Annals of Oncology 17 (Supplement 10): x191–x197, 2006
doi:10.1093/annonc/mdl259

Brain tumors: molecular biology and targeted therapies

M. E. Hegi[1,2], A. Murat[1], W. L. Lambiv[1] & R. Stupp[3]

[1]*Laboratory of Tumor Biology and Genetics, Department of Neurosurgery, University Hospital Lausanne (CHUV), Lausanne;* [2]*National Center of Competence in Research (NCCR) Molecular Oncology, Swiss Institute of Experimental Cancer Research (ISREC), Epalinges;* [3]*Multidisciplinary Oncology Center, University Hospital Lausanne (CHUV), Lausanne, Switzerland*

introduction

Glioblastoma is the most common and most malignant form of primary brain tumors in adults. It frequently develops *de novo* but may also evolve over years from a lower grade astrocytoma [1]. Recent combined modality treatments have led to some improvement in survival. However, most patients will still succumb to their disease (median survival to 15 months and 2-year survival rates of 26%) [2, 3].

Correlative molecular studies and animal models have identified numerous molecular alterations and biological processes involved in initiation and progression of glioma. However, the underlying molecular mechanisms and pathways involved are complex and remain poorly understood. The accumulation of multiple aberrations in regulatory processes enables tumor cells to bypass the effects of many of the available therapies. Molecular alterations underlying such mechanisms comprise aberrations on the genetic level, e.g. point mutations in key genes or respective amplifications and deletions [1], or are a consequence of epigenetic modifications. The latter include aberrant methylation of CpG islands in the regulatory sequence of genes, as well as changes in chromatin structure mediated by mechanisms such as histone acetylation. Hypermethylation of CpG islands in the promoter of genes leads to loss of expression (inactivation), while loss of methylation in normally silenced genes may cause inappropriate expression (e.g. *IGF2* gene), both with tumor promoting effects [4, 5]. Individual molecular tumor profiles are heterogeneous implying that different therapeutic approaches will be necessary for successful personalized treatments.

A first step towards the design of novel therapies is the identification and better understanding of relevant molecular mechanisms driving the aggressive biological behavior that subsequently may be targeted in respective treatment approaches. Hanahan and Weinberg [6] categorized the principle biological requirements for tumor formation as follows: (i) self sufficiency in growth signals, (ii) insensitivity to anti-growth signals, (iii) limitless replicative potential, (iv) sustained angiogenesis, (v) evasion from apoptosis and (vi) tumor infiltration and metastasis. The molecular aberrations conferring these properties in tumors are diverse, although they show some characteristic tumor type and malignancy grade dependent patterns. Molecular tumor profiles may become helpful tools to device therapeutic strategies aiming for individually tailored treatments.

molecular profiles indicate biological features differentiating glioma subtype and tumor grade

Glioma subtypes exhibit distinct molecular profiles (Figure 1) [1, 7–9]. A hallmark of primary glioblastoma is the amplification of epidermal growth factor receptor gene (*EGFR*) that is often associated with deletion of the *CDKN2A*$^{p16/Arf}$ gene encoding two tumor suppressors, p16 an inhibitor of CDK4 and p14^{ARF} a negative regulator of MDM2. These two aberrations are mutually exclusive with mutations in the *TP53* tumor suppressor gene that represent a hallmark in the evolution of secondary glioblastoma [10, 11]. Primary and secondary glioblastoma, although histologically undistinguishable, are distinct disease entities that occur in different age groups. Childhood glioblastoma, frequently situated in the brain stem, form a third pathogenetically distinct group from their adult counterparts [12, 13].

Oligodendroglioma, characterized by combined loss of heterozygosity on chromosomes 1p and 19q, rarely exhibit mutations in the *TP53* gene or amplification of *EGFR,* but in the majority have an epigentically inactivated O^6-methylguanine-DNA methyltransferase (*MGMT*) repair gene that may explain their particular sensitivity to alkylating agent therapy.

The distinct genomic aberrations translate into characteristic differences of global gene expression profiles of glioma subtypes (Figure 2) [14, 15]. Interestingly, differential gene expression profiles distinguishing primary glioblastoma from low grade astrocytoma, and surprisingly also from secondary glioblastoma identified a group of correlated genes related to angiogenesis and hypoxia. This may guide the use of anti-angiogenic treatment strategies [14].

Most glioblastoma (80%) exhibit inactivation of the p14^{ARF}/TP53/MDM2 pathway concurrent with abnormalities in G1-transition control. The latter is a consequence of aberration of either *RB1*, *CDK4*, *CDKN2A* or *CDKN2B*^{p15} genes involved in the same pathway of cell cycle control [16]. Associations of genetic aberrations such as *EGFR* amplification and concurrent deletion of the *CDKN2A*$^{p16/Arf}$ gene locus [7] are suggestive of a cooperative effect. This hypothesis is further supported by *in vivo* and *in vitro* models, and may have relevant consequences for resistance to respective targeted treatments [17, 18].

As molecular data on glioma accumulates, attempts are made to identify predictors of prognosis and response to therapy.

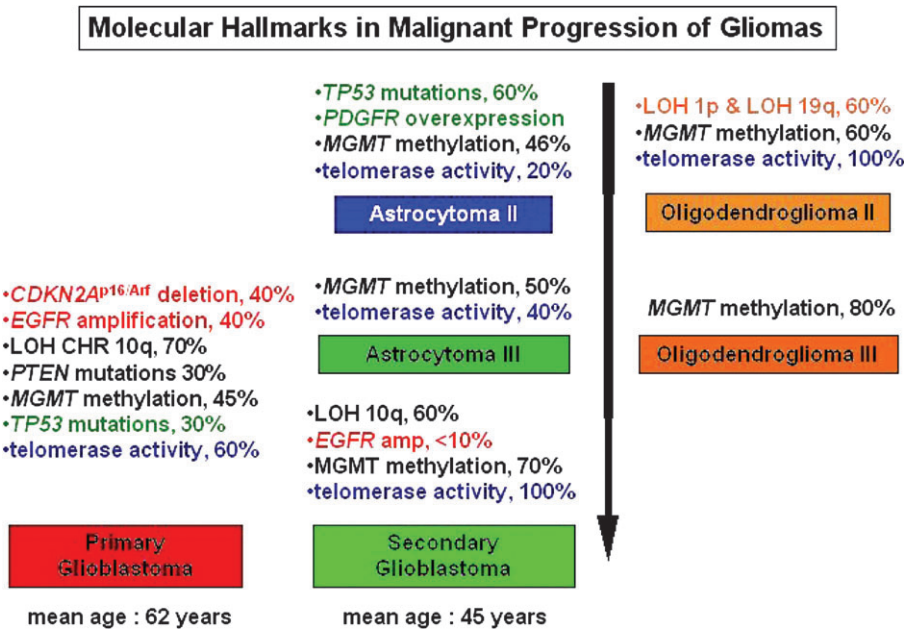

Figure 1. Distinct pathogenetic pathways characterize malignant progression of gliomas. A hallmark of primary glioblastoma is the amplification of the *EGFR* that is often associated with deletion of the *CDKN2A*$^{p16/Arf}$ gene encoding two tumor suppressors. These aberrations are mutually exclusive with mutations in the *TP53* tumor suppressor gene that represent a hallmark in the evolution of secondary glioblastoma. Primary and secondary glioblastoma occur in different age groups. Combined loss of heterozygoity on chromosomes 1p and 19q are characteristic for oligodendroglioma. They rarely exhibit mutations in the *TP53* gene or amplification of the *EGFR*. Information is compiled from the literature in particular from the [1, 7–9, 62].

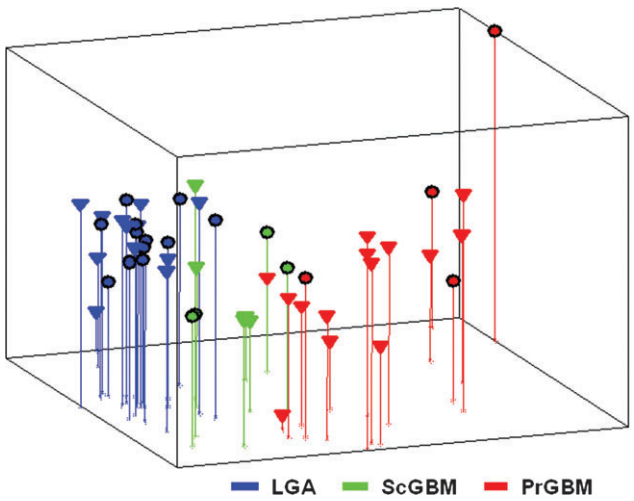

Figure 2. Gene expression profiles differentiate glioma subtypes. MDS (multidimensional scaling) based on overall gene expression (1185 genes) of 51 astrocytic gliomas. The color code indicates the tumor subtype according to histological and clinical criteria. LGA, low grade astrocytoma; ScGBM, secondary glioblastoma; PrGBM, primary glioblastoma. With permission from [19].

Recent comprehensive molecular profiling efforts provided evidence for the existence of molecular subtypes of glioblastoma that may differ in their clinical behavior [19–28]. Several prognostic factors emerged from these and other retrospective studies. However, the insights gained will require validation in independent data sets and prospective clinical trials to determine their predictive value for response to specific therapeutic interventions.

prognostic molecular markers improve diagnostic precision

Tumor histology and malignancy grade guide treatment decisions while taking into account clinical parameters such as age, performance status and extent of tumor resection, are all important prognostic factors. Identification of molecular prognostic markers will be crucial for the design of future trials. However, many markers may just reflect a different natural history and not necessarily contribute to decisions for individual patient management. Nevertheless, molecular markers may greatly improve diagnostic precision and characterize tumor entities with distinct biological behavior.

The best example is the identification of oligodendroglioma with loss of heterozygosity on chromosomes 1p and 19q (LOH 1p/19q). This tumor type is associated with high response rates to chemotherapy and a prolonged survival [8, 29].

Recently, the outcome of anaplastic oligoastrocytoma and oligodendroglioma treated within two independent randomized trials with a similar design was reported by the Radiotherapy Oncology Group (RTOG) and the European Organization of Research and Treatment of Cancer (EORTC) [30, 31]. In these trials PCV-chemotherapy was administered either before (RTOG) or after radiotherapy (EORTC), respectively. Combined losses on chromosomes 1p and 19q represented a favorable prognostic marker and characterized a distinct glioma entity independent of whether initial treatment included PCV chemotherapy or not. Patients with oligodendroglioma with LOH 1p/19q had a much better outcome than patients without LOH 1p/19q. Chemotherapy prolonged progression-free survival in all patients but failed to improve overall survival, even the subset of patients considered the most sensitive to

chemotherapy with LOH 1p/19q. Hence we need to conclude that LOH 1p/19q is primarily a prognostic factor describing a distinct pathologic entity. Its determination improves the accuracy of morphologic diagnosis overcoming subjective interobserver differences that have severely limited comparability between institutions and treatments [32]. Consequently, the ongoing European and Canadian trial (EORTC 22033/26033) for low grade glioma stratifies the patients for 1p deletions in the tumor.

targeted treatments

As pathogenetic factors are diverse, multiple treatment strategies are possible. The integration of clinical and molecular information has provided a list of mechanisms that may be targeted. Aspects of selected treatment strategies are highlighted below. Otherwise reference is given to recent comprehensive reviews of the respective fields.

DNA-repair-driven therapeutics

Epigenetic silencing of the *MGMT* gene encoding a DNA repair enzyme was recently found to be of predictive value for benefit from the alkylating agent temozolomide (TMZ) in a randomized clinical trial for newly diagnosed glioblastoma [33]. The trial had shown that the addition of concomitant and adjuvant (maintenance) TMZ to radiotherapy (RT) improved the 2-year survival rate from 10% in the RT group to 26% for TMZ/RT, setting a new standard of care for glioblastoma patients [3]. Subsequent analysis of the patient's tumor tissues revealed that benefit of the addition of TMZ chemotherapy was basically confined to patients whose tumors had a methylated *MGMT* promoter [33]. At 2 years, 46% of the patients treated with TMZ/RT and whose tumors were *MGMT* methylated survived, compared to only 14% for the patients with unmethylated tumors. High expression of the MGMT repair enzyme is known to reverse part of the treatment effect of alkylating agents by rapidly repairing O^6-methyl guanine, the biologically most important lesion for triggering a cytotoxic response [34, 35]. Since the MGMT protein is a suicide repair enzyme that gets inactivated when the alkyl group from the lesion is transferred to the active site of the enzyme, depletion of the enzyme from the tumor cells may be used as a therapeutic strategy to improve sensitivity to alkylating agents. One approach uses a non-cytotoxic substrate of MGMT, such as O^6-BG (O^6-benzylguanine) or PaTrin-2 [O(6)-(4-bromothenyl)guanine] [36] that may deplete cells of the repair enzyme. Phase I clinical trials have evaluated O^6-BG in association with TMZ or carmustine (BCNU) [37, 38]. An alternative strategy is to use an intensified, dose-dense TMZ administration schedule aiming at depleting MGMT by continued exposure. This approach is currently undergoing clinical testing within a large randomized international Intergroup trial (RTOG0525/EORTC26052–22053) [39]. Patients are stratified prior to randomization by their *MGMT* methylation status. [For additional information, see http://www.rtog.org or http://www.eortc.org or contact the study chairs: Mark Gilbert (mrgilbert@mdanderson.org) or Roger Stupp (roger.stupp@chuv.ch).] The cytotoxic property of the O^6-methyl guanine lesion that persists in the DNA in absence of functional MGMT, depends on an intact mismatch repair (MMR) system that eventually triggers the signaling pathways leading to cell death [40, 41]. Thus, best response is expected in patients whose tumor is deficient for MGMT, but proficient for MMR. In adult glioblastoma there is no evidence for lack of MMR, in contrast to pediatric glioblastoma [13]. However, additional resistance factors exist, and may include abrogation of apoptosis pathways and deregulated survival signaling that need to be identified and subsequently overcome.

Other approaches aim at rescuing or increasing sensitivity to alkylating agent therapy by inactivating base excision repair (BER) [42]. Approaches have been to use methoxyamine that inhibits apurinic/apyrimidinic endonuclease (APE)-mediated cleavage by binding to AP-sites; while inhibition of Poly(ADP-ribose)polymerase-1 (PARP) impairs the recruitment of BER proteins [43]. This approach has been effective in breast cancer models with either defective BRCA1 or BRCA2 that are both involved in repair of double strand breaks [44]. In glioblastoma cell lines or respective xenograft models PARP inhibitors proved to be particularly effective when MMR was deficient [45, 46]. The redundancy in the targeted pathway, in this case repair, has to be overcome in order to achieve a successful treatment response. Hence, targeting repair processes might be a promising strategy for improving efficacy of alkylating agent chemotherapy.

small molecule inhibitors

Over the last years small molecule drugs have been developed specifically inhibiting aberrantly activated signaling pathways. These pathways may not only be activated in the tumor cells themselves, but also in the tumor stroma and tumor-associated endothelial cells [47]. Thus targeting of respective pathways operative in different compartments, or inhibitors of multiple tyrosine kinases, such as PTK-787, may have synergistic effects and thereby enhance treatment efficacy. Evaluation of these small molecule drugs, including peptides, polypeptides and antibodies specifically targeting signaling pathways aberrantly activated in gliomas will be central to future clinical trials (recently reviewed in [48]) (Figure 3). Based on molecular profiles of glioblastoma, the EGFR- and PI3K-pathway and their downstream partners represent particularly attractive targets [49, 50]. Promising are also strategies that particularly aim at angiogenesis [47] such as inhibitors of vascular endothelial growth factor (VEGF), vascular endothelial growth factor receptor (VEGFR), or inhibitors of integrins [51]. However, based on modest responses in trials with single agents, and encouraging synergistic effects in preclinical studies, combination treatments are warranted, combining cytotoxic agents plus one or several targeted drugs (or molecules targeting multiple pathways) [52, 53]. Many new targeted drugs, or combinations thereof, are currently tested or have been tested in phase I or II trials [53, 54]. One limitation in the clinic is the frequent interaction with the commonly used enzyme-inducing anti-epileptic drugs.

Since these strategies aim (mainly) at specific molecular targets it is mandatory to establish molecular profiles of the

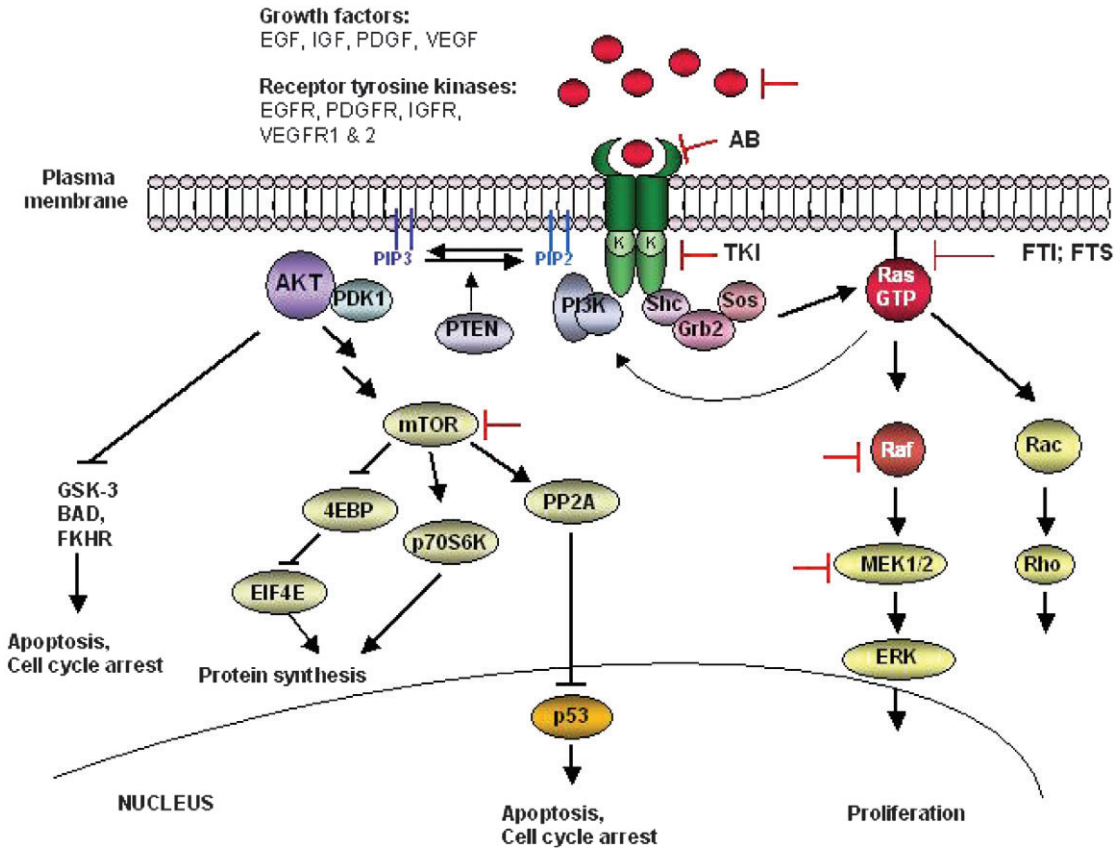

Figure 3. Signaling pathways and their inhibitors. Simplified diagram for signaling pathways operative in glioma or in tumor epithelial cells and respective inhibitors thereof (see also Table 1). The red trait signifies site of specific inhibition. EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; IGF, insulin-like growth factor; IGFR, insulin-like growth factor receptor; PDGF, platelet-derived growth factor; PDGFR, platelet-derived growth factor receptor; VEGF, vascular endothelial growth factor; VEGFR1 & 2, vascular endothelial growth factor receptor 1 and 2; PI3K, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homolog; PDK1, phosphatidylinositol-dependent kinase; AKT, v-akt murine thymoma viral oncogene homolog; mTOR, mammalian target of rapamycin; 4EBP, eukaryotic initiation factor 4E (EIF4E)-binding protein; p70S6K, ribosomal protein, S6 kinase 70kD; PP2A, protein phosphatase 2A; GRB2, growth factor receptor-bound protein 2; SOS, Ras; Raf; MEK, mitogen-activated ERK kinase; and ERK, extracellular-regulated kinase; TKI, tyrosine kinase inhibitor; AB, specific monoclonal antibodies; FTI, Farnesyltransferase inhibitors.

tumors for evaluation of correlations with treatment response. Since the EGFR represents a particularly attractive target it has been extensively evaluated in malignant glioma. Retrospective correlative studies suggested that response to EGFR tyrosine kinase inhibitors did not depend solely on the presence of the target, an activated EGFR [54]. Mellinghof et al. [55] proposed that co-expression of EGFRvIII mutant and PTEN in the tumor were crucial for response to EGFR inhibitors, while in a study by Haas Kogan et al. [56] none of the responders expressed EGFRvIII, but the response was associated with *EGFR* amplification and expression, and low levels of PKB/Akt phosphorylation. Of note, most of these molecular studies were performed on tumor tissue obtained at the initial diagnosis and may not reflect the molecular pattern at recurrence after multiple prior treatment. Molecular analysis of few tumor tissue samples, available after resection performed during and after prior therapy with the EGFR inhibitors (erlotinib or gefitinib), seemed to suggest inefficient inhibition of EGFR phosphorylation [57]. Thus, the molecular signature relevant for response to this treatment remains to be elucidated and will need more in-depth molecular analysis to elucidate the molecular mechanisms responsible for these unexpected, additional treatment resistances.

It follows that prospective trials for targeted treatments need to be designed with an integrated translational research component allowing for future molecular selection of patients potentially benefiting from a certain agent or treatment regimen and identifying the relevant pathways that may need to be targeted in addition, in order to improve antitumor activity. Thus, common to all ongoing or planned trials is the absolute necessity of availability of tumor material for molecular profiling (paraffin-embedded or ideally fresh-frozen) in order to establish molecular criteria for the choice of individually tailored treatment approaches in the future.

outlook: gene signature guide drug choice

Novel approaches have recently been described identifying gene expression signatures indicative of oncogenic pathways that may be specifically targeted for therapy [58, 59]. Using this

Table 1. Targeted treatments[a]

Target	Name	
Tyrosine kinase inhibitors		
EGFR	Gefitinib	ZD1839, Iressa
EGFR	Erlotinib	OSI-774, Tarceva
EGFR	Cetuximab	C225, Erbitux
EGFR		EKB569
EGFR & ErbB-2	Lapatinib	GW-572016
EGFR & VEGFR		AEE788
VEGFR & EGFR		ZD6474
PDGFR & c-kit	Imatinib mesylate	ST1571, Gleevec
PDGFR & VEGFR		PTK787
PDGFR & VEGFRs, c-kit, Flt-3	Sunitinib	SU11248
PDGFR & VEGFRs, c-kit, Flt-3		AG-013736
PDGFR		SU101
PDGFR		MLN518
Raf, VEGFRs, PDGFR	Sorafenib	Bay 43–9006
VEGFR		AZD2171
Kinase inhibitors		
MEK		Cl-1040
MEK		UO126
MEK		PD0325901
VEGF inhibitors		
	Bevacizumab	Avastin
	VEGF Trap	
	Pegabtanib	
Farnesyl transferase inhibitors		
	Tipfarnib	R115777, Zarnestra
	Lonafarnib	SCH66336, Sarasar
mTOR inhibitors		
	Temsirolimus	CCI-779
	Everolimus	RAD001
	Sirolimus	Rapimmune; Wyeth AP23573
Histone deacetylase inhibitors		
	Depsipeptide	FK228
	Suberoylanilide hydroxamic acid	
DNA repair		
MGMT		O^6-benzyl guanine
MGMT	PaTrin-2	O(6)-(4-bromothenyl)guanine
PARP		INO-1001
BER	AP-sites	methoxyamine
Other		
integrins avb3 & avb5	Cilengitide	EMD 121974
Hsp90	17-allylamino-geldanamycin	
Proteasome inhibitor	Bortezomib	PS-341, Velclade
PKC inhibitor	Tamoxifen	

[a]Compilation of the literature, references as mentioned in the text, in particular the review by Kesari et al. [48].

concept Solit et al. identified B-raf mutations as predictive for sensitivity to MEK-inhibition in tumor xenograft models, while mutations in ras that are further upstream and signals in addition to other growth promoting pathways led only to partial response. Thus, gene expression profiles may not only identify activated oncogenic pathways but also indicate the presence of redundant pathways that could be responsible for unexpected resistance to treatment with small molecule drugs, despite confirmed presence of the activated target. In the future it might become feasible to deduce, from the tumor derived gene expression profile of a patient, which oncogenic pathways are activated and to devise a respective rational combination treatment. This would conclude the current 'one fits all' treatment strategies based on subjective categorization of morphologic tumor features and some patient characteristics. Further advances may come from new mouse glioma models that recapitulate aberrant molecular pathways relevant in human glioma, or xenograft models of human primary tumors that may prove to be very useful for testing new small molecule drugs preclinically [60, 61].

At present, the test for the *MGMT*-methylation status represents the only predictive factor for benefit from treatment that has been evaluated in a clinical trial, but requires confirmation in ongoing prospective clinical trials. In the individual management of glioblastoma patients this information may direct the choice for alkylating agent chemotherapy, or suggest the addition of alternative treatment modalities, not depending on MGMT processing. Hopefully, new predictive factors will become available within the next years to stratify patients according to their individual molecular profiles to respective targeted therapies.

references

1. Ohgaki H, Dessen P, Jourde B et al. Genetic pathways to glioblastoma: a population-based study. Cancer Res 2004; 64: 6892–6899.
2. Stupp R, Dietrich P-Y, Ostermann Kraljevic S et al. Promising survival for patients with newly diagnosed glioblastoma multiforme treated with concomitant radiation plus temozolomide followed by adjuvant temozolomide. J Clin Oncol 2002; 20: 1375–1382.
3. Stupp R, Mason WP, van den Bent MJ et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 2005; 352: 987–996.
4. Esteller M, Corn PG, Baylin SB, Herman JG. A gene hypermethylation profile of human cancer. Cancer Res 2001; 61: 3225–3229.
5. Baylin SB, Ohm JE. Epigenetic gene silencing in cancer—a mechanism for early oncogenic pathway addiction? Nat Rev Cancer 2006; 6: 107–116.
6. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000; 100: 57–70.
7. Hegi ME, zur Hausen A, Ruedi D et al. Hemizygous or homozygous deletion of the chromosomal region containing the p16INK4a gene is associated with amplification of the EGF receptor gene in glioblastomas. Int J Cancer 1997; 73: 57–63.
8. Cairncross JG, Ueki K, Zlatescu MC et al. Specific genetic predictors of chemotherapeutic response and survival in patients with anaplastic oligodendrogliomas. J Natl Cancer Inst 1998; 90: 1473–1479.
9. Hiraga S, Ohnishi T, Izumoto S et al. Telomerase activity and alterations in telomere length in human brain tumors. Cancer Res 1998; 58: 2117–2125.
10. Fulci G, Labuhn M, Maier D et al. p53 gene mutation and ink4a-arf deletion appear to be two mutually exclusive events in human glioblastoma. Oncogene 2000; 19: 3816–3822.
11. Watanabe K, Tachibana O, Sata K et al. Overexpression of the EGF receptor and p53 mutations are mutually exclusive in the evolution of primary and secondary glioblastomas. Brain Pathol 1996; 6: 217–223.

12. Sure U, Rüedi D, Tachibana O et al. Determination of *p53* mutations, EGFR overexpression, and loss of p16 expression in pediatric glioblastomas. J Neuropathol Exp Neurol 1997; 56: 782–789.
13. Alonso M, Hamelin R, Kim M et al. Microsatellite instability occurs in distinct subtypes of pediatric but not adult central nervous system tumors. Cancer Res 2001; 61: 2124–2128.
14. Godard S, Nozaki M, Stupp R et al. Gene expression profiling of human glioblastoma. Eur J Cell Biol 2000; 79: 177.
15. Huang H, Okamoto Y, Yokoo H et al. Gene expression profiling and subgroup identification of oligodendrogliomas. Oncogene 2004; 23: 6012–6022.
16. Ichimura K, Bolin MB, Goike HM et al. Deregulation of the p14ARF/MDM2/p53 pathway is a prerequisite for human astrocytic gliomas with G1-S transition control gene abnormalities. Cancer Res 2000; 60: 417–424.
17. Bachoo RM, Maher EA, Ligon KL et al. Epidermal growth factor receptor and Ink4a/Arf: convergent mechanisms governing terminal differentiation and transformation along the neural stem cell to astrocyte axis. Cancer Cell 2002; 1: 269–277.
18. Lachat Y, Diserens AC, Nozaki M et al. INK4a/Arf is required for suppression of EGFR/DeltaEGFR(2–7)-dependent ERK activation in mouse astrocytes and glioma. Oncogene 2004; 23: 6854–6863.
19. Godard S, Getz G, Delorenzi M et al. Classification of human astrocytic gliomas on the basis of gene expression: a correlated group of genes with angiogenic activity emerges as a strong predictor of subtypes. Cancer Res 2003; 63: 6613–6625.
20. Nutt CL, Mani DR, Betensky RA et al. Gene expression-based classification of malignant gliomas correlates better with survival than histological classification. Cancer Res 2003; 63: 1602–1607.
21. Freije WA, Castro-Vargas FE, Fang Z et al. Gene expression profiling of gliomas strongly predicts survival. Cancer Res 2004; 64: 6503–6510.
22. Nigro JM, Misra A, Zhang L et al. Integrated array-comparative genomic hybridization and expression array profiles identify clinically relevant molecular subtypes of glioblastoma. Cancer Res 2005; 65: 1678–1686.
23. Rich JN, Hans C, Jones B et al. Gene expression profiling and genetic markers in glioblastoma survival. Cancer Res 2005; 65: 4051–4058.
24. Misra A, Pellarin M, Nigro J et al. Array comparative gemomic hybridization identifies genetic subgroups in grade 4 human astrocytoma. Clin Cancer Res 2005; 11: 2907–2918.
25. Liang Y, Diehn M, Watson N et al. Gene expression profiling reveals molecularly and clinically distinct subtypes of glioblastoma multiforme. Proc Natl Acad Sci USA 2005; 102: 5814–5819.
26. Bredel M, Bredel C, Juric D et al. Tumor necrosis factor-alpha-induced protein 3 as a putative regulator of nuclear factor-kappaB-mediated resistance to O6-alkylating agents in human glioblastomas. J Clin Oncol 2006; 24: 274–287.
27. Schwartz SA, Weil RJ, Thompson RC et al. Proteomic-based prognosis of brain tumor patients using direct-tissue matrix-assisted laser desorption ionization mass spectrometry. Cancer Res 2005; 65: 7674–7681.
28. Iwadate Y, Sakaida T, Hiwasa T et al. Molecular classification and survival prediction in human gliomas based on proteome analysis. Cancer Res 2004; 64: 2496–2501.
29. Smith JS, Perry A, Borell TJ et al. Alterations of chromosome arms 1p and 19q as predictors of survival in oligodendrogliomas, astrocytomas, and mixed oligoastrocytomas. J Clin Oncol 2000; 18: 636–645.
30. Cairncross G, Seiferheld W, Shaw E et al. An intergroup randomized controlled clinical trial (RCT) of chemotherapy plus radiation (RT) versus RT alone for pure and mixed anaplastic oligodendrogliomas: Initial report of RTOG 94–02. J Clin Oncol, ASCO Annual Meeting Proceedings (Post-Meeting Edition) 2004; 22 (14S): Abstr 1500.
31. van den Bent MJ, Delattre J-Y, Brandes AA et al. First analysis of EORTC trial 26951, a randomized phase III study of adjuvant PCV chemotherapy in patients with highly anaplastic oligodendroglioma. J Clin Oncol 2005; 23 (Suppl 16): 114s (Abstr 1503).
32. Burger PC. What is an oligodendroglioma? Brain Pathol 2002; 12: 257–259.
33. Hegi ME, Diserens AC, Gorlia T et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. New Engl J Med 2005; 352: 997–1003.
34. Ochs K, Kaina B. Apoptosis induced by DNA damage O6-methylguanine is Bcl-2 and caspase-9/3 regulated and Fas/caspase-8 independent. Cancer Res 2000; 60: 5815–5824.
35. Gerson SL. MGMT: its role in cancer aetiology and cancer therapeutics. Nat Rev Cancer 2004; 4: 296–307.
36. Clemons M, Kelly J, Watson AJ et al. O6-(4-bromothenyl)guanine reverses temozolomide resistance in human breast tumour MCF-7 cells and xenografts. Br J Cancer 2005; 93: 1152–1156.
37. Quinn JA, Desjardins A, Weingart J et al. Phase I trial of temozolomide plus O6-benzylguanine for patients with recurrent or progressive malignant glioma. J Clin Oncol 2005; 23: 7178–7187.
38. Friedman HS, Pluda J, Quinn JA et al. Phase I Trial of Carmustine Plus O6-Benzylguanine for Patients with Recurrent or Progressive Malignant Glioma. J Clin Oncol 2000; 18: 3522–3528.
39. Stupp R, Hegi M, van den Bent M et al. Changing paradigms—an update on the multidisciplinary management of malignant glioma. The Oncologist 2006; 11: in press.
40. Karran P. Mechanisms of tolerance to DNA damaging therapeutic drugs. Carcinogenesis 2001; 22: 1931–1937.
41. Stojic L, Cejka P, Jiricny J. High doses of SN1 type methylating agents activate DNA damage signaling cascades that are largely independent of mismatch repair. Cell Cycle 2005; 4: 473–477.
42. Liu L, Taverna P, Whitacre CM et al. Pharmacologic disruption of base excision repair sensitizes mismatch repair-deficient and -proficient colon cancer cells to methylating agents. Clin Cancer Res 1999; 5: 2908–2917.
43. Haince JF, Rouleau M, Hendzel MJ et al. Targeting poly(ADP-ribosyl)ation: a promising approach in cancer therapy. Trends Mol Med 2005; 11: 456–463.
44. Bryant HE, Schultz N, Thomas HD et al. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. Nature 2005; 434: 913–917.
45. Tentori L, Portarena I, Torino F et al. Poly(ADP-ribose) polymerase inhibitor increases growth inhibition and reduces G(2)/M cell accumulation induced by temozolomide in malignant glioma cells. Glia 2002; 40: 44–54.
46. Cheng CL, Johnson SP, Keir ST et al. Poly(ADP-ribose) polymerase-1 inhibition reverses temozolomide resistance in a DNA mismatch repair-deficient malignant glioma xenograft. Mol Cancer Ther 2005; 4: 1364–1368.
47. Ferrara N, Kerbel RS. Angiogenesis as a therapeutic target. Nature 2005; 438: 967–974.
48. Kesari S, Ramakrishna N, Sauvageot C et al. Targeted molecular therapy of malignant gliomas. Curr Oncol Rep 2006; 8: 58–70.
49. Chakravarti A, Zhai G, Suzuki Y et al. The prognostic significance of phosphatidylinositol 3-kinase pathway activation in human gliomas. J Clin Oncol 2004; 22: 1926–1933.
50. Cully M, You H, Levine AJ, Mak TW. Beyond PTEN mutations: the PI3K pathway as an integrator of multiple inputs during tumorigenesis. Nat Rev Cancer 2006; 6: 184–192.
51. Ruegg C, Hasmim M, Lejeune FJ, Alghisi GC. Antiangiogenic peptides and proteins: from experimental tools to clinical drugs. Biochim Biophys Acta 2006; 1765: 155–177.
52. Beuvink I, Boulay A, Fumagalli S et al. The mTOR inhibitor RAD001 sensitizes tumor cells to DNA-damaged induced apoptosis through inhibition of p21 translation. Cell 2005; 120: 747–759.
53. Goudar RK, Shi Q, Hjelmeland MD et al. Combination therapy of inhibitors of epidermal growth factor receptor/vascular endothelial growth factor receptor 2 (AEE788) and the mammalian target of rapamycin (RAD001) offers improved glioblastoma tumor growth inhibition. Mol Cancer Ther 2005; 4: 101–112.
54. Rich JN, Reardon DA, Peery T et al. Phase II trial of gefitinib in recurrent glioblastoma. J Clin Oncol 2004; 22: 133–142.
55. Mellinghoff IK, Wang MY, Vivanco I et al. Molecular determinants of the response of glioblastomas to EGFR kinase inhibitors. N Engl J Med 2005; 353: 2012–2024.
56. Haas-Kogan DA, Prados MD, Tihan T et al. Epidermal growth factor receptor, protein kinase B/Akt, and glioma response to erlotinib. J Natl Cancer Inst 2005; 97: 880–887.

57. Lassman AB, Abrey LE, Gilbert MR. Response of glioblastomas to EGFR kinase inhibitors. N Engl J Med 2006; 354: 525–526; author reply 525–526.
58. Bild AH, Yao G, Chang JT et al. Oncogenic pathway signatures in human cancers as a guide to targeted therapies. Nature 2006; 439: 353–357.
59. Solit DB, Garraway LA, Pratilas CA et al. BRAF mutation predicts sensitivity to MEK inhibition. Nature 2006; 439: 358–362.
60. Giannini C, Sarkaria JN, Saito A et al. Patient tumor EGFR and PDGFRA gene amplifications retained in an invasive intracranial xenograft model of glioblastoma multiforme. Neuro-oncol 2005; 7: 164–176.
61. Hu X, Holland EC. Applications of mouse glioma models in preclinical trials. Mutat Res 2005; 576: 54–65.
62. Bello MJ, Alonso ME, Aminoso C et al. Hypermethylation of the DNA repair gene MGMT: association with TP53 G:C to A:T transitions in a series of 469 nervous system tumors. Mutat Res 2004; 554: 23–32.