Amplification of the EGFR/CEP7 Gene Using FISH on a Paraffin-Embedded Section

April 2013
1 GENERAL INFORMATION

1.1 Submitting Company, Institution or Organization:
McGill University Health Centre

1.2 Date of Application: June 29, 2012

1.3 Date of Notice: April 2, 2013

Note:
This notice is based on the scientific and commercial information (submitted by the requestor(s) and on a complementary review of the literature) according to the data available at the time that this test was assessed by INESSS.

2 TECHNOLOGY, COMPANY AND LICENCE(S)

2.1 Name of the Technology: Analysis of Epidermal Growth Factor Receptor (EGFR) Gene Amplification with Respect to the Number of Copies of Chromosome 7 (CEP7) Using Fluorescence in Situ Hybridization (FISH).

2.2 Brief Description of the Technology

With FISH, the locus/chromosome balance can be analyzed visually using fluorescence microscopy (Fontaine et al., 2008). In this case, determination of the EGFR/CEP7 ratio is based on matching a fluorescent probe specific to the EGFR gene locus and a control probe, specific to the centromere of chromosome 7 (CEP7). The objective is to verify if there is an increase in the number of copies of EGFR (amplification) with respect to the number of copies of chromosome 7 (see Figure 1).

Figure 1. Analysis of EGFR Amplification and Enumeration of Chromosome 7 Using FISH

Figure adapted from Horbinski et al., 2011b.
2.3 Company or Developer

Dr. Marie-Christine Guiot, a pathologist at MUHC, according to the original protocol published by (Brat et al., 2004)\(^1\).

Pretreatment of slides: Vysis Paraffin Pretreatment kit I (Abbott Laboratories, Limited).
Probes: Vysis EGFR/CEP7 FISH Probe Kit (Abbott Laboratories, Limited).

2.4 Licence(s): Establishment licence granted by Health Canada to Abbott Laboratories, Limited (#210).

2.5 Patent, if Applicable

2.6 Approval Status (Health Canada, US FDA)

Pretreatment of slides

Health Canada Licence: FISH SPECIMEN PRETREATMENT REAGENT KIT #79946 (Abbott Molecular Inc.)

Probes

Health Canada Licence: VYSIS EGFR/CEP7 FISH PROBE KIT #81666 (Abbott Molecular Inc.)

2.7 Weighted Value: 250.44.

3 CLINICAL INDICATIONS, PRACTICE SETTINGS, AND TESTING PROCEDURES

3.1 Targeted Patients

Individuals with oligodendroglioma (ODG) or glioblastoma (GBM).

3.2 Targeted Diseases

According to the Central Brain Tumor Registry of the United States (CBTRUS), 90,828 cases of primary glioma were diagnosed in the United States between 2005 and 2009, accounting for 80% of primary malignant tumours of the brain and central nervous system (Dolecek et al., 2012). Glioblastoma accounts for 54% of cases of glioma, while ODG accounts for 6.2% of cases. The annual incidence of GBM and ODG is 3.2 cases and 0.3 cases per 100,000 population, respectively (Dolecek et al., 2012). According to the World Health Organization (WHO) classification published in 2007 which clusters gliomas into four grades (WHO I to IV), GBM is grade IV, while pure and mixed (oligastrocytoma) ODGs are WHO grade II or III, depending on whether or not they are an anaplastic form: anaplastic oligodendroglioma (AO) and anaplastic oligoastrocytoma (AOA) (Louis et al., 2007). With respect to GBM and ODG, the five-year survival rates are 4.7% and 79.1%, respectively.

3.3 Number of Patients to Be Tested

McGill University Health Centre reports, through the requestor, that it makes 150 to 200 diagnoses of glioma per year. The volume of tests expected for the next three years has been estimated at 300 to 350 tests, or more than 100 per year.

---

\(^1\) Personal electronic communication with Dr. Marie-Christine Guiot (January 9, 2013).
3.4 Medical Specialties Involved (and Other Professionals, If Applicable)

Radiation oncology, hemato-oncology and neuropathology.

3.5 Testing Procedure

Surgical resection or biopsy specimens where the tissue is fixed with formaldehyde and embedded in paraffin.

4 TECHNOLOGY BACKGROUND

4.1 Nature of the Diagnostic Technology

Complementary (diagnostic). The current Index contains several tests of this kind.

4.2 Brief Description of the Current Technological Context

Epidermal growth factor receptor amplification can be found in 50% of GBM and in 15% to 20% of WHO grade 3 glioma (AO, AOA and AA) (Furnari et al., 2007). Copy number amplification of the EGFR gene ranges from just a few to several thousand in each nucleus. Several techniques can reveal amplification, including quantitative polymerase chain reaction (qPCR), comparative genomic hybridization (CGH) and FISH. The level of EGFR protein expression can be assessed by immunohistochemistry (IHC).

4.3 Brief Description of the Advantages Cited for the New Technology

EGFR amplification is a genetic signature that is almost exclusive to GBM and the presence of this characteristic increases with patient age (Ohgaki et al., 2004). The EGFR\textsuperscript{amp} and del(1p/19q) statuses are mutually exclusive; the latter is a signature of ODG (Idbaih et al., 2011). There are several histological subtypes of GBM: pleomorphic (26%), gemistocytic (25%), oligodendrogial component (15%), small-cell (27%), gliosarcoma (2%), giant-cell (1%) and mixed (Hatanpaa et al., 2010). Benefits of EGFR status testing by FISH would include: reaching a diagnosis of GBM when there are insufficient histological criteria to do so, such as by differentiating high-grade ODG from small-cell GBM; differentiating primary GBM from recurrent GBM; and better determining the prognosis for WHO III tumours. As brain tumour tissue is usually fixed with formaldehyde and preserved in paraffin blocks, FISH can be performed simply and quickly on this type of sample, regardless of tissue age (Jha et al., 2011; Woehrer et al., 2011).

4.4 Cost of the Technology and Options

Slide examination requires a fluorescence microscope equipped with the proper filters (Carl Zeiss Canada). Additionally, as the fluorescent signal fades over time, a suitable image documentation system is required for archiving data. The cost of the equipment and kits is not known.

5 EVIDENCE

5.1 Clinical Relevance (Utility and Validity) and Analytical Validity

5.1.1 Other Tests Replaced

Does not apply.
5.1.2 Diagnostic and Prognostic Value of $EGFR^{\text{amp}}$ Status Assessed by FISH

Anaplastic ODG and Small-Cell GBM Differential Diagnosis

In 2004, Korshunov et al. published a cohort study involving 114 patients with high-grade glioma (HGG) with uniform small-cell morphology. The main objective was to verify the diagnostic and prognostic values of various genetic markers associated with glioma, including $EGFR$. Within the group of small-cell tumours, the authors found a great variety of genetic profiles and clinical outcomes. Four separate subsets were identified: 13 patients whose tumour did not involve any genetic alteration and whose 5-year overall survival rate (5-year OSR) was 83%; 20 patients with del(1p/19q) or del 19q status (5-year OSR: 59%); 35 patients whose tumour showed p16 and/or PTEN without $EGFR$ amplification (5-year OSR: 8%); and 46 patients with $EGFR^{\text{amp}}$ status (5-year OSR: 0%). Multivariate analysis showed that for small-cell HGG, $EGFR^{\text{amp}}$ status is an independent factor for poor prognosis, while the absence of genetic aberration and del(1p/19q) status are factors for favourable prognosis (see Table 1). Perry et al. also concluded that $EGFR^{\text{amp}}$ status is a distinctive marker for small-cell astrocytoma and predicts aggressive biological behaviour as with GBM.

5.1.3 Diagnostic and Prognostic Value of $EGFR^{\text{amp}}$ Status (WHO III)

The histopathological diagnosis of GBM (WHO IV) requires anaplastic glial cells, increased mitotic activity, and vascular proliferation with or without necrosis (Louis et al., 2007). Interobserver variation is rare with a diagnosis of GBM, but it has been well described in cases of low-grade glioma (van den Bent, 2010). The hypothesis that $EGFR$ status is a complementary diagnostic tool for WHO grade III tumours and can better predict their biological behaviour was verified.

Table 1 presents a few studies whose objective was to verify the independent prognostic value of $EGFR^{\text{amp}}$ status in cohorts of patients with anaplastic astrocytic or oligodendroglial tumours (WHO III). Other than the Korshunov study involving a group of tumours classified morphologically as WHO grade III or IV, none of the identified studies was able to establish, with statistical significance, an independent prognostic value associated with $EGFR^{\text{amp}}$ status for WHO grade III tumours (see Table 1).

5.1.4 Primary GBM Distinguished from Secondary GBM by $EGFR^{\text{amp}}$ Status

Primary GBM, with no previous clinical manifestations or proof of evolution from a low-grade astrocytoma (secondary GBM), accounts for 90% of cases and occurs primarily in the elderly (Barker et al., 2012). The fact that $EGFR$ amplification is observed only in GBM suggests that it is a late event in the tumour progression of astrocytoma (Hoang-Xuan et al., 2005). Benito et al. (2010) investigated the association between the presence or absence of $EGFR$ amplification and the clinical and genetic characteristics of a series of 45 primary GBMs. More than half the cases showed $EGFR$ amplification (53%). No survival benefit was associated with this characteristic (data not shown). Secondary GBM accounts for approximately 10% of cases and develops in younger patients (under 50 years of age). It often harbours mutations of the $TP53$ and $IDH1$ genes. The frequency of PTEN deletion and $EGFR$ amplification is low. Several studies confirmed these data, which distinguish de novo GBM from secondary GBM (Appin et al., 2013; Watanabe et al., 1996).
In summary, \textit{EGFR} amplification remains essentially a diagnostic marker, as \(EGFR^{\text{amp}}\) status does not seem to provide additional prognostic information, probably because of its strong association with a diagnosis of GBM (Horbinski et al., 2011b). In anaplastic ODG, the poor prognosis associated with \(EGFR^{\text{amp}}\) status seems to be in fact a misdiagnosis of small-cell GBM (Fallon et al., 2004).
# Table 1: Prognostic Values of $EGFR^{\text{amp}}$ Status Assessed Using FISH

<table>
<thead>
<tr>
<th>Study Design</th>
<th>$EGFR^{\text{amp}}$ Status Assessed</th>
<th>Prognostic Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Fuller et al., 2003) (Cohort study, diffuse OA of various grades)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RT + CT, N=70, 37 years (12-78), follow-up for 3.3 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Variables</td>
<td>OS (months)</td>
<td>Multivariate HR (death) (N=70)</td>
</tr>
<tr>
<td>Grades: WHO 2, 3 and 4</td>
<td>NA, 73 and 12; $p &lt; 0.0001$</td>
<td>II vs. III: 3.2; $p=0.03$ (59)</td>
</tr>
<tr>
<td>&lt; 40 years, 40-59 years, ≥ 60 years</td>
<td>96, 73 and 4; $p &lt; 0.0001$</td>
<td>II vs. IV: 5.6; $p=0.002$ (45)</td>
</tr>
<tr>
<td>$EGFR^{\text{amp}}$: yes vs. no</td>
<td>16 vs. 96; $p=0.0007$</td>
<td>1.06; $p=0.0001$</td>
</tr>
<tr>
<td>Chr. 7$^{\text{poly}}$: yes vs. no</td>
<td></td>
<td></td>
</tr>
<tr>
<td>del 1p/19q: yes vs. no</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No alterations: yes vs. no</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Korshunov et al., 2004) (Cohort study, high-grade small-cell gliomas)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RT + CT, N=114, 47 years (19-72), follow-up for 117 months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Variables</td>
<td>5-year OS (%)</td>
<td>Multivariate HR (death) (N=114)</td>
</tr>
<tr>
<td>&lt; 50 years vs. ≥ 50 years</td>
<td>37 vs. 9; $p=0.004$</td>
<td>NS</td>
</tr>
<tr>
<td>$EGFR^{\text{amp}}$: yes vs. no</td>
<td>0 vs. 41; $p &lt; 0.0001$</td>
<td>3.28; $p=0.001$</td>
</tr>
<tr>
<td>Chr. 7$^{\text{poly}}$: yes vs. no</td>
<td>5 vs. 55; $p &lt; 0.0001$</td>
<td>NS</td>
</tr>
<tr>
<td>del 1p/19q: yes vs. no</td>
<td>54 vs. 17; $p &lt; 0.0001$</td>
<td>−2.79; $p=0.01$</td>
</tr>
<tr>
<td>No alterations: yes vs. no</td>
<td>83 vs. 12; $p &lt; 0.0001$</td>
<td>−3.47; $p=0.0006$</td>
</tr>
<tr>
<td>(Dehais et al., 2006) (Cohort study, AA: 9%, OA: 56% and AOA: 35%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RT + CT, N=156, 46 years (20-83), median follow-up of 57.3 months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Variables</td>
<td>Median OS (months)</td>
<td>Multivariate RR (death) (N)</td>
</tr>
<tr>
<td>&gt; 46 years vs. ≤ 46 years</td>
<td>25 vs. 41; $p=0.012$</td>
<td>1.73; $p=0.018$ (156)</td>
</tr>
<tr>
<td>OA vs. AOA and AA</td>
<td>67 vs. 29; $p=0.0002$</td>
<td>0.54; $p=0.01$ (156)</td>
</tr>
<tr>
<td>$EGFR^{\text{amp}}$: yes vs. no</td>
<td>17 vs. 39; $p=0.0002$</td>
<td>NS (150)</td>
</tr>
<tr>
<td>del 1p/19q: yes vs. no</td>
<td>99 vs. 29; $p=0.0002$</td>
<td>0.30; $p=0.0025$ (143)</td>
</tr>
<tr>
<td>EORTC 26951 RT vs. RT + PCV, N=368, 48 years (19-68), follow-up for 117 months.</td>
<td>14%/19%</td>
<td>Variables</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>&lt; 50 years vs. ≥ 50 years</td>
<td>Not assessed</td>
<td>HR: 1.85; p=0.002 (84)</td>
</tr>
<tr>
<td>EGFR&lt;sup&gt;amp&lt;/sup&gt;: yes vs. no</td>
<td>2.68; p &lt; 0.0001</td>
<td>NS (29)</td>
</tr>
<tr>
<td>Chr. 7&lt;sup&gt;polys&lt;/sup&gt;: yes vs. no</td>
<td>1.94; p=0.0002</td>
<td>HR: 1.65; p=0.02 (59)</td>
</tr>
<tr>
<td>del 1p/19q: yes vs. no</td>
<td>0.12; p &lt; 0.0001</td>
<td>HR: 0.12; p &lt; 0.0001 (100)</td>
</tr>
<tr>
<td>del 1p: yes vs. no</td>
<td>0.57; p=0.01</td>
<td>HR: 0.57; p=0.01 (56)</td>
</tr>
<tr>
<td>Necrosis: yes vs. no</td>
<td>HR: 2.00; p=0.0004</td>
<td>HR: 2.00; p=0.0004 (92)</td>
</tr>
</tbody>
</table>

Abbreviations: AA = anaplastic astrocytoma; AOA = anaplastic oligoastrocytoma; Chr. 7<sup>polys</sup> = chromosome 7 polysomy; CT = chemotherapy; HR = hazard ratio, univariate or multivariate analysis; N = number of patients; NA = not achieved; NS = not statistically significant; OA = oligoastrocytoma; OS = overall survival; PCV = procarbazine, lomustine (CCNU) and vincristine; PFS = progression-free survival; RT = radiation therapy; vs. = versus.
5.2 Clinical Validity

<table>
<thead>
<tr>
<th>Component</th>
<th>Presence</th>
<th>Absence</th>
<th>Not Applicable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specificity</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive predictive value (PPV)</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative predictive value (NPV)</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Likelihood ratio (LR)</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>ROC Curve</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Accuracy</td>
<td></td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>

Sensitivity, Specificity, PPV and NPV

From a series of 750 glioma follow-ups, Horbinski et al. (2011a) showed a strong correlation between EGFR<sup>amp</sup> status and the level of EGFR expression in IHC (p < 0.0001). When gliomas that showed moderate to strong immunoreactivity were combined, EGFR IHC was able to identify all cases with EGFR amplification viewed by FISH (100% sensitivity), and it also displayed 99% NPV. As several cases that showed moderate or strong immunoreactivity presented no amplification, specificity and PPV were 39% and 59%, respectively.

As previously shown, EGFR amplification is considered a diagnostic marker for patients with high-grade glioma when cellular morphology is insufficient to diagnose GBM. Assessment of EGFR<sup>amp</sup> status from paraffin sections using FISH is a conventional technique (Hyytinen et al., 1994) in broad clinical use (Horbinski et al., 2011a). EGFR<sup>amp</sup> status assessed by FISH is also used as a stratification factor in several recently completed or ongoing clinical studies, including the NCT00337883<sup>3</sup> study, whose purpose was to compare the efficacy of erlotinib, a tyrosine kinase inhibitor (TKI), in patients with GBM. Others include phase 2 randomized controlled trials NCT01520870<sup>3</sup> and NCT00301418<sup>4</sup>, whose objectives are also to assess the efficacy of TKI in patients with recurrent AA or GBM.

No studies were found that compared the prognostic value of EGFR<sup>amp</sup> status assessed using FISH to other methods.

5.3 Analytical (or Technical) Validity

<table>
<thead>
<tr>
<th>Terms</th>
<th>Presence</th>
<th>Absence</th>
<th>Not Applicable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Repeatability</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Reproducibility</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Analytical sensitivity</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Analytical specificity</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Matrix effect</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Concordance</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Correlation</td>
<td></td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>

The term “EGFR amplification” refers to an increase in the EGFR gene copy number. In cases of malignant glioma, polysomy 7 is responsible for amplification of DNA, not just the EGFR gene. Although amplification of a gene often precedes overexpression of the transcript (Coulibaly et al., 2010), amplification of the gene should not be confused with overexpression of the protein (Schwab, 1999). In oncology, immunohistochemistry allows for the production level of an oncoprotein like EGFR to be assessed effectively.

Interobserver Reproducibility
Using a series of 170 histological specimens and 153 cytological specimens from lung and non-lung tumours, two different observers assessed the mean copy number (MCN) of EGFR using FISH (Zlobec et al., 2010). Interobserver agreement ($r = 0.99$, cytological specimens; $r = 0.89$, histological specimens). The average difference in MCN between observers was $-0.003$ (95% CI, $-0.05$ to $0.05$) and $0.008$ (95% CI, $-0.09$ to $0.11$) for cytology and histology, respectively (Bland–Altman).

Concordance and Correlation Between Tests
Immunohistochemistry versus FISH: Spearman test, $p < 0.0001$ (Horbinski et al., 2011a) and $p = 0.0004$ (Guillaudeau et al., 2009).
Polymerase chain reaction versus FISH: 89% concordance and a kappa correlation coefficient of 0.73 (Smith et al., 2001).

5.4 Recommendations for Listing in Other Jurisdictions
The European Society for Medical Oncology (ESMO) published guidelines for managing patients with malignant glioma (Stupp et al., 2010). Epidermal growth factor receptor status was not taken into account.

The National Comprehensive Cancer Network (NCCN) published a report regarding the clinical relevance of several tumour markers associated with glioma. Epidermal growth factor receptor status was not taken into account (Febbo et al., 2011).

6 ANTICIPATED OUTCOMES OF INTRODUCING THIS TEST

6.1 Impact on Human and Material Resources
No study was found.

6.2 Economic Impact of Including this Test in the Health and Social Services System
No economic impact or cost-benefit study was found.

6.3 Main Organizational, Ethical, Social, Legal, and Political Issues
Quality assurance is a major issue. Standard criteria for interpreting results must be defined. The development of tyrosine kinase inhibitors and antibodies directed against EGFR is a growing avenue of research. Several molecules have demonstrated effectiveness against other cancers. Consequently, the clinical usefulness of assessing EGFR status should be considered from the perspective of whether or not effective treatment is available to block the biological action of EGFR amplification (Martin et al., 2009).
7  INESSS NOTICE IN BRIEF

Amplification of the *EGFR/CEP7* Gene Using FISH on a Paraffin-Embedded Section

Status of the diagnostic technology

- Established
- Innovative
- Experimental (for research only)
- Replacement for technology: ____________, which is becoming obsolete

INESSS recommendation

- Include in the Index
- Experimental (for research only)

Do not include in the Index:

Given the data currently available, the test lacks maturity for clinical use.
No target treatment based on test results.
Very interesting for categorizing patients for clinical trials.

- Reassess

INESSS decision regarding any required work

- Draw connection with listing of drugs, if it is a companion test
- Produce an optimal use manual
- Produce indicators, if close monitoring is required
- None
REFERENCES


