes and miRNAs regulate complex transcriptional networks. Nevertheless, the multitude of single molecular alterations converges into a manageable number of common signaling pathways. As such a context-dependent interpretation of individual molecular changes appears helpful for developing efficient targeted therapeutic strategies.

Figure 3. Candidate mechanisms and molecules for targeted therapies. A multitude of single molecular alterations converge into relatively common signaling pathways that are highly interconnected and that may be targeted by available agents. Features of tumor and endothelial cells have been combined in this figure and angiogenic pathways, which are clinically most relevant at this time, are overrepresented.