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Genomic discoveries in adult astrocytoma

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Abstract

Astrocytomas are the most common glial tumor of the central nervous system. Within this category, glioblastoma is the most prevalent and malignant primary brain tumor. Glioblastoma can arise *de novo*, or through progression from lower-grade lesions, but is uniformly associated with poor outcomes despite surgical resection, chemotherapy, and radiation therapy. Recent genomic discoveries have provided new insight into gliomagenesis and have identified key genetic alterations that have diagnostic, prognostic and predictive capacity. Numerous molecular classification schemes have been proposed to sort tumors into clinically meaningful categories to guide treatment. However, creating therapy targeted towards these alterations has been made challenging by the redundancy of essential signal transduction pathways affected in these tumors, intratumoral heterogeneity, and the hypermutated profiles of recurrent tumors. Future treatment strategies will require a personalized approach with consideration of the unique genetic profile of a specific tumor and the use of multimodality therapies.

Introduction

In 2007, the WHO proposed a classification system for tumors of the central nervous system (CNS), categorizing a heterogeneous group of cancers into discrete types based on histopathologic criteria. Grade I lesions included pilocytic astrocytoma, grade II consisted of diffuse astrocytoma; grade III of anaplastic astrocytoma, and grade IV of glioblastoma (GBM) [1]. GBMs are the most common primary malignant brain tumors; within GBMs, the majority arise *de novo* and are termed primary GBMs, and those that progress from lower-grade lesions and undergo transformation are secondary GBMs. Although this classification system has been used to guide prognostication and management, outcomes for GBM remain dismal, with median survival at 15–20 months [2]. Recently, however, our understanding of CNS tumors has been revolutionized by genomic studies. The identification of driver mutations in gliomas has already allowed for improved patient prognostication, and there is hope these new insights into gliomagenesis will generate novel and specific strategies for treatment [3].

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Molecular alterations in astrocytomas

IDH1/2

Mutations of the isocitrate dehydrogenase (*IDH1* and *IDH2*) gene are thought to occur early in gliomagenesis and drive cancer progression [4]. Although detected in 70–80% of grade II and III gliomas and secondary GBMs, *IDH1* alterations rarely occur in primary GBM [5••]. The predominant causative single nucleotide polymorphism (SNP) results in an arginine to histidine substitution at codon 132 (R132H); *IDH1* mutations are strongly associated with *TP53* mutations in low-grade astrocytomas [4,6,7••]. The wild-type form catalyzes the reaction of isocitrate to α-ketoglutarate, but the mutated form results in the overproduction of 2-hydroxyglutarate (2HG) [8]. 2HG has been shown to induce DNA damage, prevent differentiation in hematopoietic cells, promote carcinogenesis. In addition, it also increases levels of and stabilizes hypoxia-inducible factor subunit HIF-1α, which is thought to promote tumor growth [9••,10,11,12]. In addition to effects on cellular metabolism, *IDH1* mutations are associated with a global DNA hypermethylation phenotype, and result in blockade of histone demethylation and prevention of cell differentiation [13•,14,15,16••]. Even when accounting for tumor grade, patients with tumors that possess *IDH1* mutations experience longer survival and have improved overall prognoses [17].

ATRX

Loss of expression mutations of the alpha-thalassemia/mental retardation syndrome X-linked (*ATRX*) gene have been described in the majority of grade II and III astrocytomas and secondary GBMs, but are rare in primary GBM [18•,19]. In addition to being a member of the SWI/SNF family of chromatin remodeling proteins, *ATRX* mutations are associated with an alternative lengthening of telomeres (ALT) phenotype [18•,20]. Indeed, the molecular basis of ALT appears to obligate inactivation of either *ATRX* or its binding partner *DAXX*. Jiao *et al.* also found that 99% of the tumors with *ATRX* mutations had co-occurring mutations of *IDH1*, and 94% with both *ATRX* and *IDH1* alterations had *TP53* mutations; alterations in *ATRX* were almost mutually exclusive with 1p/19q co-deletions, which are characteristic of oligodendrogliomas. Subsequent studies have reaffirmed these findings, and found that patients with tumors harboring *ATRX* mutations experienced longer times to treatment failure compared to those without such alterations [20].

EGFR

Amplification of the epidermal growth factor receptor (*EGFR*) gene has been reported in 40% of GBMs. Of these, 20–30% express a variant produced from the deletion of exons 2–7, EGFRvIII, a constitutively active receptor that is unable to bind ligand and results in continuous activation of cell growth and anti-apoptotic pathways [21]. Activation of *EGFR* in gliomas also occurs through gain-of-function mutations and double minute chromosomes [22]. There have been several reports noting glioma dependence on *EGFR* activation, citing that interruption of *EGFR* signaling results in short-term inhibition of glioma growth [23,24]. Recently, Frattini *et al.* described translocations of *EGFR* and in-frame fusion to either septin 14 (*SEPT14*) or phosphoserine phosphatase (*PSPH*) in 7% of GBMs [25•]. In 3% of GBMs, fibroblast growth factor receptor 1 (*FGFR1*) inversion and in-frame fusion to the coding domain of transforming acidic coiled-coil 1 (*TACC1*) results in a constitutively

active protein (FGFR-TACC) [26••]. Although these fusion events appear to be rare, the resulting fusion proteins are promising therapeutic targets.

TERT

Mutations of the telomerase reverse transcriptase (*TERT*) promoter which result in increased telomerase expression have been observed in several human cancers; they have been found in the majority of primary GBM but are less common in lower-grade gliomas and secondary GBMs [27,28]. *TERT* promoter mutations appear to be mutually exclusive with *ATRX* mutations and activation of the ALT pathway, highlighting two distinct mechanisms for telomere maintenance in cancer cells. Recently, a new SNP near the telomerase RNA component (TERC) gene was found to be potentially associated with increased risk of glioma. This SNP and a previously identified risk loci near *TERT* demonstrated a correlation with longer telomeres [29,30]. The association between *TERT* promoter status and patient survival was not significant when *IDH1* mutation status was accounted for [27].

Molecular classification systems

Although the WHO classification system has been universally used to guide diagnosis, treatment, and prognostication, the variability in the histologic appearance of gliomas has made uniform tumor grading challenging. By contrast, the above described genomic alterations appear to segregate consistently, providing insight into gliomagenesis and suggesting approaches for the molecular categorization of astrocytomas. For example, despite the fact that primary and secondary GBMs appear identical histologically, they are distinct in terms of their genetic signatures. Primary GBM is associated with *EGFR* amplifications and phosphatase and tensin homolog (*PTEN*) deletions, which are uncommon in lower-grade astrocytomas. These tumors also are associated with amplifications of platelet derived growth factor receptor a (*PDGFRA*), *MET, CDK4, MDM2*, and *MDM4*; mutations of phosphatidylinositol-3-OH kinase (*PI3K*); mutations and deletions of *TP53, CDNK2A/ARF, CDKN2A/p16, RB1*, and *NF1* [5••,31]. By contrast, secondary GBMs are characterized by mutations in *IDH1* and *TP53*. In one study, of the 80% anaplastic astrocytomas and secondary GBMs with *IDH1* and *IDH2* mutations, only 3% had mutations in *EGFR, PTEN, CDKN2A* and *CDKN2B*; in the 18% of tumors with wild-type *IDH1* and *IDH2*, 74% exhibited alterations in *EGFR, PTEN, CDKN2A* and *CDKN2B* [7••,31]. As lower-grade astrocytomas also display mutations of *IDH1* and *TP53*, these lesions likely progress to secondary GBM by accumulating new genetic alterations.

Genetic signature

Comprehensive genetic profiling studies have sought to use the divergent molecular profiles of gliomas to create new schema for tumor classification to be used in diagnosis, prognostication, and prediction [32]. In 2010, a study from The Cancer Genome Atlas described four distinct subgroups of GBM based on their molecular profile: (1) proneural, characterized by alterations in *PDGFRA* and *IDH1*; (2) classical, with mutations in *EGFR*, (3) mesenchymal, with mutations in *NF1*, and (4) neural, which did not exhibit a distinct genetic profile. The mesenchymal and classical subgroups displayed improved survival with aggressive therapy (temozolomide (TMZ) and radiation), but no benefit was seen in those

with proneural tumors [33••]. Of gliomas classified as proneural, examination of DNA methylation patterns has led to further subcategorization, with some tumors displaying a glioma-CpG island methylator (G-CIMP) phenotype [13•,34]. G-CIMP are highly associated with *IDH1* mutations, and resultingly, were associated with a more favorable prognosis. Subsequent studies found that IDH1 mutation alone was sufficient to induce the G-CIMP phenotype [35••].

Jiao *et al.* has since proposed another classification model in which tumors with *IDH1* and *ATRX* mutations are 'I-CF glioma;' *IDH1, CIC*, and *FUBP1* with 1p/19q loss are 'I-A glioma;' and gliomas without the previously noted mutations with multiple other genetic alterations are 'I-X' gliomas. This categorization not only aids in diagnosis and eliminating the histologically challenging diagnosis of oligoastrocytoma, but also has prognostic value, as those with I-CF gliomas experienced a median survival of 96 months, compared to 51 months in I-A gliomas and 13 months in I-X gliomas [18•]. A similar classification system using *IDH1* and *IDH2* and *TERT* has been proposed by Killela *et al.* They demonstrated that patients with gliomas with *IDH1/2* mutations experienced a median survival of 57 months and those with *TERT* mutations and *IDH1/2* mutations had a median survival of 125 months, in contrast to those with *TERT* mutations only, with a median survival of 11.5 months [28].

Although these proposed classification systems are valuable for diagnosis and prognostication, no one schema has emerged as more compelling than the others. Upon further examination, one predominant pattern appears: IDH1/2 mutations appear to be the fundamental genetic change by which other alterations segregate and is consistently predictive of a more favorable patient outcome. The use of other markers may allow for more nuanced prognostication, but they appear secondary to the effect driven by IDH1/2 (Figure 1).

Signal transduction pathways

The pattern of genetic alterations in astrocytomas also allows for tumor classification by the affected signal transduction pathway. TCGA described three core pathways altered in the majority of GBM: RTK/RAS/PI3K, p53, and Rb [36••]. Brennan *et al.*, through targeted proteomic analyses of 27 glioma samples, found three distinct subclasses with mutually exclusive alterations: (1) EGFR activation, (2) PDGFR activation, and (3) loss of *NF1* expression [37]. A subsequent study has proposed classifying gliomas by *EGFR* and *PDGFRA* expression and patterns of gene-coexpression upon demonstrating that gliomas that expressed *EGFR* and associated genes were associated with a significantly poorer prognosis, compared to tumors expressing *PDGRFA* and those with low expression of both [38]. Although the majority of gliomas have alterations in these pathways, recent studies have demonstrated the existence of alternate pathways that ultimately also result in tumor cell proliferation and escape from apoptosis. Heterozygous deletions of the NF-κB inhibitor-α (*NFKBIA*) gene were found in 25% of GBMs, which were mutually exclusive with *EGFR* amplifications. These two alterations appear to converge on the same downstream pathway, as patients with tumors that demonstrate either alteration experience shorter survival times compared to those who have neither [39]. In addition, Morris *et al.* recently identified inactivating mutations of *FAT1*, which binds β-catenin and antagonizes the Wnt signaling

pathway, in 20% of GBMs [40]. The identification of these driver mutations and dysregulated core pathways has suggested potential therapeutic targets.

Strategies for targeted therapy

Although the standard of care for high-grade astrocytomas consists of surgical resection, chemotherapy (typically with TMZ), and radiotherapy, the treatment course for lower-grade lesions can vary. Most patients receive surgical resection, but use of additional therapies depends on both tumor and patient factors. Despite multimodality therapy, however, outcomes for GBM remain poor [3]. Given the prevalence of *IDH1* alterations and its role as a driver mutation, there has been considerable interest in using inhibitors of mutant *IDH1* as therapy. Rohle *et al*. identified a selective inhibitor of the R132H-IDH1 mutant, which was capable of inducing differentiation and impair tumor growth *in vitro* and *in vivo* with *IDH1*-mutant gliomas [41•]. They did not observe any effect of the inhibitor on DNA methylation; however, treatment of *IDH1*-mutant glioma cells with the DNA methyltransferase inhibitor decitabine resulted in reversal in mutant IDH*1*-induced methylation and caused similar effects on cell proliferation and differentiation [42]. There are ongoing phase I studies examining the effects of IDH inhibitors in IDH mutant gliomas (NCT02193347).

With the prevalence of alterations in the RTK/PI3K/Akt pathway in astrocytomas, specific therapy targeting EGFR signaling once held great promise. Erlotinib and gefitinib, first-generation EGFR inhibitors, as well as newer agents such as cetuximab and lapatinib, have not demonstrated a significant treatment benefit in trials [43–47]. Glioma resistance has been primarily attributed to redundancy in signaling pathway dysregulation in GBM, and the convergence of the effect of numerous alterations on the same downstream pathways [48]. Consistent with this idea, loss of *PTEN* predicts treatment failure with EGFR inhibitors, while patients with tumors without *PTEN* and *TP53* alterations experience improved survival [49]. The importance of *PTEN* status in determining response to EGFR inhibitors has been reaffirmed in subsequent studies [23,50]. Effectors downstream of RTKs have also been targeted; the PI3K inhibitor PX-866 has been shown to attenuate glioma cell growth *in vitro* and *in vivo*, and is currently in clinical trials [2,51]. Mammalian mTOR inhibitors, including sirolimus and everolimus, have also been studied in clinical trials for GBM, but have also not demonstrated improvements in outcomes when alone or in combination with EGFR inhibitors [52].

Another treatment strategy that holds the promise is immunotherapy, which has seen success in the treatment of melanoma, renal cell carcinoma, and prostate carcinoma. Though the CNS was once thought to be a space of immune privilege, it is now clear that gliomas are not immunologically silent [53]. However, the interaction between the immune system and gliomas is a complex one, in which gliomas are able to create a state of immunosuppression to evade destruction by effector cells. The development of tumor-specific antigen vaccines is an area of active investigation in glioma, and new studies point to the potential of immune checkpoint therapies for use in tumors with high mutational burdens (Box 1).

Treatment challenges

The difficulties in designing targeted therapy can be attributed to numerous factors. Originally named glioblastoma multiforme for its variable histologic appearance, GBM has become the exemplar of intratumoral heterogeneity in cancer. Not only are there cell subpopulations that display distinct phenotypes with tumors, with respect to self-renewal capabilities and response to treatment, but single-cell studies have demonstrated there can be tremendous variability at the genetic, transcriptional, and functional levels [54,55••]. Patel *et al.* and others have described mosaic expression of RTK and mutually exclusive expression of *EGFR* variants between cells [48,56]. Using the classification system proposed by Verhaak *et al.*, they found that although tumors may overall demonstrate a predominant proneural, classical, or mesenchymal profile, all tumors had cells belonging to each of the proposed subtypes. Within the predominantly proneural tumors, more subtype heterogeneity was associated with decreased survival [33••,55••].

Several models have been proposed to explain both the initiation and the maintenance of intratumoral heterogeneity. Although their existence has not been definitively proven, cancer stem cells (CSCs) are one mechanism through which tumor heterogeneity can be continuously maintained. In this hierarchical model, a subpopulation of CSCs are capable of self-renewal and differentiation into cells with varying phenotypes [57–61]. Recent studies have suggested that there may not be a discrete subpopulation of CSCs, but the expression of stem cell-related genes may exist on a continuum in tumor cells [55••]. Earlier studies have also highlighted the necessary role of the tumor microenvironment and interactions between tumor cells in maintaining heterogeneity; cells with *EGFR* mutations are able to potentiate *EGFR* expression among *EGFR*-wild type GBM cells *via* paracrine cytokine signaling [62].

The mechanisms that generate striking intratumoral heterogeneity undoubtedly play a crucial role in the emergence of resistance to chemotherapy in recurrent tumors. A subpopulation of CSC-like cells were implicated in promoting tumor recurrence after treatment with TMZ in a mouse model of glioma [58]. This CSC model is not mutually exclusive with the hypothesis of clonal evolution, where cells accumulate varying mutations, and under the selective pressure of treatment, allow for the survival of certain cell populations. Novel somatic mutations in the mismatch repair gene *MSH6* in recurrent GBM have also been identified, suggesting a mechanism underlying resistance to alkylating agents [63]. Additionally, exome sequencing of grade II gliomas at diagnosis and recurrence revealed that the majority of recurrences did not display driver mutations in genes including *TP53* and *ATRX*, which were present in the initial tumor. Recurrences that arose after treatment with TMZ also revealed new mutations in the RB and PI3KAkt/mTOR pathways [64]. Both studies have described hypermutated genetic profiles after recurrences with TMZ treatment, demonstrating the ability of treatment to drive tumor evolution.

Conclusions and future directions

Our understanding of the genetic alterations driving astrocytoma initiation and progression has advanced dramatically with the advent of next-generation sequencing technologies. These alterations can have profound effects on cells, activating signal transduction pathways

that result in uncontrolled proliferation, altering cell metabolism to promote cell growth, effecting global DNA methylation changes and chromatin remodeling, and activating mechanisms to maintain telomere length. Several molecular classification models have been proposed by examining the divergent molecular profiles of astrocytomas of different classes, all with prognostic significance. Insight into this complex genetic landscape is not only valuable from a diagnostic and prognostic perspective, as these genetic markers have been applied to provide molecular data to complement histopathologic data, but they have also revealed potential targets for rational therapeutic design. However, targeted therapies have so far had disappointing results in clinical trials; their limited success may be the result of several mechanisms and suggests areas for future investigation. First, the core dysregulated pathways in glioma appear to be cooperative and to converge on the same downstream effectors and there also exist alternative pathways that yield the same ultimate effect. Future therapeutic strategies cannot rely on the inhibition of one effector, but multiple targets at multiple signaling levels must be blocked to obtain a meaningful change in clinical outcomes. Furthermore, these therapies must be used on highly selected patient populations, which necessitates an understanding of the global genetic landscape of various tumor types, as well as patient-specific alterations that will enable matching the appropriate therapy to each individual. Finally, a deeper understanding of the marked intratumoral heterogeneity displayed by glioblastoma and judicious use of therapies is needed to prevent the evolution of recurrent tumors that are treatment-resistant.

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Box 1

Immunotherapy: a promising treatment strategy

Targeted therapy for glioblastoma remains an elusive goal, but there is hope that immunotherapeutic strategies may finally achieve treatment specificity. One category of immunotherapeutic agents, including peptide and dendritic cell (DC) vaccines, have been devised to target tumor-specific and tumor-associated antigens [65]. The most prominent example in gliomas is EGFRvIII; the earliest studies of rindopepimut (PEPvIII-KLH; CDX-110), a 14-amino acid peptide (PEPvIII) conjugated to keyhole limpet hemocyanin (KLH), demonstrated significantly improved patient outcomes in combination with radiation therapy and chemotherapy. A Phase II trial of this agent alone and a Phase III trial in combination with GM-CSF are currently underway (NCT00458601; NCT01480479). DC vaccines typically involve the administration of autologous DCs from patients previously pulsed with tumor lysates or tumor-specific antigens. ICT-107 and DCVax®-L are polyvalent DC vaccines currently in clinical trials (NCT01280552; NCT00045968). Another strategy that augments the host immune system response against tumor tissue is that of immune checkpoint blockade. Cytotoxic T lymphocyte antigen-4 prevents the co-stimulatory interaction between T-cells and antigen presenting cells, effectively inhibiting the immune response against certain antigens. This mechanism functions to prevent the development of autoimmunity, but is exploited by glioma cells, which overexpress CTLA-4. Ipilimumab, a monoclonal CTLA-4 antibody, was approved for the treatment of melanoma in 2010. The programmed cell death-1 (PD-1) pathway is another immune checkpoint which appears to negatively regulate T-cell responses. Nivolumab, a monoclonal antibody against PD-1, is currently in Phase III clinical trials for a variety of solid tumors. A Phase II trial comparing combination nivolumab and ipilimumab with bevacizumab in patients with recurrent glioblastoma is currently underway (NCT02017717). A recent report posited that the efficacy of immune checkpoint therapies depends in part on tumor mutational burden. Champiat and colleagues found that the mutational frequencies of solid tumors correlated with response to anti-PD-1 therapies; of the tumor types for which these therapies have been tested, melanoma and lung squamous cell carcinoma have the greatest frequencies of somatic mutations reported in the literature and also display the most robust responses to immune checkpoint therapies [66•]. The theory that the greater the mutational burden, and therefore, the more tumor neoantigens produced and more immunogenic the tumor, the better the response immune checkpoint therapies is particularly interesting to consider with glioblastoma, which is known for its intratumoral heterogeneity.

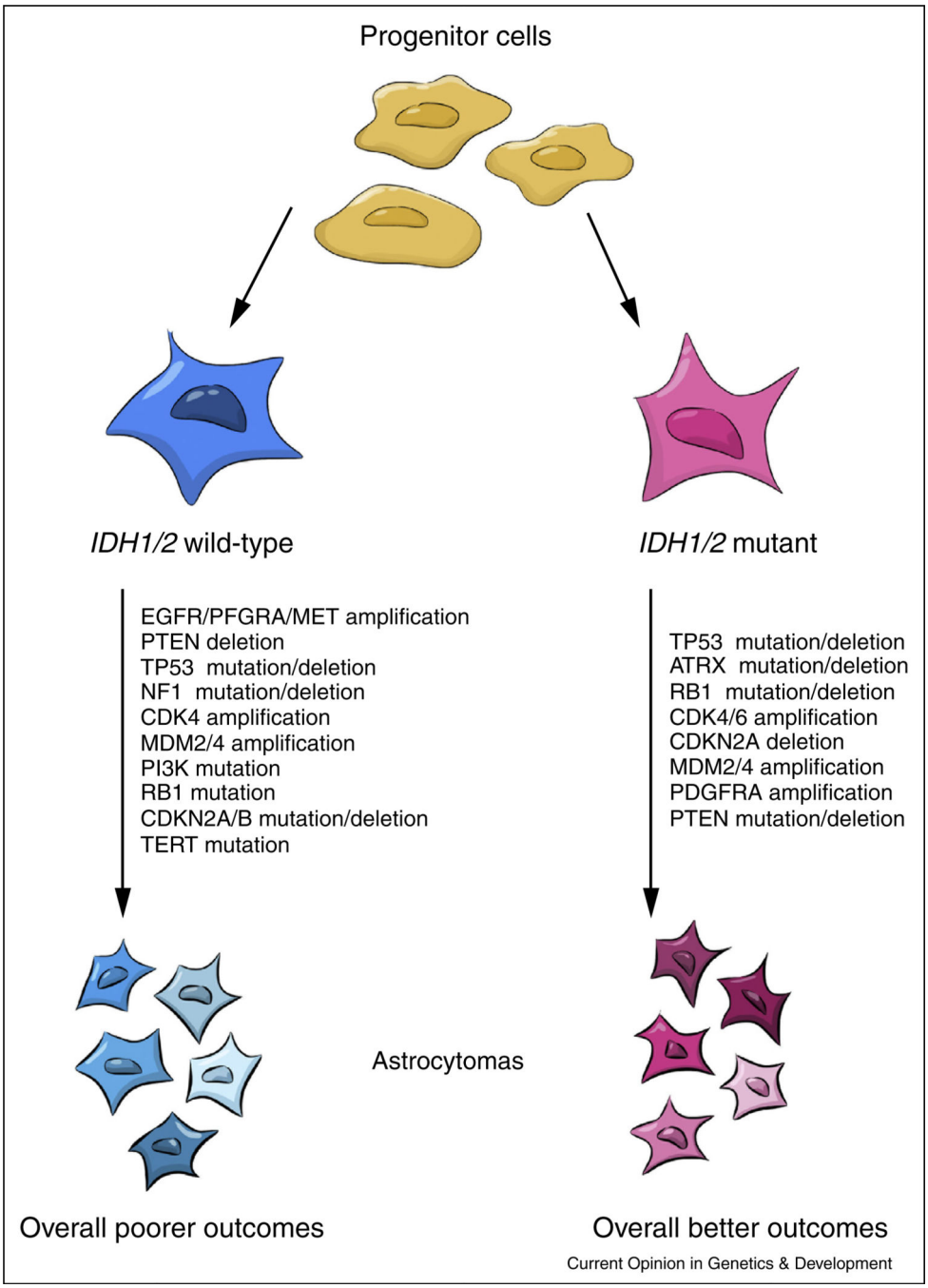

Figure 1. *IDH1/2* mutations occurs early in gliomagenesis and is the fundamental prognostic marker

The molecular classification systems that have been proposed are based on a number of different markers, the most fundamental of which may involve *IDH1/2*. *IDH1/2*-mutant and *IDH1/2*-wild type tumors follow two distinct genetic pathways with divergent clinical outcomes.