Influence of polymorphisms on EGFR targeted therapy in non-small-cell lung cancer

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1. ABSTRACT

Non-small-cell-lung cancer (NSCLC) is the leading cause of cancer-related deaths. However, chemotherapy has reached a therapeutic plateau and deals with significant toxicity. Novel anticancer treatments to neutralize specific molecules or genes involved in cancer development (“targeted-therapy”) are being developed to reduce side-effects and improve outcome. The epidermal-growth-factor receptor (EGFR) is over-expressed in NSCLC and emerged as an attractive target. Two classes of anti-EGFR agents (tyrosine-kinase-inhibitors and monoclonal antibodies) have shown clinical activity, depending on EGFR mutations and expression. However, clinical outcome, including tolerability, can not always be explained by these biomarkers. Thus, the identification of novel biomarkers is a viable area of research. Germline polymorphisms can be easily assessed, and polymorphisms in EGFR, AKT1 and ABCG2 have been correlated with outcome and toxicity in NSCLC patients given anti-EGFR therapies. However, there is lack of unanimity in findings, influenced by differences in study design/analysis, and the prognostic/predictive role of these polymorphisms needs to be evaluated within prospective studies. Finally, there is a critical need to conduct more studies on the relation of genotype with drug concentration/activity.

2. INTRODUCTION

Non-small-cell lung cancer (NSCLC) is the most common form of lung cancer, representing 85% of all lung cancer cases. Approximately two-thirds of NSCLC patients are diagnosed at an advanced stage (1), for which platinum-based regimens are standard first line treatment (2). However, the currently approved NSCLC treatment of platinum-based chemotherapy has shown limited efficacy and significant toxicity. Pooled data from older randomized trials of cisplatin-based chemotherapy versus best supportive care showed that cisplatin-based chemotherapy was associated with a modest improvement in overall survival (OS) (3). In more recent randomized trials, new cytotoxic drugs such as paclitaxel, docetaxel, vinorelbine, or gemcitabine in combination with a platinum compound have shown an absolute 15-20% improvement of survival in favor of chemotherapy vs. best supportive care. In particular, the one-year survival rate for best supportive care was 11-17% vs. 30-35% for chemotherapy, which prolonged median survival by 3-4 months (4). However, none of the last generation doublets was shown to be superior to the others and they all seemed to have reached the therapeutic plateau, with objective response rates of 30% to 40%, median survival time of 8 to 10 months, and 1-year survival rate of 30% to 40% (5). Indeed, a four-arm randomized phase III trial demonstrated no substantial
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differences in response rate, time to progression (TTP) and OS among paclitaxel (24-hour infusion)-cisplatin, docetaxel-cisplatin, paclitaxel-carboplatin and gemcitabine-cisplatin combination (5). The dose limiting toxicity profile of these regimens, as well as response rates not exceeding 40%, warrant novel strategies and new combination regimens against NSCLC.

Recent advances in our understanding of the molecular basis of NSCLC have enabled the development of new, rationally designed, targeted antitumor agents. In particular, the epidermal-growth-factor receptor (EGFR) pathway has emerged as the major target for the inhibition of NSCLC progression, and two main categories of EGFR-targeting therapeutic agents (tyrosine kinase inhibitors (TKI) and monoclonal antibodies) are being actively investigated in multiple clinical trials as a single agent or in combination with other agents (http://www.clinicaltrials.gov/).

The EGFR-TKIs gefitinib and erlotinib have been approved for the treatment of patients with locally advanced or metastatic NSCLC after failure of at least one prior chemotherapy regimen (6,7). Furthermore, gefitinib has been recently registered in Europe for the first-line treatment of patients with EGFR activating mutations (8). This approval is based on the data of the Phase III IPASS study, which exceeded its primary objective, demonstrating superior progression-free survival (PFS), greater objective response rate (ORR), improved tolerability and significant quality of life benefits for gefitinib compared to carboplatin/paclitaxel doublet chemotherapy in clinically selected first-line patients in Asia (9). In particular, PFS was significantly longer for gefitinib than doublet chemotherapy in patients with EGFR mutation positive tumors, and significantly longer for doublet chemotherapy than gefitinib in patients with EGFR mutation negative tumors. These results represent a milestone toward personalized medicine in NSCLC oncology.

However, 1) identification of additional factors could help in adapting individualized therapy especially for patients with a low frequency of somatic mutations (i.e. Caucasians), 2) gefitinib and erlotinib have also antitumor activity in some patients whose tumors don’t carry drug-sensitizing mutations in the EGFR gene (10), and 3) in the IPASS trial gefitinib also demonstrated a more favourable tolerability profile than chemotherapy, but there is still a large and unpredictable interindividual variability in toxicity of anti-EGFR agents. Therefore, several studies focused on other potential molecular biomarkers to predict the responsiveness and toxicity to EGFR inhibitors. Increased copy number of the EGFR gene may be one such marker (11), as may be the presence of amphiregulin, which is a ligand that binds to and activates EGFR (12). Similarly, emerging data suggest that resistance to EGFR-inhibition may be also due to other mutations in EGFR, as well as activation of proteins downstream of the receptor (K-Ras), tumor dedifferentiation (so-called epithelial-mesenchymal-transition, EMT), and other cell surface proteins, such as cMET (13-17). Nonetheless, all these changes do not completely explain the variable clinical outcomes, and identification of other biomarkers of sensitivity/resistance may help in optimal patient selection.

Assessing germline genetic polymorphisms as either predictive or prognostic markers is very appealing, especially in the advanced NSCLC setting, when diagnosis is usually done from small needle biopsy samples and tumors are either not resected or resected after neoadjuvant therapy, so that the handling of tumor material can be problematic. Polymorphisms are inherited genetic variants harboured by all the cells of the body. A genotype represents a static value unable to change in response to a different situation, such as exposure to chemotherapy, and it may not reflect all changes in tumor DNA, such as loss of heterozygosity. However, previous studies showed no differences in SNPs analyzed in tumor and normal tissues (18). Therefore, their analysis can be easily performed in blood tissue and is easier to adopt in the routine clinical setting than tumor gene expression arrays, which need core needle biopsies of patient’s tumors with immediate freezing, laser microdissection and subsequent sophisticated infrastructure.

Several germ-line DNA variations of EGFR and other genes have been associated with clinical outcome and this review focuses on the relationship between these candidate germline polymorphisms and the response and toxicity to EGFR inhibitors in NSCLC.

2.1. EGFR pathway

One of the most important mechanisms in signalling pathways in cells is the phosphorylation of proteins carried out by protein kinases (19). These proteins are involved in the regulation of cell proliferation, migration, differentiation, metabolism and apoptosis. In particular, tyrosine kinases, which catalyze the phosphorylation of tyrosine amino acid residues, are highly regulated in the cell as they have important regulatory effects in cell homeostasis and signalling pathways. There are two classes of tyrosine kinases: receptor tyrosine kinases and cellular tyrosine kinases. Receptor tyrosine kinases have an intracellular catalytic tyrosine kinase domain, a hydrophobic transmembrane domain and an extracellular ligand binding domain. Dimerization of the two receptor tyrosine kinases occurs when the ligand bind to the receptors. This results in the phosphorylation of the tyrosine residues of the intracellular catalytic domains which leads to an active conformation and results in the activation of signalling pathway within the cell.

EGFR, also known as HER1 or ErbB1, is a member of the ErbB receptor tyrosine kinase family (Figure 1A) and is expressed in almost all adult human tissues, with the exception of hematopoietic cells (20). This glycoprotein of 170-kD (1186 amino acids), encoded by a gene in the short arm of chromosome 7 (7p12.1-12.3), consists of an extracellular ligand-binding domain, a hydrophobic transmembrane domain, and an intracellular tyrosine kinase (TK) domain. Several ligands are known to bind to the EGFR, including EGF, transforming growth factor-alpha, amphiregulin, heparin-binding EGF, betacellulin, and epiregulin. Activation of this pathway
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Figure 1. A. The members of the HER family are: HER1, also known as EGFR (epidermal growth factor receptor) and ErbB1; HER2, also known as c-neu and ErbB2; HER3, also known as ErbB3; and HER4, also known as ErbB4. B. Receptor dimerization, or pairing, is an essential requirement for HER function and for the signaling activity of all HER receptors. This process can occur between 2 different receptors from the HER family (heterodimerization, e.g., HER1 and HER3) or between 2 of the same receptors (homodimerization, e.g., HER1 and HER1). Stimulation by a specific ligand confers a specific dimerization profile that is tissue specific or tumor specific. Dimerization results in activation of the kinase domain, transphosphorylation, and the induction of intracellular signaling cascades that mediate cell growth and survival. Two important signaling pathways activated by the HER family dimers are the PI3K/Akt pathway that promotes tumor cell survival, and the mitogen-activated protein kinase (MAPK) pathway that stimulates proliferation.

occurs via extracellular ligand binding to an EGFR monomer, inducing a conformational change and leading to receptor dimerization (Figure 1B). EGFR can form a homodimer with another EGFR monomer or a heterodimer with another receptor of the ErbB family. Dimerization induces TK domain activation and autophosphorylation of the tyrosine residues. The activated kinase phosphorylates other proteins, evoking cellular proliferation, migration, differentiation, inhibition of apoptosis, angiogenesis, and metastasis. The activating ligand and coreceptor to which EGFR dimerizes, determines which signaling pathway gets activated, while the signaling is “switched off” by internalization of the receptor/ligand complexes. Main pathways include the mitogen-activated protein kinase (MAPK) PI-3K/Akt and signal transducer and activator of transcription (STAT) pathways. The Ras-Raf-MEK-MAPK pathway stimulates cell proliferation, angiogenesis, inhibits apoptosis, and increases metastasis. The PI-3K/Akt pathway affects cell survival, metabolism, and proliferation, and inhibits apoptosis. The STAT-pathway also regulates the process of cell proliferation, differentiation and apoptosis (21).

2.2. EGFR targeted therapy

High EGFR expression is common in a number of epithelial tissues and in various solid tumor types, where EGFR overexpression correlates with more aggressive disease, poorer prognosis and reduced radio-/chemosensitivity (22-24). In particular, overexpression of EGFR is detectable in 40-80% of NSCLC, where it is more likely to occur in squamous cell carcinoma (70%), followed by adenocarcinoma (50%) and, to a lesser extent, in large-cell carcinoma (25). EGFR plays a crucial role in cellular proliferation, differentiation, apoptosis and survival (26).

Against this background, EGFR was identified as an attractive target for development of novel anticancer drugs. There are two classes of anti-EGFR agents with clinical activity, the monoclonal antibodies directed at the extracellular domain of the receptor, preventing ligand-dependent or independent activation and downstream signalling, and the EGFR-TKIs, orally available, low molecular weight compounds that compete with ATP for binding to the receptor’s intracellular TK pocket, blocking the catalytic activity and autophosphorylation and the following cellular effects (26). Both classes target the same receptor and the subsequent downstream effects of the EGFR-pathway, but their mechanism of action and specificity are different, and may contribute to the observed differences in efficacy and toxicity profiles, as well as when they were combined with traditional chemotherapeutic agents.

2.2.1. Tyrosine kinase inhibitors (TKIs)

TKIs targeting EGFR tyrosine kinases used in NSCLC are listed in Table 1. The main focus of the present review will be on gefitinib and erlotinib, which have been already approved for the treatment of patients with locally advanced or metastatic NSCLC after failure of at least one prior chemotherapy regimen, while gefitinib is also registered for first-line treatment of patients with EGFR activating mutations (6-8). They are orally bioavailable and they selectively and reversibly bind to the ATP-binding site of the EGFR intracellular TK domain. They have a common chemical backbone structure and show similar disposition characteristics in humans after administration, with a similar bioavailability, of approximately 60% (27,28). However, gefitinib and erlotinib have wide pharmacokinetic variability in cancer patients, and several pharmacokinetics parameters of gefitinib and erlotinib are different (27,29,30). Administration of erlotinib at approved daily dose (150mg) achieved approximately 3.5 fold higher steady-state plasma concentration than gefitinib with the recommended dose (250mg). Food intake and administration of the drugs with food might also increase erlotinib bioavailability. Therefore, gefitinib has lower bioavailability and higher systemic clearance than erlotinib. In vitro studies also showed that erlotinib is less susceptible than gefitinib to metabolism by major liver enzymes and higher plasma erlotinib exposure is achieved despite administration of a lower erlotinib daily dose when compared with gefitinib (30). Finally, the approved erlotinib dose is administered at its maximum tolerated dose while the gefitinib dose is one third of its maximum tolerated dose (31). Gefitinib and erlotinib may also have different drug-drug interaction properties. In particular, it has been shown that administration of a single dose of rifampicin (a potent CYP3A4 – isoenzyme involved in the
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Table 1. Tyrosine kinase inhibitors (TKIs) of the EGFR tyrosine-kinase family used in NSCLC

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Tyrosine kinase target</th>
<th>Cancer target</th>
<th>Clinical status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gefitinib (Iressa, ZD1839)</td>
<td>EGFR</td>
<td>NSCLC, breast</td>
<td>Approved</td>
</tr>
<tr>
<td>Erlotinib (Tarceva, OSI774)</td>
<td>EGFR</td>
<td>NSCLC</td>
<td>Approved</td>
</tr>
<tr>
<td>Lapatinib (Tykerb, GW572016)</td>
<td>ErbB1, ErbB2</td>
<td>NSCLC, breast, gastric</td>
<td>Approved (breast)</td>
</tr>
<tr>
<td>Canertinib (CI 1033)</td>
<td>EGFR, ErbB2, ErbB3, ErbB4</td>
<td>NSCLC, breast</td>
<td>Phase I/I</td>
</tr>
<tr>
<td>EKB-569</td>
<td>EGFR, ErbB2</td>
<td>NSCLC, colorectal</td>
<td>Phase II</td>
</tr>
<tr>
<td>BIBW2992</td>
<td>EGFR, ErbB2</td>
<td>NSCLC, breast</td>
<td>Phase II</td>
</tr>
</tbody>
</table>

Table 2. Monoclonal antibodies directed against EGFR used in NSCLC

<table>
<thead>
<tr>
<th>Agent</th>
<th>Type</th>
<th>Clinical status</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMC-C225 (cetuximab/erbitux)</td>
<td>Chimeric IgG1</td>
<td>Approved</td>
</tr>
<tr>
<td>ABX-EGF (panitumumab)</td>
<td>Human IgG2</td>
<td>Approved (colon)</td>
</tr>
<tr>
<td>EM7 2000 (matuzumab)</td>
<td>Humanized IgG1</td>
<td>Phase II</td>
</tr>
<tr>
<td>MDX-447</td>
<td>Humanized</td>
<td>Phase I/I</td>
</tr>
<tr>
<td>TheraCIM h-R3</td>
<td>Humanized</td>
<td>Phase I</td>
</tr>
<tr>
<td>Mab 806</td>
<td>Anti-EGFR VIII</td>
<td>Phase I</td>
</tr>
</tbody>
</table>

metabolism of gefitinib - inducer) significantly reduces gefitinib systemic exposure by 83%, while administration of irtraconazole (a potent CYP3A4 inhibitor) significantly increases it by 78% (32). Erlotinib is extensively metabolized, predominantly by CYP3A4, and to a lesser extent by CYP1A2 and the inducible isof orm CYP1A1 (30), with 75% of the metabolites excreted by the biliary system.

2.2.2. Monoclonal antibodies

Several mAbs directed against EGFR are FDA-approved for different tumor types, while others are in clinical trials (Table 2). The most extensively studied mAb is the chimeric IgG1 antibody cetuximab, whose binding to EGFR, competitively inhibits EGF binding, thereby blocking EGFR activation and promoting receptor internalization and degradation (33). Phase II and III clinical trials have shown promising results in the first-line treatment of advanced NSCLC. In particular, the phase III FLEX trial demonstrates that cetuximab in combination with vinorelbine/cisplatin improved the overall survival (OS) in untreated advanced NSCLC patients expressing EGFR compared to chemotherapy alone (11.3 vs 10.1 months; \(P=0.044\)) (34).

Another Phase III trial (BMS099) showed that adding cetuximab to the taxane/carboplatin combination marginally prolonged PFS (4.40 vs 4.24 months; \(P = 0.236\)). However, the primary endpoint, i.e. significant improvement in PFS, was not achieved. Similarly, the difference in OS did not reach statistical significance, while the only significant improvement was in ORR overall response rate (25.7% vs 17.2%; \(P=0.007\)) (35). However, although no statistically significant difference was observed in the PFS, there was a 1-month improvement in the OS after cetuximab addition. Therefore, this agent is recommended by NCCN in combination with vinorelbine/cisplatin for advanced NSCLC patients expressing EGFR (36). Furthermore, cetuximab seems to be safe in use, and therefore it may be a worthwhile option for patients who are not optimal candidates for other treatments (37).

3. Polymorphisms

Polymorphisms are inherited differences in DNA that are stable and found at a frequency of >1% among the individuals in a population. The simplest type is the single nucleotide polymorphism (SNP), which is a single base difference between genome sequences that occurs approximately every 1 kb in the human genome. Additional types of polymorphisms are represented by variable number of tandem repeats, also known as minisatellites, which consist of multiple copies of repeated DNA sequences (0.1–10 kb) distributed within the human genome, and microsatellite repeats, a simpler but more common variant of minisatellites, in which a sequence of up to four nucleotides are repeated many times (38). Genetic polymorphisms are often associated with reduced activity of the encoded protein, although there are allelic variants that encode proteins with enhanced activity (39).

Therefore, polymorphisms can be a sensitive indicator of biological factors that affect both 1) response of the tumor to the treatment, either in terms of tumor shrinkage or survival benefit (predictive factors), and 2) patients’ outcome, independently from the type of administered treatment (prognostic factors). Furthermore, polymorphisms of drug metabolizing enzymes, such as thiopurine S-methyltransferase and uridine diphosphate glucuronyltransferase have already been included in FDA-approved tests to predict toxicity of 6-mercaptopurine and irinotecan, respectively (40).

Polymorphisms in EGFR, AKT1 and ABCG2 have been correlated with outcome and toxicity in NSCLC patients given anti-EGFR therapies (41,42). However, there is lack of unanimity in findings, influenced by small sample sizes, and differences in study design/analysis. Additionally, there is a critical need to conduct more studies to establish univocal genotype-to-phenotype relationships and validate the screening methodologies, in order to define the best strategy to stratify patients on the basis of their likelihood of response and drug tolerability. For example, the best strategy for the screening of dihydropyrimidine dehydrogenase (DPD) deficiency in patients treated with fluoropyrimidines remains an unsolved question, despite countless clinical reports evidencing the deleterious impact of DPD impairment in patients on fluorouracil or capecitabine intake. This is mostly the result of unclear genotype-to-phenotype relationships with DPD epigenetic regulations, along with
the complete lack of consensus about the best technical way to evaluate DPD status, that has prevented the health authorities to recommend DPYD genetic testing so far (43,44). Contradictory genotype-to-phenotype relationships have been also reported when studying the role of cytidine deaminase (CDA) A79C polymorphism and outcome after gemcitabine (45,46). Besides, ethnicity could play a major role in the incidence of different polymorphism, such as for CDA 79A/C and 208G/A SNPs in Caucasians and African and Asian population, respectively (47).

Finally, more comprehensive prospective studies to determine whether genetic polymorphisms are independently predictive with anti-EGFR therapy or are simply correlated with other molecular or clinical prognostic factors are warranted. Therefore, this review will discuss the main results observed with candidate polymorphisms markers of outcome and toxicity after anti-EGFR therapy.

### 3.1. Polymorphisms affecting EGFR-TKIs

Several studies have been performed in order to answer the question whether polymorphisms in EGFR and EGFR-related genes can predict the response and toxicity to EGFR-TKI therapy in NSCLC (Table 3). Most analyses focused on polymorphisms in the region which regulates the expression of the EGFR gene. The regulatory regions of EGFR are within the S’-flanking region and intron-1, and both the EGFR -216G/T and -191CA polymorphisms are located in the transcriptional start site region of the promoter, wherein multiple nuclear regulatory affinity sites are located (48). In particular, the -216G/T polymorphism is located at an important binding site for the transcription factor Sp1, and the T allele is associated with increased EGFR mRNA expression (49). Similarly the -191CA variant has been associated with increased EGFR promoter activity and gene expression, while the A-G substitution of the R497K SNP at codon 497, resulting in a substitution of arginine by lysine, is associated with decreased EGFR activity (50,51).

The intron-1 also contains a highly polymorphic region, with 14-21 C4 nucleotide repeats (52). In vitro and in vivo studies showed that transcription activity of EGFR is inversely correlated with the length of the C4-repeat, with the longer allele 21 inducing an 80% reduction in the gene expression compared with the shorter allele 16 (53). Additionally, a constant decline of intratumoral EGFR protein expression was also observed to be associated with the increase in allele length (54).

Of note, all these polymorphisms have significant ethnic variations, with the polymorphic variants associated with increased EGFR production rare in Asians in comparison with the other populations (49,50).

Finally, other polymorphisms in the EGFR pathway include variations in AKTI, as well as in the genes encoding for CYP-enzymes and ABCG2 transporter, which might be involved in EGFR-TKIs metabolism and efflux, respectively.

#### 3.1.1. Clinical outcome

Most studies suggested that NSCLC patients treated with EGFR-TKI inhibitors respond better to therapy when they carry the short EGFR-C4 repeat genotype. Firstly, Ichihara et al. analyzed the relation between genetic factors and clinical outcome in 98 NSCLC Japanese patients treated with gefitinib (55). These patients were screened for EGFR/k-ras mutations, EGFR copy-number and the EGFR polymorphisms, including intron-1 CA repeat, -216G/T and -191CA. As reported in most studies on biomarkers of EGFR-TKIs activity, EGFR mutations were predictive factors of sensitivity to gefitinib, OS and PFS. Regarding polymorphisms, OS was prolonged in patients with the shorter C4 alleles compared with those with the long alleles (defining long C4 repeats equal or greater than 19, or the sum of two alleles greater than 39, and short C4 repeats as less than 19, or the sum of two alleles less than 39) among patients with EGFR activating mutations. This difference however was not significant (P=0.13).

In a similar study, the association of gefitinib responsiveness with the CA-repeat polymorphisms and EGFR mutations was investigated in 86 Korean patients with advanced NSCLC (56). In this study, short C4 was defined as the sum of both alleles <or =37, while long C4 was defined as sum > or =38. Again, the EGFR activating mutations were associated with response and OS. However, these mutations were more frequent in patients with high C4 repeats, but there was a trend toward higher response rate in patients harboring the short C4 repeats. Furthermore, short C4-repeat status was associated with better response and longer TTP, independent of EGFR mutations.

### Table 3. Germline genetic variants in EGFR and EGFR related genes

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Effect</th>
<th>Location</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR C4 repeat</td>
<td>EGFR gene transcription declines with increasing number of CA repeats</td>
<td>Chrom. 7, Intron 1</td>
<td>53</td>
</tr>
<tr>
<td>EGFR R497K A/G</td>
<td>The variant leads to substitution of Arginine by Lysine which is associated with decreased EGFR activity</td>
<td>Chrom. 7, Exon 13</td>
<td>50,51</td>
</tr>
<tr>
<td>EGFR -216 G/T</td>
<td>T allele associated with higher promoter activity</td>
<td>Chrom. 7, Promoter</td>
<td>49</td>
</tr>
<tr>
<td>EGFR -191 CA</td>
<td>A allele associated with increased protein production</td>
<td>Chrom 7, Exon 1</td>
<td>50,51</td>
</tr>
<tr>
<td>EGFR -161 AG</td>
<td>G allele associated with increased EGFR levels</td>
<td>Chrom. 4, 5’-UTR</td>
<td>89</td>
</tr>
<tr>
<td>AKTI-SNP4</td>
<td>A allele associated with reduced AKTI mRNA expression</td>
<td>Chrom. 7, Exon 13</td>
<td>72,81,82</td>
</tr>
<tr>
<td>ABCG2 421 GA/Q (Q41K)</td>
<td>A allele associated with reduced transport of EGFR TKIs</td>
<td>Chrom. 4, Exon 5</td>
<td>78</td>
</tr>
<tr>
<td>ABCG2 -1562 C/T</td>
<td>T allele associated with lower ABCG2 expression</td>
<td>Chrom. 4, Promoter</td>
<td>72,81,82</td>
</tr>
<tr>
<td>ABCG2 1143 C/T</td>
<td>T allele associated with lower ABCG2 expression</td>
<td>Chrom. 4, Exon 4</td>
<td>72,81,82</td>
</tr>
<tr>
<td>FcGR2A 1G (Val158Phe)</td>
<td>Val158Phe variant results in Val to Phe substitution at position 158. Val is associated with stronger binding to IgG1 and more effective ADCC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FcGR2A 2G/A (His131Arg)</td>
<td>The variant results in His to Arg at position 131 and seems to mediate ADCC more effectively</td>
<td>Chrom. 1, Exon 5</td>
<td>95</td>
</tr>
<tr>
<td>FcGR3A 1G (Val158Phe)</td>
<td>Val158Phe variant results in Val to Phe substitution at position 158. Val is associated with stronger binding to IgG1 and more effective ADCC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FcGR3A 2G/A (His131Arg)</td>
<td>The variant results in His to Arg at position 131 and seems to mediate ADCC more effectively</td>
<td>Chrom. 1, Exon 4</td>
<td>95</td>
</tr>
</tbody>
</table>
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Nie et al. also reported higher response rate in Chinese patients with shorter CA-repeat status, defined as any allele less than equal to 16 (57). In this analysis they evaluated the relation between the EGFR polymorphisms R497K, intron-1 C4 repeats and the clinical outcome of 70 NSCLC patients treated with gefitinib. Their results also showed that patients with shorter CA repeats had higher EGFR expression and prolonged survival compared to those with high CA-repeat status. In contrast, no correlation has been found between the R497K polymorphisms and EGFR expression or clinical outcome.

Another study evaluated the EGFR 216G/T, -191C/A, intron-1 and R497K polymorphisms in 92 Caucasians affected by advanced NSCLC and treated with gefitinib (58). Shorter CA repeats (defined as “S”=16 or less) were associated with improved PFS and OS. The EGFR -216 G/T variant was also associated with longer PFS.

Similar results were reported by Tiseo et al., who analyzed 91 Caucasians NSCLC patients treated with gefitinib for EGFR mutations, K-ras mutations, EGFR gene copy number and C4 intron-1 polymorphisms (59). In agreement with previous data, gender, non-smoking status, skin toxicity and EGFR mutations were associated with clinical response. Furthermore, the non-smoking status and the intron-1 EGFR (CA)16 status, including at least one (CA)16 allele, were significantly correlated with survival.

However, no association of EGFR intron-1 CA repeats with clinical outcome was observed in the largest pharmacogenetic analysis in NSCLC Caucasian patients (N=175) treated with gefitinib, grouping patients both with 1) combined CA repeat length on both alleles of<35 versus>35, and 2) a CA repeat length on both alleles of <18 versus all other (60). The specimens of these patients were also analyzed for EGFR -216G/T and -191C/A polymorphisms, and patients with the G-C haplotype had significantly lower response rate.

In our analysis of 96 NSCLC Caucasians patients treated with gefitinib, we also observed that EGFR activating mutations were significantly correlated with response, and longer TTP and OS, while the EGFR intron-1 CA (for which patients were classified as S/S, L/L and S/L if the number of repeats was ≤16 on both alleles, >16 on both alleles and ≤16 in one allele and >16 in the other), -216G/T, -191C/A and R497K were not correlated with clinical outcome (61).

Finally, a recent study used a whole gene-based tag-SNP approach in order to investigate the association between different polymorphisms in EGFR and therapeutic outcome and survival in 84 advanced NSCLC patients treated with gefitinib (62). Only the novel EGFR polymorphisms rs2293347 (D994D) and intron-1 C4 were associated with therapeutic response. Indeed, the rs2293347 GG or shorter CA repeat genotype, defined as ≤16 CA repeats, had a significantly higher response than the rs2293347 G4 or A4 or the longer C4 repeat genotype. The rs2293347 GG genotype was also correlated with a longer PFS compared with the rs2293347 G4 or A4 genotype, whereas the clinical benefit was even more with the combination of rs2293347 GG and shorter CA-repeat status.

All these controversial findings might be explained by the small sample size and retrospective nature of most studies, by the interethnic differences and by the different definitions used for key variables, such as ‘short’ and ‘long’ intron-1 CA repeat, and ‘clinical outcome’, evaluating response, clinical benefit, TTP, PFS or OS. Furthermore, C4 repeat allele sum of greater than 35 was associated with improved OS in the absence of therapy with an EGFR-TKI, which is a reversal of expectations in TKI-treated patients, suggesting also the potential role of this polymorphism as prognostic factor (63).

Clinical response to EGFR-TKIs may also be influenced by changes affecting downstream EGFR signal transducers. The serine-threonine kinase Akt is a central player in the PI3K-oncogenic pathway, involved in anti-apoptosis, or pro-cell proliferation effects. Retrospective studies showed that patients with phospho-Akt-positive tumors had a better response, disease-control rate, and TTP, suggesting that gefitinib may be more effective with basal Akt activation (64,61). However, in the ONCOBELL prospective trial, only the EGFR FISH, and not Akt immunohistochemistry, predicted response to gefitinib (65). Recent studies discovered an oncogenic mutation in the AKTI subunit which stimulates Akt signalling and induces cellular transformation, but its rare incidence suggests that it may not play a role in NSCLC development or response to EGFR-TKIs (66). A candidate gene approach focusing on apoptotic pathways identified two functional polymorphisms (AKTI-SNP3 and SNP4) affecting the expression and activity of Akt (67). The haplotype including these polymorphisms was associated with lower protein levels in tissues from Caucasians, and contributed to the lowest apoptotic response of EDB-transformed lymphoblastoids to radiation (67,68). Our recent study in 96 Caucasians showed that the AKTI-SNP4 A/A genotype was associated with shorter OS (61). Given the small number (N=6) of patients harboring the AKTI-SNP4 A/A genotype, in order to evaluate whether other poor prognostic factors could potentially explain their short survival, we checked carefully their baseline demographic and biological characteristics, which were similar to the average of the studied population. Furthermore, at multivariate analysis, the AKTI-SNP4 polymorphism remained an independent predictive parameter of progression and death risk. However, a recent trial reported that other genetic variations in AKTI were associated with increased recurrence and significantly shorter survival in esophageal cancer patients treated with regimens including fluoropyrimidines, platinum compounds and taxanes, but not with gefitinib, suggesting that genetic variations in the PI3K/AKT pathway may be prognostic and/or predictive factors of drug response (69). In order to evaluate whether the AKTI-SNP4 polymorphism was a candidate biomarker predictive of drug activity or a prognostic factor, we used a population of advanced NSCLC who were treated only with pemetrexed or carboplatin-pemetrexed regimen,
without receiving EGFR-TKIs as salvage therapy. The lack of correlation between the AKT1-SNP4-A/A genotype and survival in these gefitinib-untreated patients suggested that it is not a prognostic factor, whereas it might be a predictive factor of gefitinib activity. Finally, to gain further insight into the mechanisms behind our findings, we performed in vitro studies showing a significant association with both AKT1 mRNA expression and gefitinib IC50s (Figure 2), in agreement with the clinical results. However, these results have still to be validated in a larger cohort of patients, in prospective multicenter trials, as well as additional case-control studies.

3.1.2. Toxicity

Treatment for advanced NSCLC is palliative in nature. In patients with a good performance status, first-line treatment with platinum-based combination chemotherapy should lead to prolonged OS and improvement in symptoms. However, these regimens are limited by drug toxicity and targeted therapies were developed also to reduce side-effects. The specificity of this class of agents for the target results in a much more favorable safety profile than most standard chemotherapy agents, with fewer non-specific toxicities and no hematopoietic effects. The major adverse effects specific to EGFR-TKIs are the development of a rash, primarily on the face, neck, and upper torso, and the diarrhoea. To date, little is known about the etiology of these effects, and there is a high level of interpatient variability. This could be due to the methods used to assess and categorize rash and diarrhoea, pharmacokinetic or pharmacodynamic differences, but also pharmacogenetic heterogeneity of patient populations (70). Therefore several studies evaluated the correlation between selected polymorphisms and toxicity induced by EGFR-TKIs.

Huang et al. focused on the genetic factors associated with skin rash in 52 patients with NSCLC treated with gefitinib, analyzing EGFR intron-1 CA repeat status and the EGFR SNPs -216C/T, -191C/A, and R521K. In this study, only the intron-1 CA repeat polymorphism was correlated with grade 2-3 skin rash, observed in 21% of patients with LL genotype (19-22 repeats), 31% S/L genotype (15-18 repeats) and 71% with S/S genotype (<15 repeats) (71). Of note, early grade-2/3 rash was correlated with tumor response, while the EGFR intron-1 CA repeat genotype was not significantly correlated with response (P=0.35).

Similarly, the EGFR -216 G/T variant was associated with a significantly higher risk of both rash and diarrhoea in 92 NSCLC patients treated with gefitinib (58).

However, other studies reported different data on the association between EGFR-TKI treatment with skin rash and diarrhoea. For example, an integrated analysis of genotypic/pharmacokinetic variability showed a strong association, independent from erlotinib plasma concentration, between diarrhoea and the two linked EGFR promoter polymorphisms (-216G/T and -191C/A) in 80 NSCLC, head-and-neck and ovarian cancer patients (72). In contrast, skin rash was associated with the intron-1 CA repeat polymorphism and erlotinib concentration (P=0.044). We observed similar results in our uniform population of 96 NSCLC patients treated with gefitinib, with grade >1 diarrhoea occurring significantly more frequently in patients harboring the EGFR -191C/A and A/A, the EGFR -216G/G and the EGFR R497K A/A variants. These results might be explained by the pathophysiology of anti-EGFR-induced diarrhoea, which is thought to result from excessive chloride secretion,
inducing a secretory diarrhoea. Therefore, the diarrhoea might result from the higher EGFR expression in the intestinal lumen associated with the EGFR promoter polymorphisms variants, as suggested by Rudin et al. (72). In contrast the A allele in the R497K polymorphism is associated with alterations in EGFR ligand binding, and studies in colorectal cancer tissues showed a decreased phosphorylation of EGFR, while no differences were detected for EGFR mRNA expression (73). However, the R497K polymorphism was also associated with decreased activation of c-Myc, whose activity is also downregulated by the Escherichia coli heat stable enterotoxin STA, a major causative agent of secretory diarrhoea (74).

Rudin et al. (72) also studied polymorphisms in ABCG2, CYP3A4, and CYP3A5, showing that the variants of the ABCG2 -15622C/T and 1143C/T polymorphisms were associated with lower ABCG2 production and higher erlotinib concentration. ABCG2 is a half transporter member of the major family of ATP-binding cassette (ABC) transporters. ABCG2 overexpression is commonly associated with resistance to a wide range of structurally and mechanistically unrelated anticancer agents including camptothecins, anthracyclines, and antifolates (75). Emerging data suggest that the EGFR-TKIs are able to interact with ABCG2. Of note, gefitinib is transported by ABCG2 at clinically achievable concentrations (≤1 µM), while at higher drug concentration (>1 µM) gefitinib is no longer a substrate but rather an inhibitor of the transporter (76). Therefore, ABCG2 expression has an important impact on gefitinib resistance phenotypes both in vitro and in vivo (77). Additionally, considering that 1) ABCG2 is highly expressed in the gastrointestinal tract where it plays a role in the regulation of the uptake of several xenobiotics and that 2) gefitinib is an orally active compound, one might expect also an important role for ABCG2 in the absorption and elimination of this drug.

Several common SNPs in the ABCG2 gene have been described that might have an important impact on ABCG2 protein expression, function and localization. In particular, the nonsynonymous SNP ABCG2 421C/A resulting in a glutamine to lysine amino acid change at position 141 (Q141K) has been associated with markedly decreased levels of ABCG2 protein expression and/or activity and with higher accumulation of both gefitinib and erlotinib (78). Interestingly, Cusatis et al. (79) reported a strong association between the ABCG2 421C/A polymorphism and diarrhoea in gefitinib-treated NSCLC patients. In particular, they showed that 7 (44%) of 16 patients heterozygous for ABCG2 421C/A developed diarrhoea, versus only 13 (12%) of 108 patients homozygous for the wild-type genotype. The authors suggested that the reduced protein levels and altered ATPase activity of the ABCG2 421C/A variant in the intestine might affect the oral absorption and/or elimination pathways of gefitinib thereby increasing the steady-state gefitinib plasma concentrations leading to the diarrhoea.

However, Rudin et al. (72) found no correlation between the ABCG2 421C/A polymorphism and diarrhoea or skin rash in erlotinib-treated patients. Also Akasaka et al. (80) found no association between this polymorphism and diarrhoea and gefitinib-treated Japanese NSCLC patients. Consistently, we did not find any association between the ABCG2 421C/A polymorphism and gefitinib-induced toxicity in a population of 94 Caucasians affected by NSCLC (81). In contrast, in the same population, we observed a correlation between the ABCG2 -15622C/T polymorphism and the ABCG2 (1143C/T, -15622C/T) haplotype with moderate-severe diarrhoea. These results are in line with the previously reported association of the ABCG2 TT haplotype with increased toxicity (any toxicity ≥grade 2) in sunitinib-treated patients (82), and together with the data on the association of this haplotype with increased erlotinib exposure (72) suggest that the TT genotype is associated with a lower expressed and/or less active ABCG2 protein, which thereby affects the elimination of TKIs and increases the drug-induced toxicity.

Finally, Rudin et al. (72) studied the common A-G transition within intron 3 of CYP3A4*1B, CYP3A4*1C, CYP3A5*1B, CYP3A5*2B, CYP3A5*3 as well as the common A-G transition in the 5′ regulatory region of CYP3A4 (CYP3A4*1B), which affected the activation or inactivation of several anti-cancer agents. CYP3A4 polymorphisms were marginally associated with skin rash. Individuals with lower CYP3A4 expression (A/A) were more likely to develop rash than those with higher CYP3A4 levels (A/G and G/G; P=0.077). Similarly, the CYP3A4*3 G polymorphism was also marginally associated with grade ≥2 rash (P=0.094, dominant model) and any grade diarrhoea (P=0.062).

3.2. Polymorphisms affecting anti-EGFR antibodies

Very little information is available about biomarkers which might predict cetuximab responsiveness and/or toxicity in NSCLC. The FLEX phase III trial, showing an OS benefit of cetuximab in addition to chemotherapy in first-line treatment of NSCLC, was performed in patients selected for EGFR expression (34). However, most studies have not shown an association between cetuximab activity and EGFR expression, as detected by immunohistochemistry (83). K-ras mutations are associated with decreased Response rates and an absence of survival benefit from EGFR monoclonal antibodies in colorectal cancer (84). However, in the Bristol-Myers Squibb 099 trial, which compared carboplatin plus paclitaxel with carboplatin plus paclitaxel and cetuximab in 676 patients with advanced NSCLC, the presence of K-ras mutations was not associated with a lower benefit from cetuximab (85).

Regarding polymorphisms potentially affecting clinical outcome after cetuximab, investigations in colorectal cancer and head and neck squamous cell carcinoma patients treated with cetuximab may provide some insight which should be useful also in lung cancer, as described in the following paragraphs. Indeed, cetuximab is approved for the treatment of metastatic colorectal cancer (in combination with irinotecan as second-line treatment of patients refractory to irinotecan, and as a single agent for patients with mCRC who cannot tolerate irinotecan) and of head and neck cancer (in combination with radiation
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therapy for treatment of locally or regionally advanced squamous cell carcinoma of the head and neck, and as a single agent for patients with recurrent or metastatic squamous cell carcinoma of the head and neck who have failed prior platinum-based therapy. Similarly, the fully humanized IgG2 anti-EGFR monoclonal antibody Panitumumab (table 2) is approved for the treatment of EGFR-expressing metastatic colorectal cancer patients who have failed prior therapy with fluoropyrimidine-, oxaliplatin-, and irinotecan-containing chemotherapy.

3.2.1 Clinical outcome

Studies of EGFR pathway polymorphisms associated with response to cetuximab have given contradictory results with disparate findings from a few small series of patients.

For instance, Goncalves et al. have shown that PFS and OS were significantly improved in colorectal cancer patients expressing the variant of EGFR R521K in exon 13 after treatment with cetuximab/irinotecan in a population of 32 EGFR-positive metastatic colorectal cancer patients (86). Structural analysis of the molecular interaction between the Fab fragment of cetuximab and the extracellular domain of EGFR revealed that amino acid exchanges at critical interaction sites dramatically influenced binding affinity not only of EGF itself but also of cetuximab. However, because the effect of an arginine-to-lysine exchange at codon 521 had not yet been tested, the interaction of the EGFR-R521K genotype with cetuximab binding affinity remains unresolved.

Graziano et al. have studied different genetic variants, including EGF 61A>G, EGFR -216G/T, 497G/A, intron-1 CA repeats, cyclin-D1 870A/G and Fc R IIIa-158VF and RIIIa 131G/A in 110 colorectal cancer patients treated with cetuximab and irinotecan. These patients were treated after second-line, irinotecan-based chemotherapy, with irinotecan with cetuximab, administered as weekly or every 2 weeks in 33 and 77 patients, respectively. The results showed a significant association between longer OS and EGF intron 1 S/S (defined as less than 17-repeat allele, while L-allele has ≥17 CA-repeats), and EGF 61G/G genotypes (87).

The EGF 61G allele is transcriptionally more active than the A allele and is found to be associated with upregulated EGF levels. EGF signaling may promote a number of regulatory factors, which enhance tumor aggressiveness; therefore, the observed favorable effect of the EGF 61G/G genotype may be counterintuitive. Furthermore, the EGFR-ERBB system displays complex tunings and the presence of alternative negative signaling regulators. At specific concentrations that vary between experimental systems, EGF has been shown to induce apoptosis and growth inhibition rather than the usual growth-promoting effect (88). According to such findings, it cannot be ruled out that a functional EGF genotype, which upregulates EGF levels, may play a favorable prognostic rather than predictive influence, explaining why the EGF 61G/G genotype was associated with improved OS and not with improved PFS or response rate/skin toxicity. Notably, similar findings have been reported by Ali-Osman et al. (89), who analyzed EGF 61A/G in 332 astrocytoma patients, with the G/G carriers having significantly better survival rates than the A/A carriers.

In contrast, Zhang et al. reported that the A allele of the EGF 61 A/G polymorphism was associated with better survival (90,91), while no correlation were detected for the EGFR intron-1 CA-repeat status, which was studied by subdividing patients into two groups: 16 carriers of both CA < 20 alleles, and 18 carriers of any CA ≥20 alleles, with five missing cases.

In the 39 metastatic colorectal cancer patients treated with single-agent cetuximab, Zhang et al. also showed that patients harboring the CCND1 A870A cyclin D1 polymorphic variant had a significantly shorter OS (90). The role of cyclin D1 might be explained by several studies which suggested that the blockade of EGFR tyrosine kinase activity by cetuximab (mAb225) leads to cell cycle arrest in the G1 phase. Cyclin D1 serves as a key cell cycle regulatory protein for cell G1–S phase transition and a study in head and neck squamous cell carcinoma cell lines showed an association between deregulated cyclin D1 expression and a decrease in the efficacy of EGFR-TKIs gefitinib (92). In addition, patients who responded to erlotinib showed a marked reduction of cyclin D1 protein expression, along with much higher erlotinib tissue levels, than unresponsive patients (93). However, the data on correlation of the cyclin D1 A870G polymorphism with clinical outcome in different cancer types and in different ethnic populations are controversial and the mechanism through which patients with the cyclin D1 AA genotype are resistant to cetuximab treatment is unclear. It is possible that patients with the A allele overexpress the cyclin D1 protein, and maintenance of cyclin D1 levels is critical for patient sensitivity to cetuximab, but further in vitro studies are warranted.

Other polymorphisms associated with PFS in the colorectal cancer patients enrolled in the IMCL-0144 study were the FCGR2A-H131R and FCGR3A-F158S variants (91). These data are of interest because recent studies demonstrate antibody-dependent cell-mediated cytotoxicity (ADCC) is one of the modes of action for rituximab and trastuzumab, and Fragment c (Fc) portion of IgG1 mAb has shown to induce ADCC (94). Therefore, Fragment-c-y-receptors (FcγR) play an important role in initiating ADCC and might affect cetuximab activity. Furthermore, López-Albaitero et al. (95) demonstrated that effector cells expressing the FcγR IIIa-158VV allele were significantly (P=0.0001) more effective than those expressing FcγR IIIa-158VF and FF alleles in mediating lysis of tumor cells. Combined analysis of these two polymorphisms showed that patients with the favorable genotypes (FCGR2A, any histidine allele, and FCGR3A, any phenylalanine allele) showed a median PFS of 3.7 months, whereas patients with any two unfavorable genotypes (FCGR2A arginine/arginine or valine/valine) had a PFS of 1.1 months (P=0.004).

However, no association with clinical outcome was observed in patients categorized according to the
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*FcγR IIIa-158 VF* polymorphism in the study by Graziano et al. (87).

These controversial results suggest that several genes differently expressed as a function of recognized genetic alterations are not well clarified yet, as well as the phenotypic characteristics that subsequently occur and can contribute to determine the different clinical behavior of tumors with different genotypic features, and to the response to different treatments.

### 3.2.2 Toxicity

Predictors of cetuximab toxicity have been investigated in a few studies. Beside treatment response, Graziano et al. have found that patients carrying the *EGFR intron 1 S/S* variants were more susceptible to develop grade 2-3 skin toxicity compared with *EGFR intron-1 L/L* carriers (87).

More recently, Klinghammer et al. have analyzed the association between cetuximab/docetaxel induced skin-rash and polymorphisms in the *EGFR* gene (96). In their Phase II study, enrolling 51 patients with recurrent or metastatic squamous cell carcinoma of the head and neck, they genotyped two genetic variants of *EGFR*, namely *EGFR-R521K*, and *EGFR intron-1 CA* polymorphism, using a length of ≤16 CA repeats in the shorter allele as cutoff for the definition of two genotype groups. Their findings demonstrated a significantly increased risk of skin toxicity in patients with the *EGFR-R521K* genotype (*G/G* \(P=0.024\)) as well as a trend toward a reduced risk of tumor progression for the same patients (*P=0.08*). In contrast, no correlation was observed between *EGFR-R521K* and OS, as well as between the *EGFR intron-1 CA* repeats variants and skin toxicity, PFS, or OS.

These different results about the association of *EGFR* polymorphism with skin toxicity may arise from differences in ethnic background and treatment regimens, and suggest that the potential value of *EGFR* polymorphisms in predicting efficacy under EGFR-targeting antibody treatment has to be validated in clinical trials including larger patient cohorts.

### 4. CONCLUSIONS/PERSPECTIVES

Clinical trials have demonstrated that EGFR inhibitors are effective for treatment of a subset of patients with advanced NSCLC.

*EGFR* and *K-Ras* mutations have been associated to sensitivity/resistance to the EGFR-TKIs in NSCLC, but do not account for all clinical outcomes. Similarly, the large interindividual variability in toxicity makes the identification of novel pharmacogenetic markers to screen patients an attractive prospect.

Germline polymorphisms are easy to assess and several polymorphic variants of *EGFR* and genes involved in anti-EGF agent activity, metabolism and transport, have been studied as predictors of outcome and toxicity.

The *EGFR intron-1 CA* repeat polymorphism has been the most extensively studied, and most data suggested that NSCLC patients treated with EGFR-TKIs carrying the shorter *CA* repeat alleles respond better to therapy. This polymorphism was also correlated with grade 2-3 skin rash, but other studies showed controversial results or suggested the role of polymorphisms in *ABCG2* to predict gastrointestinal toxicity. These observations however are reported by a few studies and warrant confirmation in larger populations. Similarly a few studies evaluated predictors of cetuximab responsiveness, and further studies, including prospective trials, are urgently needed.

Finally, future research on the personalization of use of anti-EGFR agents should also address 1) the reliability of use of a more accessible tissue (i.e., blood) compared to tumor, which raises the question if germline and somatic genotypes of drug transporters, enzymes of drug metabolism and targets are representative of gene expression level or functional status in the target tumor tissues, which are often not accessible for biomarker measurement, and 2) the clinical validation of a multiple-gene approach, since the single-gene approach has important limitations which need to be overcome to build a more robust approach to patients’ genotyping.

However, thanks to the technical advancement in the development of user-friendly genotyping platforms and the widespread availability of them to the research community, the pharmacogenetic approach to treatment personalization using multiple selected/validated biomarkers may become a reality. Through these technical and cultural advancements, hopefully we will be able to accelerate the transfer of basic research findings to clinical practice and improve the selection of NSCLC patients for anti-EGFR treatment by identifying both genetically high-risk subgroup for drug-resistance or toxicity, and patients more likely to respond to these treatments.

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### 6. REFERENCES

Polymorphisms as predictors of outcome to EGFR-targeted therapies


Polymorphisms as predictors of outcome to EGFR-targeted therapies


Polymorphisms as predictors of outcome to EGFR-targeted therapies


68. Enamian ES, Hall D, Birnbaum MJ, Karayiorgou M, Gogos JA. Convergent evidence for impaired AKT1-


71. Huang CL, Yang CH, Yeh KH, Hu FC, Chen KY, Shih LW, Chang CH, Yu CJ, Shih JY, Lin ZZ, Yang PC. EGFR intron 1 dinucleotide repeat polymorphism is associated with the occurrence of skin rash with gefitinib treatment. Lung Cancer 64(3), 346-351 (2009)


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http://www.bioscience.org/current/vol16.htm