EGFR amplification and EGFRvIII mutation[1] (Non-core)

Reason/Evidentiary Support

The epidermal growth factor receptor (*EGFR*) gene at 7p12 is the most commonly amplified proto-oncogene in gliomas.[2] *EGFR* amplification is detectable in approximately 40% of IDH-wildtype glioblastomas, WHO grade IV, and is particularly common in tumours from adult patients with the classic or receptor tyrosine kinase (RTK) type 2 molecular subtype of glioblastoma.[3,4] *EGFR* amplification is commonly associated with point mutations and other genetic rearrangements, the most common of which, EGFRvIII, being detectable in about 50% of *EGFR*-amplified glioblastomas.[5,6] EGFRvIII is caused by an 801-bp in-frame deletion of exons 2 to 7 that results in a constitutively active protein lacking major parts of the extracellular receptor domain including the ligand binding site.[6] Moreover, EGFRvIII carries a unique peptide encoded by the fusion site of exons 1 and 8 that has served as a tumour-specific epitope for anti-EGFRvIII immunotherapy.[7] As *EGFR* amplification and positivity for EGFRvIII are virtually restricted to glioblastoma, IDH-wildtype, their diagnostic detection in an IDH-wildtype diffuse astrocytic glioma may support a glioblastoma diagnosis even in the absence of characteristic histological features like microvascular proliferation and/or necrosis. Detection of *EGFR* amplification or EGFRvIII positivity also may be clinically relevant as a predictive marker of response to molecularly-guided therapies targeting *EGFR* and/or EGFRvIII.[8,9]

EGFR amplification is usually seen in the majority of neoplastic cells in a given tumour and can be readily detected by FISH or CISH on routine FFPE tissue sections, although amplification levels may be heterogeneous from cell to cell. Targeted molecular techniques based on extracted tumour DNA, such as quantitative real-time PCR and MLPA, are also suitable for diagnostic detection of *EGFR* amplification. More recently, microarray-based genomic or epigenetic analyses as well as NGS approaches are increasingly being used.[10] Gene amplification (defined by a circumscribed high-level copy number gain of the *EGFR* gene at 7p12) needs to be distinguished from low-level copy number gains of chromosome 7 caused by numerical chromosomal abnormalities, in particular trisomy 7, which are not restricted to IDH-wildtype glioblastoma but also common in diffuse and anaplastic astrocytomas[11] (see also **Chromosome 7 Gain**). To date, there is no evidence that different levels of *EGFR* gene amplification (e.g., increases in copy number of 10-fold versus 100-fold) have distinct diagnostic or prognostic impact.

Detection of EGFRvIII in *EGFR*-amplified glioblastomas also can be performed at the DNA level, e.g., by MLPA, microarray-based techniques and NGS. However, detection at the mRNA or protein level using RT-PCR or immunohistochemistry with EGFRvIII-specific antibodies appears to be more sensitive.[5] This is due to the fact that EGFRvIII positivity usually shows regional heterogeneity and sometimes affects only a minor subset of the tumour cells.[5] Thus, representative sampling of tumour tissue is an important issue to avoid false-negative testing for EGFRvIII. Unfortunately, precise cut-off values for distinction between high- and low-level copy number gains have not been defined and may need to be adjusted for each testing method.

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