



# Epidermal growth factor receptor (EGFR) in the era of Precision Medicine: The tale of a perfect example of targeted therapy. A review



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## ABSTRACT

Epidermal growth factor receptor has been under the lights for the past few years as a perfect and typical example of a success story of targeted therapy. In the era of Precision Medicine, such modalities of treatment are highly implicated in the adequate choice of therapy for a wide variety of oncological diseases. This review article is about the different aspects of detection, application, and analysis of the epidermal growth factor receptor genetic mutations that constitute a key element in the initiation of targeted therapy.

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## Contents

|      |                                                                                 |     |
|------|---------------------------------------------------------------------------------|-----|
| 1.   | Introduction . . . . .                                                          | 157 |
| 2.   | EGFR prognosis in cancer . . . . .                                              | 158 |
| 2.1. | Lung cancer . . . . .                                                           | 158 |
| 2.2. | Colorectal cancer . . . . .                                                     | 158 |
| 2.3. | Glioblastoma multiforme (GBM) . . . . .                                         | 159 |
| 3.   | Applications of EGFR testing in treatment . . . . .                             | 159 |
| 3.1. | Lung cancer . . . . .                                                           | 159 |
| 3.2. | Colorectal cancer . . . . .                                                     | 159 |
| 3.3. | Glioblastoma multiforme (GBM) . . . . .                                         | 160 |
| 3.4. | Thymoma and thymic carcinomas . . . . .                                         | 160 |
| 3.5. | Head and neck squamous cell carcinoma (HNSCC) . . . . .                         | 160 |
| 4.   | Methods for EGFR mutations detection . . . . .                                  | 160 |
| 4.1. | Immunohistochemistry . . . . .                                                  | 160 |
| 4.2. | Polymerase chain reaction . . . . .                                             | 161 |
| 4.3. | Direct DNA sequencing . . . . .                                                 | 161 |
| 4.4. | High-performance liquid chromatography (HPLC) . . . . .                         | 161 |
| 4.5. | PCR, Scorpion Amplified Refractory Mutation System technology (SARMS) . . . . . | 161 |
| 5.   | Conclusion . . . . .                                                            | 161 |
|      | References . . . . .                                                            | 161 |

## 1. Introduction

Growth factors are essential for the growth and development of multicellular organisms. The biological substances bind their specific receptor in order to relay messages between cells, allowing them to communicate. Once bound, the ligand- receptor complex can transduce extracellular signals either by activating intracellular messengers or by

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directly translocating into the nucleus of the cell for direct interaction (Wieduwilt and Moasser, 2008).

The epidermal growth factor (EGF) family is a member of the receptor tyrosine kinases (RTKs) involved in signaling pathways that control angiogenesis, cell differentiation, proliferation, survival, and progression of many cancers (Clauditz et al., 2012). The EGF family of receptors are also called ErbB or HER receptors. From this family of receptors, a subfamily of four closely related receptor tyrosine kinases exists: EGFR (ErbB-1), HER2/c-neu (ErbB-2), HER3 (ErbB-3), and HER4 (ErbB-4) (Reisner, 2012). The EGFR gene is located on the short arm of chromosome 7 at position 12. The binding of the EGF to its receptor induces conformational changes within the receptor which increases the catalytic activity of the intrinsic tyrosine kinase resulting in autophosphorylation. The activated kinase then phosphorylates tyrosine residues on different cellular substrates including phospholipase C- $\gamma$ , (PLC- $\gamma$ ), mitogen-activated protein kinase (MAPK) and the ras GTPase-activating protein (GAP), which ultimately leads to an increase in catalytic activity (Voldborg et al., 1997). In other words, once bound, activated protein kinases trigger signaling pathways that modify genetic expression patterns; playing their part as essential regulators for different developmental processes. If this genetic modification is deregulated, the process becomes related to cancer (Perona, 2006). In certain cases, overexpression of the epidermal growth factors and their receptors lead to uncontrolled cell proliferation such as in tumors (Greig et al., 1988). EGFR overexpression has been described in several tumor entities. The most clinically appealing mutations appear in lung, colorectal, brain (glioblastoma multiforme), thymic, and head and neck cancers. This abnormal growth can be luckily controlled by pharmacological agents that are able to inhibit EGFR (Zükin, 2012). Two classes of anticancer therapeutics are directed against EGFR overexpression, the tyrosine-kinase inhibitors (TKIs) – gefitinib (Iressa®), erlotinib (Tarceva®), lapatinib (Tykerb®) etc....- and monoclonal antibodies- trastuzumab (Herceptin®), pertuzumab, cetuximab (Erbbitux®), panitumumab (Vectibix®) etc.... - which are available for clinical use in different combinations for different types of cancers discussed later (Zükin, 2012; Wieduwilt and Moasser, 2008). Other inhibitors are under clinical studies for use in combination with chemotherapeutic drugs in treatment. Many studies have shown that EGFR can be both an indicator for the suitability of treatment and a predictive factor of response to targeted therapy (Xue and Jin-ming, 2011). But beside this beneficial therapeutic function, EGFR overexpression has been linked to advanced disease with aggressive phenotype and overall poor outcomes (Clauditz et al., 2012). With this said, EGFR testing has its significant role at the level of malignancies. According to Zhao et al.'s study, EGFR amplification was assessed in glioma patients in addition to its association with survival. In the many cases of glioma patients tested at all ages, EGFR amplification was unfavorably associated with a poor survival rate (Zhao et al., 2012).

Uncontrolled EGFR, in light of all its hallmarks, qualifies as an entity categorized as cancer. From the standpoint of cell proliferation, apoptosis, angiogenesis, motility, and invasion, all are rendered abnormal (Modjtahedi et al., 2012). Aberrant EGFR expressions have been reported in various types of epithelial cancers. According to Modjtahedi and Essapen's review, they reported that in some studies, abnormal EGFR expression of normal or truncated forms of receptors was associated with poor prognosis and resistance to treatments. These studies lead to the development of various types of EGFR inhibitors; five have gained the US Food and Drug Administration (FDA) approval for treatment use. The type of cancer and application of treatment is indicated as follows: NSCLC (non-small-cell lung cancer) (gefitinib and erlotinib), metastatic colorectal cancer (cetuximab and panitumumab), head and neck (cetuximab), pancreatic cancer (erlotinib) and breast cancer (lapatinib) (Modjtahedi and Essapen, 2009). However, with the ongoing studies on EGFR mutations and amplifications, more drugs are being introduced and newer combinations are showing better results in terms of patient survival and progression free survival. Some of these cancers, in

association with their treatment, will be discussed in the following sections of this paper.

## 2. EGFR prognosis in cancer

### 2.1. Lung cancer

Lung cancer is the most common cause of cancer-related deaths in both men and women in the world with the highest fatality rate. Lung cancers associated with mutated epidermal growth factor receptors constitute one of its biggest subsets. Since these mutations depend on mutant EGFRs for proliferation, they show promising therapeutic value and respond to orally available EGFR tyrosine kinase inhibitors (TKIs) (Xiao-Hong Kang et al., 2013). These cancers associated with molecular aberrations account for ~50% in East Asians and ~15% in Caucasians (Suda et al., 2010).

EGFR is recognized as an important molecular target in cancer therapy. Its mutation tends to develop angiogenic metastasis and is a very important marker for therapy for non-small cell lung cancer (NSCLC). According to Lim and Togashi et al., since NSCLC responds to TKIs through gefitinib and erlotinib, these drugs give a high response rate and maintain a long progression-free survival rate (Togashi et al., 2014; Lim et al., 2014). KRAS - Kirsten Rat Sarcoma 2 viral - oncogene, on the other hand, is found in 30% of the United States patients with advanced lung adenocarcinoma. EGFR and KRAS are two main genes that can predict different outcomes in patients with stage IV lung adenocarcinomas. Results from a study by Johnson et al. showed that patients with mutations in EGFR were correlated with a longer survival period than patients with mutations in the KRAS gene. Efforts in developing therapies opposing KRAS mutations have been largely unsuccessful and the prognostic effect of KRAS remains in question (Johnson et al., 2013). Another gene involved in NSCLC is the Anaplastic Lymphoma Kinase gene (ALK). This gene is not positive in a large number of people (9%), however, in those showing positivity, the overall survival of patients was similar to that of EGFR (Grigoriu et al., 2015).

### 2.2. Colorectal cancer

Colorectal carcinoma (CRC) is the fourth most commonly diagnosed cancer in the United States. It is also the second leading cause of cancer death (Shaib et al., 2013). A large proportion of patients diagnosed with this cancer will develop metastatic disease to different organs in the future (Loupakis et al., 2015).

Colorectal carcinomas are highlighted by the laboratory testing of EGFR and KRAS - wild-type (normal) and mutant - genes. These genes help predict patient survival and response to treatment. KRAS is a key signaling protein between extracellular EGFR ligands and intracellular signaling among cells and can determine treatment options in metastatic colorectal carcinoma (mCRC) patients (Lianfeng Shan et al., 2014). Anti-EGFR therapy is almost always initiated in cases of mCRCs since 50–70% of cases are positive for EGFR (Motlagh et al., 2007). However, it has been shown in several studies that the decision on a method for treatment based on the detection of EGFR mutation alone (in mCRC cases) is not enough. KRAS assessment is a necessity and must be accounted for in that decision of methodology in treatment. As mentioned in the literature for Modjtahedi and Essapen, several trials have shown that the presence of activating KRAS mutation in patients with mCRC have shown resistance to therapy with anti-EGFR monoclonal antibodies (mAbs) (cetuximab and panitumumab). Based on these results, the American Society of Clinical Oncology has suggested that all patients with metastatic CRC who are candidates for therapy with anti-EGFR monoclonal antibodies (mAbs) have their tumor tested for KRAS mutations. Different outcomes are expected if patients test positive for wild-type or mutant KRAS. A significant number of studies have confirmed that patients with mCRC carrying mutated KRAS genes (at the level of codons 12 and 13) do not benefit from treatment with monoclonal

antibodies cetuximab and panitumumab. KRAS gene activating mutations are the main negative predictor of mCRC anti-EGFR therapy (Modjtahedi and Essapen, 2009; Carotenuto et al., 2012; Lianfeng Shan et al., 2014).

### 2.3. Glioblastoma multiforme (GBM)

Glioblastoma multiforme (GBM) is the most aggressive malignant primary brain tumor in man (Furnari et al., 2007). It is associated with a mean survival of 15 months (Stupp et al., 2005). In primary GBM samples tested through the TCGA program in a study conducted by Frank B. Furnari et al., 66% of cases were due to a receptor tyrosine kinase amplification or mutation. 50% of these RTK cases were due to amplifications and/or mutations in EGFR. Deletion mutations that involve the EGFR extracellular domain are unique to GBM. These include EGFR types I, II, III, VI, VII, VIII variants. Most notable, types VII and VIII have been confirmed to be active and highly oncogenic (Frank B. Furnari et al., 2015). Another study conducted by Gursel et al. showed that 88% of glioblastoma cases are found to consist an abnormal RTK signaling cascade. Most noteworthy is the amplification or mutation of EGFR which is found in about half of these cases; majority carrying the *EGFRvIII* mutation, an in-frame deletion of exons 2 to 7 (Gursel et al., 2012). This in-frame deletion of the wild type EGFR tyrosine kinase gives rise to a truncated receptor which is constitutively active and ligand independent. This active mutant shows GBM characteristics with increased proliferation, survival, and invasive phenotype; thus, highly associated with tumorigenesis (Gursel et al., 2012). The standard of care is surgical removal, radiotherapy, and adjuvant chemotherapy. Despite all these choices for treatment, its prognosis is still poor (Johnson et al., 2012). At the molecular level, the activation of EGFR in GBM is different than that present in lung cancer. In glioblastomas, activation is through mutation or deletion in the extracellular (EC) domain, however, in lung cancer, the mutation occurs at the level of the EGFR kinase domain. This shows that the target conformation of the EGFR inhibitors is different in glioblastoma than in lung cancer. EGFR EC mutants specific to gliomas are poorly inhibited by the EGFR inhibitors which target the active kinase conformation (present in lung cancer) (Vivanco et al., 2012). Therapies that target EGFR or its mutant active form are currently being extensively studied; most notable, TKI combinations, monoclonal antibodies, vaccines, and RNA-based agents. According to Taylor et al., data from experimental studies about these therapies are encouraging. However, drug resistance is limiting their clinical efficacy so far (Taylor et al., 2012).

## 3. Applications of EGFR testing in treatment

### 3.1. Lung cancer

Lung cancer remains a global burden with NSCLC accounting for around 80% of all lung cancer cases (Ricciuti et al., 2016). As mentioned, EGFR, KRAS, and ALK are important genes in assessing treatment options and survival among patients. However, EGFR is the most promising biomarker to date (Gao et al., 2015). According to the literature for Mascaux et al., advanced NSCLC with EGFR mutations respond much better to EGFR TKIs than with chemotherapy initiated alone. It is thus considered the first line therapy (Mascaux et al., 2011). Erlotinib and gefitinib showed high response rates, progression free survival, and better quality of life. After a median of 8–10 months, patients began developing acquired resistance to this type of therapy, showing progression of disease. Many mechanisms for this resistance have been drawn; the most widely accepted and prevalent resistant mechanism is the T790M missense mutation in the EGF receptor (Stasi and Cappuzzo, 2014). This mutation substitutes two amino acids (threonine instead of methionine) affecting the ATP binding site of the EGFR kinase domain (Tan et al., 2015).

Despite the improved response rates and progression-free survival rates, EGFR mutant NSCLC patients with the gatekeeper T790M

mutation began to emerge and started showing resistance to TKIs (if treated with). Luckily, second generation irreversible EGFR TKIs – Dacomitinib and Afatinib – began to elucidate promising results inhibiting both the wild type EGFR and EGFR T790M. Afatinib has been recently approved for first line treatment of EGFR mutant NSCLC patients; Dacomitinib remains under study (Ou and Soo, 2015).

The KRAS gene also plays an important role in driving lung tumorigenesis in NSCLC patients. Its positivity (mostly in NSCLC adenocarcinoma) confers a poor prognosis due to the lack of any targeted therapy. Years of pursuit have failed trying to formulate a drug to inhibit KRAS mutant genes in NSCLC patients. Unlike EGFR and ALK, a targeted agent against KRAS mutated genes remains elusive. ALK TKIs do exist on the market (crizotinib and ceritinib) and their outcome is similar to that of EGFR (better quality of life and survival); however, its positivity among NSCLC patients is lower (Bhattacharya et al., 2015).

In advanced NSCLC patients, accessibility of tumor samples is not always possible and satisfactory. In addition, most patients would have shown a resistance to the EGFR-TKIs. Repeated biopsies and invasive procedures would be hectic to perform on the patient if sample quality is low. Thus, newer, more accurate, and less invasive procedures should be explored to discover any new mutation or to monitor TKI response. Circulating DNA fragments carrying tumor specific sequence alterations are found in the cell free portion of the blood sample. These cell free tumor DNA particles carry a high degree of specificity to detect EGFR mutation in NSCLC samples. Several studies have been conducted on the feasibility of using circulating tumor cells (CTCs) from serum in order to diagnose EGFR tumor mutations and monitor tumor dynamics. The evidence provided by these studies suggests the potential CTCs hold in EGFR mutated NSCLC patients (Bordi et al., 2015). One of such a study was conducted by Marchetti et al. They used CTC preparations obtained by Veridex CellSearch System then subjected the samples to next generation sequencing (NGS). In all samples collected, multiple EGFR mutations were recorded suggesting CTC heterogeneity. It was concluded that CTC preparations obtained by the CellSearch System coupled with NGS represents a suitable source of tumor DNA for EGFR analysis. CTC samples are also a very sensitive and specific diagnostic tool for EGFR mutation analysis and EGFR-TKI monitoring (Marchetti et al., 2014).

### 3.2. Colorectal cancer

In a systematic review written by Jiang et al. on colorectal cancer, it was concluded that among patients receiving anti-EGFR therapy, increased EGFR gene copy number was correlated with improved survival outcomes (Jiang et al., 2013). In a recent study conducted by Loupakis et al., they showed that triplet chemotherapy (i.e. 5-fluorouracil [5-FU]/leucovorin [LV], oxaliplatin, and irinotecan; FOLFOXIRI) induction, in addition to the vascular endothelial growth factor inhibitor bevacizumab, showed significantly better objective results than doublet chemotherapy plus bevacizumab. The only problem was chemotherapy induced adverse effects (Loupakis et al., 2015). In yet another review by Yazdi et al., they showed that combining anti epidermal growth factor receptor drugs as a targeting therapy with conventional chemotherapy yielded very good results in terms of overall survival and progression free survival (Yazdi et al., 2015). The best combination therapy concluded was cetuximab and panitumumab with conventional chemotherapy. As previously discussed, despite the good prognosis upon initiating EGFR therapy, KRAS positivity can confer resistance to anti-EGFR therapy. Approximately 40% of patients with mCRC test positive for KRAS and are resistant to EGFR inhibitors (Shaib et al., 2013). In a study for Bihl et al., the authors investigated 100 colorectal carcinoma samples with known KRAS mutation status (62 mutated and 38 wild type). The results were conveyed with three different KRAS mutation testing techniques (Pyrosequencing, Dideoxysequencing, and INFINITI) to test reliability and sensitivity. For the large majority of samples, all three methods yielded similar KRAS mutational statuses. As discussed above, new molecular tests are focusing on detecting KRAS

mutation to predict the response to therapy. Besides the innovative step concerning therapeutic agents that target EGFR, it is still effective in a subset of patients. KRAS status can predict which of the patients may or may not benefit from anti-EGFR therapy because mutations in the KRAS gene are associated with poor response to anti-EGFR therapies (Bihl et al. 2012). In some KRAS negative individuals, MET gene amplification can confer resistance to anti-EGFR therapy. Hence, MET inhibitors are necessitated in individuals with secondary resistance to anti-EGFR therapies in CRC (Bardelli et al., 2013).

### 3.3. Glioblastoma multiforme (GBM)

Glioblastoma multiforme (GBM) is the most common primary brain tumor in adults. It represents the highest grade of gliomas associated with poor prognosis and short median survival range despite surgery, radiotherapy, and chemotherapy. Research on gliomas has revealed unique markers to specific histological types or to different grades of malignancies. A cohort study for Le Mercier et al. evaluated 100 GBM samples to try and classify GBM cases using only three markers (by immunostaining) EGFR, PDGFRA, and p53. The results were categorized into two subtypes as follows: “Classical-Like” (CL); characterized with positive EGFR staining and negative p53 and PDGFRA staining. The second “Proneural-Like” (PNL) subtype; characterized with positive p53 and/or PDGFRA staining. At the prognostic level, the overall survival was higher for patients belonging to PNL subtype than those belonging to CL. In addition, radiotherapy treatment alone considerably improved the overall survival for patients belonging to the PNL subtype (Le Mercier et al., 2012).

The epidermal growth factor receptor (EGFR) and platelet-derived growth factor receptor  $\beta$  (PDGFR $\beta$ ) are considered hallmarks in GBM tumor progression. As stated in Furnari's study above, half of the RTK cases detected were due to amplifications and/or mutations in EGFR. And according to Gursel et al. and Vivanco et al., *EGFRvIII* mutation was most notable, since it expressed an active truncated receptor showing no response to any external ligand with resistance to treatment (by TKIs). In a recent study conducted by Camorani and the corresponding authors, *EGFRvIII* mutants were detected and it was determined through thorough analysis that *EGFRvIII* mutants escape therapy by showing dependence on PDGFR $\beta$  signaling. The rationale of combining therapies in blocking both pathways shows some promise to the future of this disease (Camorani et al., 2015). In yet another recent study conducted by Jones et al., *EGFRvIII* mutants were associated with an increased VEGFR2 expression. This increased expression prevented cellular senescence and promoted progression into cell cycle (cancer). Luckily, VEGFR-selective tyrosine kinase inhibitor cediranib inhibited VEGFR2-mediated actions and increased senescing cells preventing cancer (Jones et al., 2015).

Throughout the various studies conducted on GBM, *EGFRvIII* mutants showed the least responsiveness to TKIs. According to Nehoff et al., individual use of TKIs on GBM *EGFRvIII* mutants showed no effect on progression of cancer or survival; however, combination of both TKIs (specifically crizotinib and dasatinib) appeared to induce apoptotic cell death and polyploidy. In addition, the migration and invasion of GBM cells were reduced in vitro (Nehoff et al., 2015). Hence, recent experimentations always bring newer information to doctors and scientists in regard to GBM and better drugs- or combinations- are being provided (or at least suggested) which can help prolong patient survival and cut tumor progression.

### 3.4. Thymoma and thymic carcinomas

A thymoma is a rare anterior mediastinal tumor of the thymus gland. The course of disease can range from idle to aggressive, challenging the choice and course of treatment. Thymomas are classically classified into three histological types, A, B, and C (Marx and Muller-Hermelink, 1999). Molecular characteristics can also be attributed to this disease, but no relation exists between EGFR staining and histologic type. Historically,

thymomas and thymic carcinomas have been treated surgically with post-operative radiation therapy to reduce the risk of recurrence. However, induction chemotherapy and molecular targeted agents may also be appropriate for thymic carcinomas since its behavior resembles that of lung cancer instead of its ‘benign form’ (thymoma/invasive thymoma) (Komaki and Gomez, 2014).

The treatment of thymic carcinoma and invasive thymoma has typically involved induction chemotherapy of three cycles of cyclophosphamide, doxorubicin, cisplatin, and prednisone, to be followed by surgery. However, targeted therapy has led to the emergence of a variety of approaches to test for the ability to improve disease outcome. Molecular characterization of thymomas and thymic carcinomas based on overexpression of markers has improved the protocol of treatment in the majority of patients. For example, thymic carcinomas overexpress cKIT (CD117) and VEGFR. Thymomas and thymic malignancies are quite uncommon in their expression of the EGF receptor; their appearance is only limited to a small proportion of Asian patients (Pan et al., 2004; Sasaki et al., 2001; Kelly, 2013). To date, anti-EGFR inhibitors tested on the small proportion of patients has led to unsatisfactory and disappointing results. Cetuximab has been tested by Farina et al. and Palmieri et al. on thymic carcinoma patients and they both reported some activity from two single case reports from patients who overexpressed EGFR (Farina et al., 2007; Palmieri et al., 2007). Despite this information reported on thymic treatment, evidence from the literature suggests otherwise. EGFR TKIs and monoclonal antibodies cannot be recommended to treat patients with thymic malignancies (Kelly, 2014).

### 3.5. Head and neck squamous cell carcinoma (HNSCC)

Despite extensive study done on head and neck cancer - specifically squamous cell carcinoma (HNSCC) - EGFR remains the only non-chemotherapeutic molecular target that can be targeted with biologic therapy. More than 90% of HNSCC cases overexpress the EGFR gene; however, this overexpression is correlated with a bad outcome in patients. Currently, the only approved antibody to EGFR in HNSCC is cetuximab (Hansen and Siu, 2013). Cetuximab used alone as monotherapy or in conjunction with radio or chemotherapy prolonged patients' lives and increased overall survival. However, the response rate to adding cetuximab to radio or chemotherapy was only 10 to 20% which is much lower than initially expected. Many studies have investigated a mechanism of resistance to cetuximab therapy which was explained by many genetic and epigenetic factors. It was concluded by Wang et al., that persistent activation of PI3K/AKT/mTOR signaling (intracellular signaling pathway important in cell cycle regulation) in most HNSCC lesions might have led to the emergence of cetuximab resistance in treatment of these lesions (Wang et al., 2014). Another study conducted by Boeckx et al. displayed microarray results of HNSCC cases which showed resistant cells exhibiting RAS-MAPK pathway signaling. This contributed to the drug resistance initially present. An interruption in this pathway can help overcome the cetuximab resistance present in HNSCC cases (Boeckx et al., 2014).

## 4. Methods for EGFR mutations detection

The following section describes some sensitive and specific methods implicated in EGFR mutation detection. They are usually used to detect more commonly known mutations of EGFR in NSCLC patients, but are not restricted to these types of patients only.

### 4.1. Immunohistochemistry

Immunohistochemistry (IHC) is carried out using specific antibodies to detect specific mutations. In cases of lung adenocarcinoma, specific antibodies that are able to detect mutant EGFR proteins are used for the detection of the EGFR mutation using cytology and small biopsy specimens. IHC methodology can be used as a screening method to detect

patients that can benefit from TKI therapy, since EGFR mutation status is the best predictor of response to TKIs in lung adenocarcinoma (Hasanovic et al., 2012). Immunohistochemical analyses are routinely carried out in clinical laboratories since they can detect protein expression level or any protein modification. In clinical practice, this technique is the most widely used probably due to preserving tumor morphology and being more cost-effective than extracting DNA (Eberhard et al., 2008).

#### 4.2. Polymerase chain reaction

Polymerase chain reaction (PCR) is a rapid method used to amplify the amount of a particular DNA sequence. PCR can be extensively modified and has a wide range of application on the detection of EGFR mutations (Powledge, 2004).

Reverse Transcription PCR (RT-PCR) is used to determine the expression of a gene and does not require post-PCR sample handling, decreasing sample contamination.

Quantitative PCR (Real-time PCR) is a method used to quantify the amount of PCR product in a sample. This technique uses fluorescent dyes containing DNA probes, such as TaqMan, to measure the amount of product (Valasek and Repa, 2005).

The mutant-enriched PCR is a rapid and sensitive technique which can detect one mutant gene among a pool of wild type genes (around  $10^3$  to  $10^4$  copies). It eliminates the pool of wild type genes and enriches the mutated genes in a two-step PCR reaction. Several data have indicated that this assay could be used in pleural effusion for EGFR mutation screening in inoperable advanced NSCLC patients (Obradovic and Jurisic, 2012).

#### 4.3. Direct DNA sequencing

Direct sequencing of the PCR product is the most commonly used method to study the mutational state of EGFR. The two main drawbacks that exist are low sensitivity and risk of contamination in handling post-PCR products (Angulo et al., 2012). In addition, length of procedure, amount, and type of sample limit the effectiveness of this technique in modern clinical labs.

#### 4.4. High-performance liquid chromatography (HPLC)

Denaturing high performance liquid chromatography (DHPLC) is another method used in the detection of EGFR mutation. In a study conducted by Jänne, NSCLC specimens were analyzed by HPLC on the Transgenomic WAVE HS system (Jänne et al., 2006). This is a rapid method for EGFR mutation screening with 100% sensitivity and without false negatives. It can also detect clinically relevant mutations in small diagnostic specimens. In the study performed by Jänne, 178 NSCLC specimens were screened for mutations in exons 18 to 21 of EGFR and were analyzed by a DNA endonuclease, SURVEYOR, which cleaves mismatched heteroduplexed DNA. For this analysis, both frozen and formalin-fixed, paraffin-embedded (FFPE) tumor specimens were prepared (Jänne et al., 2006). Results as per the study showed that in comparison with sequencing, the sensitivity and specificity of the present method were 100% and 87%. SURVEYOR analysis detected 7 (4%) mutations that were not previously detected by direct sequencing.

Tan Min Chin developed a partially denaturing HPLC (pDHPLC) assay in order to detect a large range of sequence variants with high sensitivity in an inexpensive and standardized manner. The results also showed a better detection limit and lower cost and time requirement than direct sequencing (Chin et al., 2007).

#### 4.5. PCR, Scorpion Amplified Refractory Mutation System technology (SARMS)

This technique is highly selective in detecting a low percentage of a mutant allele in a background of wild-type DNA. The principle of this

method utilizes two technologies, ARMS and Scorpions – for detection of mutations in real-time PCR. Mutation-specific amplification is achieved by ARMS using Taq DNA polymerase. Specific mutated sequences are amplified. The detection of these amplified sequences is done by Scorpions. They are bifunctional molecules containing a PCR primer covalently linked to a probe (that holds a fluorophore). In other words, it signalizes the presence of a mutation (EGFR RQV PCR Kit Handbook, 2010).

After reviewing the literature, we deduced that most of these methods are not used alone. Many combinations were used to detect EGFR mutations. Immunohistochemical analysis (IHC) was not used individually as a single method, but very often in combination with direct sequencing and/or fluorescence in situ hybridization (FISH). Also, HPLC was analyzed in conjunction with direct sequencing, or with some additional methods like PCR, Scorpion Amplified Refractory Mutation System technology (SARMS) (Qin et al., 2011; Obradovic and Jurisic, 2012). In light of EGFR detection methods, methods with high sensitivity, such as mutant-enriched PCR assay, DHPLC, and PCR, Scorpion ARMS are being used in the field of gene mutation analysis.

### 5. Conclusion

Many cancers have been characterized molecularly by specific biological markers. The importance of such characterization holds in its potential to change a designated prognosis. However, other markers, if present, change that same prognosis into a bad outcome.

In both lung and colorectal cancer, EGFR positivity is correlated with a better outcome than if absent. Gefitinib and erlotinib are simple anti-EGFR TKI therapies that can counteract lung cancer (NSCLC); cetuximab and panitumumab are also anti-EGFR monoclonal antibodies that can counteract colorectal cancer. However, KRAS positivity can hinder this process and render the therapy ineffective in both types of cancer patients. Other mechanisms have also been drawn to show the emergence of drug resistance.

In glioblastoma multiforme, *EGFRvIII* mutation is the most prominent mutant that shows resistance to TKIs. In these mutants, *EGFR* activation is through mutation or deletion in the extracellular (EC) domain of the receptor. EGFR EC alterations specific to gliomas are poorly inhibited by the EGFR inhibitors which target the active kinase conformation. The target conformation of the EGFR inhibitors is different in glioblastoma than in lung cancer rendering all therapies useless. Newer drugs and newer combinations are being discovered that can help limit cancer proliferation in GBM.

Thymic malignancies are poor in their EGFR expression, but new data shows some sensitivity to anti-EGFR therapy. However, in reference to the literature, EGFR TKIs and monoclonal antibodies are not recommended to treat patients with thymic malignancies.

Lastly, EGFR positivity in head and neck squamous cell carcinoma portrays a bad outcome in patients because of the many resistant pathways present in that disease. Cetuximab combined with radio or chemotherapy is not enough to stop the progression of the disease and can minimally prolong overall survival.

It is important to mention that the advancement in technology is supporting the detection of EGFR mutations that will, in special clinical and surgical situations, eliminate the need for a re-biopsy to be performed. The news of the circulating tumor cells and cell-free DNA analysis are very promising and encouraging in terms of sensitivity levels of detection and are, therefore, considered as the future of biomarkers analysis and patient follow-up. *EGFR*, is only one of the best examples to be cited.

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