Pharmacology and Clinical Development of New Molecularly Targeted Agents

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Introduction

Definition of Molecularly Targeted Antitumor Agents

Pharmacology can be defined as the study of substances that interact with living systems through molecular and chemical processes, especially by binding to regulatory factors and inhibiting or activating physiological body processes [1]. Such deliberate therapeutic applications may be considered the proper role of medical pharmacology, which is the science of substances used to treat human diseases.

For decades, the pharmacological treatment of cancer has used cytotoxic (i.e., cell-killing) therapy, which has been termed cancer chemotherapy [2]. Cancer chemotherapy is curative in subsets of patients who present with advanced disease, including germ cell cancer, small cell lung cancer, and ovarian cancer. Although treatment is not curative for most of the solid tumors, there has been a significant improvement in progression-free survival (PFS). These results also facilitated the study of adjuvant chemotherapy, leading to survival prolongation in a number of cancer types, and helped foster further trials in different clinical settings. Moreover, several of the most active chemotherapy regimens are being used in the neoadjuvant setting to reduce the size of the primary tumor allowing improved surgical outcome as well as preservation of vital organs, such as for anal, bladder, breast, gastroesophageal, rectal, 31 head and neck cancers, and osteogenic and soft 32 tissue sarcomas [3].

However, from its introduction, cancer chemotherapy has been encumbered by its poor selectivity because most antineoplastic drugs are toxic not only to tumor cells but also to important populations of the body's nonneoplastic cells, such as the fast-replicating cells of blood compartment, skin cells, and gastrointestinal tract lining cells. The resulting problems of unwanted side effects are compounded by difficulties in predicting the desired effectiveness of chemotherapy in individual patients. This unsatisfactory situation and the development of technology leading to the sequencing of the genome have driven intensive researches and development over the last few decades toward more specific and less toxic anticancer drugs that block the growth and spread of cancer by interfering with specific molecules involved in tumor growth and progression [4]. Because scientists refer to these molecules as "molecular targets," targeted cancer therapies are sometimes called "molecularly targeted therapies" or similar names. Several results of these efforts have reached the clinic, and

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many more are now in preclinical testing. Common to all these targeted therapies is their interaction with defined molecules present on cancer cells, which adds various degrees of increased selectivity to their toxic effects. As a consequence, detecting the target molecule on tumors before therapy holds great diagnostic potential for predicting the efficacy of the drug and personalizing therapy. Ideal anticancer drugs would indeed eradicate cancer cells without harming normal tissues. Unfortunately, no currently available agents meet this criterion, and clinical use of these drugs involves multiple challenges including the appearance of new toxicities [5], the need for biomarkers, the need of validation of genomic tests, and the evolution of cancer molecular imaging. Therefore, this chapter aims to present translational scientists and clinicians with an integrated critical view on the pharmacology (i.e., pharmacodynamics, pharmacokinetics, and pharmacogenetics), as well as on the clinical development (and related emerging problems) of the molecularly targeted antitumor agents in solid tumors.

Beyond Clinicopathological Typing: New Pharmacological Targets for Individualized Treatments

Factors such as disease stage, performance status, age and co-morbidity provide a crude discrimination of prognosis in many tumors. These clinical prognostic factors represent surrogate markers of clinical behavior and could be useful for predicting patient prognosis and guiding anticancer treatment [6]. For example, mediastinal lymph node involvement and the number of metastatic lymph nodes are important adverse prognostic factor in surgically treated stage IIIA non-small-cell lung cancer (NSCLC) [7]. Similarly, there is a significant difference in survival when the visceral pleura is involved. Indeed, visceral pleural invasion was observed more frequently in biologically aggressive tumors and, by multivariate analysis, this invasion proved to be a significant independent predictor of poor prog-

nosis in NSCLC patients with or without lymph node involvement [8]. Therefore, in most solid tumors, the therapeutic strategy is based on the tumor type and stage as well as on the health status of the patient at diagnosis. Several data suggested that the efficacy or toxicity of anticancer treatments is also influenced by the histologic subtype. This differential therapeutic efficacy based on histologic subtype is well documented for pemetrexed in advanced or metastatic NSCLC, where a phase III trial showed that patients with nonsquamous histology had a survival benefit when treated with cisplatin/pemetrexed versus cispaltin/gemcitabine, while the reverse was observed in patients with squamous histology [9]. On the basis of these results, the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMEA) approved pemetrexed for the use in the first-line treatment of advanced nonsquamous NSCLC.

However, the treatment of certain cancers has been revolutionized in recent years by the introduction of novel drugs designed to target specific molecular factors implicated in tumor behavior. These novel targeted therapies are based on advances in our understanding of key cellular networks and genetic nodal points around which tumors could arise and progress [10]. Genome characterization efforts have indeed highlighted the importance of "driver" somatic alterations that activate crucial oncoproteins originating tumor with a pivotal dependency. Single-agent therapeutic regimens especially designed to intercept deregulated dominant oncogenes have proven to be effective treatment in these "oncogene addicted" tumors [11]. Notable examples include imatinib, a tyrosine kinase inhibitor (TKI) in KIT-positive gastrointestinal stromal tumors, trastuzumab, a humanized monoclonal antibody (mAb) against human epidermal growth factor receptor (HER)-2 in women with HER2-positive breast cancer, sunitinib, a multitargeted TKI that inhibits both angiogenic pathways (i.e., vascular endothelial growth factor receptor and platelet-derived growth factor receptor) and direct pro-oncogenic pathways (e.g., stem-cell factor receptor and FMS-like tyrosine kinase-3),

in metastatic renal cell carcinoma (RCC). In particular, the epidermal growth factor receptor (EGFR) has been successfully targeted either by mAbs or small molecules inhibiting the tyrosine kinase domain. The mAb cetuximab blocks the extracellular domain of EGFR, thereby competing with the ligands, resulting in the inhibition of the receptor. This mAb, which is approved for the treatment of advanced colorectal cancer, has also been approved as first-line treatment combined with platinum-based chemotherapy in EGFR-positive NSCLC patients with good performance status [12, 13]. The EGFR-TKI gefitinib has been approved by the FDA and EMEA as upfront therapy replacing chemotherapy in late-stage NSCLC patients harboring activating-EGFR mutations [14]. Similarly, the manageable toxicity, along with its efficacy, makes the EGFR-TKI erlotinib an important option as maintenance therapy, and both erlotinib and gefitinib are also the only drugs of proven efficacy in the third-line setting for patients with NSCLC previously treated with chemotherapy [15]. Another example of targeted therapy is the antiangiogenic agent bevacizumab, in combination with carboplatin-paclitaxel or any platinum-based chemotherapy, which has been recently approved as first-line treatment for patients bearing tumors with nonsquamous histology [16]. More recently, the anaplastic lymphoma kinase (ALK) inhibitor crizotinib has been approved by the FDA for the treatment of locally advanced or metastatic NSCLCs with EML4-ALK translocation fusions [17]. A number of other molecular aberrations have been identified including PIK3CA mutations, IGF-1R overexpression, c-MET amplification or overexpression, or alterations in key signaling pathways, such as RAS/RAF/MEK and phosphoinositide-3 kinase (PI3K)/Akt/mTOR [18]. Several other drugs aimed to interact with these aberrant molecules are actively being investigated in the clinic, including the BRAF inhibitor sorafenib, the Src/Abl inhibitor dasatinib, and many others [11–19].

Main Targets and Pharmacodynamics of Molecularly Targeted Antitumor Agents

Pharmacodynamics is the study of the biochemical and physiological effects of drugs on the body, including the mechanisms of drug action and the relationship between drug concentration and effects. The incorporation of pharmacodynamic analyses is increasingly important in phase I clinical trials investigating whether the novel targeted agents are able to reach their targets and exert their effect in a desirable way. In contrast to the traditional nonspecific cytotoxic antiproliferative agents, which often have a small therapeutic window, steep dose–toxicity curve and an efficacy assumed to be somehow related to toxicity, molecularly targeted agents usually show less toxicity, a wider therapeutic window and an efficacy more related to growth inhibition than to tumour shrinkage. Therefore, using some representative examples of different classes of molecularly targeted agents, this chapter discusses the main pharmacological targets and mechanisms of action of such drugs, including possible suggestion for the optimization of the pharmacological studies to improve their development in the context of cancer care [20].

Agents Targeting Growth Factor Receptors

Receptor tyrosine kinases play important roles in animal development and their deregulation has been linked to several diseases, including cancer. The best example is the known role of the ERBB/HER family of receptors in the pathophysiology of breast, gastric, colorectal, lung, head, and neck tumors. There are four members of the HER family: EGFR, also termed ERBB1/HER1, HER2/Neu/ERBB2, HER3/ERBB3, and HER4/ERBB4. Activation of these receptors occurs by dimerization upon ligand binding (Fig. 3.1), and can be altered in different tumor types [21].
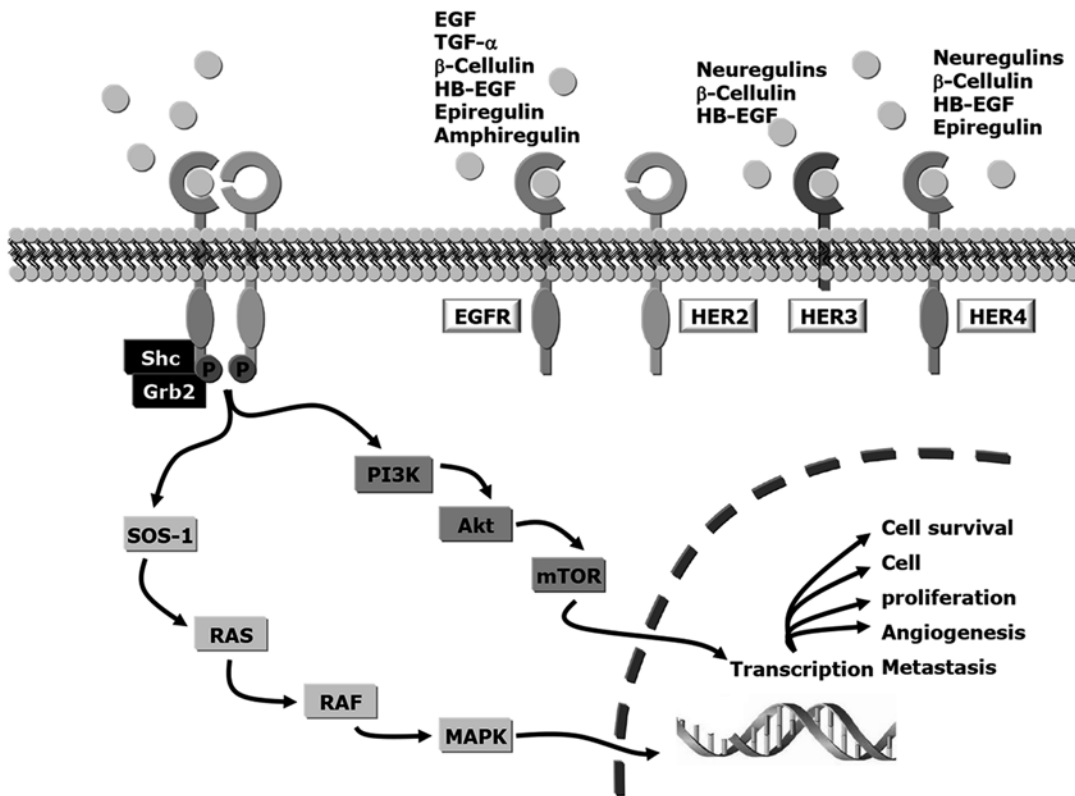
Fig. 3.1 EGFR signaling pathways: Signaling pathways and epidermal growth factor tyrosine kinase receptors involved in the tumorigenesis of NSCLC. *Akt* protein kinase B, *EGF* epidermal growth factor, *EGFR* epidermal growth factor receptor, *hb-EGF* heparin binding EGF, *MAPK* mitogen-activated protein kinase, *PI3K* phospha-tidylinositol-3-kinase, *Raf* v-raf 1 murine leukemia viral oncogene homolog 1, *Ras* retrovirus-associated DNA sequences, *SOS* Son of sevenless, *TGF* transforming growth factor, *mTOR* mammalian target of rapamycin, *FGF* fibroblast growth factor, *VEGF* vascular endothelial growth factor, *Grb2* growth factor receptor-bound protein 2

Given the relevance of these receptors in cancer, multiple strategies to target HER family members have been used, but only two have successfully reached the clinic, namely antibodies (mAb) designed against the extracellular domain of the receptors, and small TKIs which interact with the intracellular domain.

Three mAbs against HER receptors are approved for the treatment of solid tumors: cetuximab and panitumumab against EGFR and trastuzumab against HER2. Cetuximab is a chimeric monoclonal anti-EGFR antibody that contains human constant domains and rodent variable domains, while panitumumab is a fully human antibody. Trastuzumab is a humanized antibody in which human sequences replace all rodent sequences except for the complementary determining regions (CDRs) which are responsible for binding to HER2 [22]. The mechanism of action of mAbs against HER receptors is thought to involve many processes, several of which depend on the region of the receptor recognized by the antibody. Stimulation of HER endocytosis and removal of HER receptors from the cell surface upon interaction with the mAbs is expected to represent a common event in the action of these treatments [23]. This reduces the total amount of the cell surface receptors and leads to reduced signaling. Another important action of the mAbs is to facilitate the attack of the tumoral cells by the immune system. The importance of the immune reaction in the mechanism of action of anti-

HERs mAbs has been demonstrated by elegant studies using mice deficient for the antibody receptor FcγRIII. Loss or blockade of the FcγRIII receptor on leucocytes severely impairs the antitumor effect of trastuzumab in vivo, indicating involvement of Fc-receptor-dependent mechanisms in the action of trastuzumab [24]. This immunological effect may also explain the clinical benefit of combining antibodies to the same molecule, but which act on different epitopes, as has been recently reported for the trastuzumab and pertuzumab combination in breast cancer [25]. Similarly, skin rash, which is one of the clinical markers of cetuximab activity, may be related to the inflammatory skin reaction mediated by this type of cytotoxic response. Cetuximab and panitumumab interact with subdomain III of the EGFR, which is a region where EGF binds to the receptor. Therefore, cetuximab is expected to impede adequate binding of EGF ligands to the cognate receptor, blocking ligand-mediated receptor activation. Trastuzumab interacts with subdomain IV of HER2. This interaction does not prevent ligand-induced HER2 oligomerization and activation. However, when the ligand is expressed as a transmembrane molecule, its ability to activate HER receptors is profoundly compromised by trastuzumab [26]. This finding is relevant since tumors fed by transmembrane growth factors of the heregulin subfamily could be targeted by trastuzumab, even in the absence of HER2 overexpression. Pertuzumab, which binds subdomain II of HER2, has been created to interfere with receptor dimerization, and, as mentioned above, has recently shown clinical efficacy [27].

Anti-HER receptor antibodies may cause an arrest of the cell cycle in G1 through induction of the cyclin-dependent kinase inhibitor p27. In addition, these agents are known to inhibit angiogenesis [28]. Trastuzumab and cetuximab also suppress DNA repair capacity through unknown pathways, contributing to the ability of the antibody to enhance the antitumor effect of DNA-damaging agents such as cisplatin [29].

In the clinical setting, trastuzumab has been approved for the treatment of metastatic and adjuvant breast cancer in combination with a taxane-based chemotherapy. In the pivotal clinical trial in metastatic breast cancer, the combination of trastuzumab with paclitaxel showed an increase in survival compared with paclitaxel alone [30]. Of note in that study the arm combining trastuzumab with anthracyclines showed an unacceptable cardiac toxicity limiting the use of trastuzumab with this type of chemotherapy. In different clinical phase II studies, trastuzumab has been combined with different chemotherapies including vinorelbine, gemcitabine, or capecitabine among others, showing different ranges of clinical activity [31, 32]. In the adjuvant setting, trastuzumab has been combined with taxanes and platinum-based regimens to avoid the concomitant administration with anthracyclines, and is also given after finishing chemotherapy to complete a total treatment of 1 year [33]. In gastric cancer, trastuzumab has recently been approved for the treatment of the metastatic disease in combination with cisplatin and a fluoropyrimidine. This randomized phase III trial, showed an increase in survival with the combination of trastuzumab and chemotherapy versus chemotherapy alone [34].

Regarding the mAbs against EGFR, such as cetuximab or panitumumab, they have been approved for the treatment of metastatic colorectal cancer, either alone or in combination with chemotherapy for patients who do not harbor mutations at the K-RAS gene [35]. Patients harbouring mutations of this molecule were resistant to EGFR inhibition by cetuximab or panitumumab; therefore, these therapies are limited to patients with wild-type K-RAS tumors. Oxaliplatin, irinotecan, and chemotherapies based on 5-fluorouracil are the most frequent drugs used when combining these antibodies [36]. Cetuximab is also approved, based on an increase in survival, for the treatment of locally advanced head and neck cancer in combination with radiotherapy and for the metastatic disease in combination with platinum-based chemotherapy. As can be seen, most of these antibodies are used in association with chemotherapies, being the platinum compounds the most used agents [37].

The second category of targeted agents in the clinical setting includes the small TKIs, which are chemical entities that neutralize the kinase

activity by binding to the enzymatic region of the receptor. These compounds are particularly attractive because of their oral availability. In addition, they are able to block receptors with molecular alterations, such as truncations of their extracellular domain, which prevent the action of anti-HER antibodies [38]. In general, TKIs act on the adenosine triphosphate (ATP)-binding domain of the kinase region, competing with ATP for the interaction with the receptor. Inhibition of the TK activity has been a successful therapeutic approach for the treatment of several tumors with pathological activation of HER receptors, and the EGFR-TKIs erlotinib and gefitinib have been incorporated into treatment paradigms for patients with advanced NSCLC, while the small EGFR-HER2 TKI lapatinib has been approved for the treatment of metastatic breast cancer in combination with capecitabine. Regarding the latter, a pivotal trial showed an increase in PFS with the combination compared with capecitabine alone [39]. Ongoing studies are currently evaluating the role of lapatinib in the adjuvant setting given in combination with chemotherapy, trastuzumab or both. In addition, lapatinib is also approved in hormone receptor positive HER2 overexpressing metastatic breast cancer in combination with letrozole [40].

Despite four large phase III trials failed to demonstrate any survival advantage from the combination of EGFR-TKIs with chemotherapy in first-line treatment, the identification of somatic EGFR mutations, followed by retrospective analyses and prospective trials with EGFR-TKIs in selected patients, explained the previous conflicting results and defined the stage for more specific use of these agents [14, 15]. Of note, erlotinib is also approved in metastatic pancreatic cancer based on a slight increase in overall survival. However, this small benefit has questioned its clinical use [41].

Two types of HER-TKIs have been described, depending on their interaction properties. Reversible inhibitors, such as erlotinib, gefitinib, or lapatinib bind to the ATP-binding pocket of the kinase region of the receptors, and can be released from this region after washing out of the drug. In contrast, inhibitors such as neratinib or canertinib irreversibly bind to the receptor, and they are thus expected to impede the function of the HER receptor even after washing out of the drug. Recovery of the HER receptors in the latter instance depends on neosynthesis by the cell machinery. The in vitro efficacy of the irreversible inhibitors is higher than the one of the reversible inhibitors. However, reversible inhibitors may result less toxic [42]. In addition to the ATP-competitive inhibitors, it is expected that future noncompetitive or mutant selective inhibitors will be useful to fight resistance to the actual agents. The experience acquired with TKIs targeting EGFR in lung cancer indicates that mutations which reverse affinity of the ATP-binding pocket represent a mechanism of resistance to HER inhibitors. In particular, about 50% of NSCLC tumors from patients that initially respond to EGFR-TKIs harbor secondary mutations that cause resistance, mainly the T790M mutation in exon 20. These mutations allow ATP to bind to the ATP-binding pocket with higher affinity than small TKIs. This would cause displacement of the inhibitors form the ATP-binding pocket by intracellular ATP [43]. To potentially overcome the issue of resistance, next-generation TKIs are being developed. Examples of irreversible TKIs include afatinib (BIBW 2992), dacomitinib (PF-00299804), or neratinib (HKI-272). Afatinib is being evaluated in a phase IIb/III trial in metastatic lung cancer patients that failed to a first line or second line of treatment including chemotherapy and gefitinib or erlotinib. A recent study showed that afatinib significantly improved PFS in a population enriched for the presence of mutations in EGFR [44].

The above-mentioned studies demonstrated that many molecularly-targeted agents are not expected to be clinically effective in common cancers. Therefore, conventional phase I/II trials may be unable to distinguish agents that modulate intended targets from those that do not. In contrast, a clinical pharmacodynamic trial can potentially identify those investigational agents that deserve full clinical development using evidence of target modulation in human malignancy as the basis for this decision. In particular, when coupled with measurement of achieved drug level in

a tumor biopsy, phase 0 pharmacodynamic trials can provide important information about investigational agents that fail to modify their candidate targets [45]. This may occur by distinguishing those agents that fail to achieve adequate intratumoral levels to affect the target, from those that do not affect a target in situ despite reaching adequate intratumoral drug levels. Because the purpose of a phase 0 pharmacodynamic clinical trial is to obtain evidence of drug action on its molecular target in a clinical setting, the results of the pharmacodynamic assessment may become the primary, and sometimes sole, objective of the phase 0 protocol. This represents an important paradigm shift from the historical practice of conducting correlative studies in oncology trials, in which clinical pharmacodynamics evaluations should be integrated in early clinical investigations using available tissue specimens for molecular evidence of drug-induced changes.

However, phase 0 trials with pharmacodynamic endpoints require reliable, validated assays to measure target modulation. Assay methodology determining target modulation should therefore be optimized in preclinical models using clinical procedures and tissue handling, processing, and storage procedures standardized prior to clinical trial initiation [46]. These will establish, for example, whether the amount of tissue obtained from an 18-gauge percutaneous needle biopsy is sufficient to reliably measure target modulation, or confirm that the sample handling procedures followed in an interventional radiology suite will not impair the evaluation of target effects. These tests require extensive resources, sophisticated and sensitive tools, and an integrated multidisciplinary team, limiting the feasibility of performing phase 0 trials only at some institutions.

Agents Targeting Key Downstream Signaling Pathways

Despite the promising results obtained with the currently used targeted therapies against growth factor receptors in extending the life expectancy of selected patients with specific solid tumors, their capability in preventing resistance is still limited. The growing knowledge about the key players in downstream pathways, including signaling cascades such as the PI3K/AKT/mTOR and the HGF-Met, makes them attractive targets for the development of new therapies that can reduce or even prevent resistance. In particular, recent preclinical data have shown that combination therapy between inhibitors of different signaling pathways might circumvent resistance against some drugs and constitute a more effective therapeutic strategy [47, 48]. Therefore, in this section, we will briefly discuss the mitogen-activated protein kinase (MAPK) cascades, which are among the most prominent pathways involved in tumor progression, and the recent advances in the development of pathway-targeting inhibitors, which might successfully be used as effective anticancer agents. In particular, The ERK1/2 MAPK pathway (usually termed as the "canonical" MAPK cascade) is composed of three MAP kinase kinase kinases (MAPK-KKs) (A-Raf, B-Raf and Raf-1), two MAPKKs (MAPK ERK kinases 1/2, MEK1/2) and two terminal MAPKs (ERK1/2). The available evidence supports that this pathway—rather than being a three-tiered linear pipeline which transduces signals from the cell surface to the nucleus—involves a number of inter-players, unravelling a complex network of spatio-temporal activators and inhibitors [49]. Upon surface receptor activation, adaptor proteins (i.e., growth factor receptor-bound protein 2, Grb2) lead to the activation of GTPases belonging to the Ras family (i.e., K-Ras, N-Ras, H-Ras). Activated Ras proteins can interact with and activate members of the Raf kinase family. Regarding the canonical MAPK cascade, Ras binding is sufficient to activate B-Raf, while Raf-1 (C-Raf) and A-Raf have to go through a more complex series of activation steps. Once activated, all Raf proteins are capable of activating MEK proteins, although B-Raf is the most efficient in the task. Raf kinases bind MEK and phosphorylate two serines in the MEK activation loop during a single interaction. Two mammalian MEK isoforms have been described (i.e., MEK1/2), usually considered as a unique protein due to a large sequence identity, although

recent analyses have pointed out slight differences in their regulatory pattern [50, 51].

Moreover, the traditional view of the canonical MAPK cascade as an axis that simply transduces signaling through growth factor receptors, Ras, Raf, MEK, and ERK has been extensively reviewed in the last decades, as numerous spatiotemporal modulators of the pathway have been described. First of all, several scaffold proteins have been evidenced, each one able to modulate the final ERK1/2 activity localization. Kinase suppressor of Ras-1 (KSR1) has long been recognized as the main scaffold protein for the cascade, being capable of binding all kinase members of the pathway and thus greatly accelerating and sustaining signal transduction [52]. Other scaffold proteins such as the similar expression to Fgf genes (Sef), the IQ motif-containing GTPase-activating protein 1 (IQGAP1), and the leukocyte-specific protein-1 (LSP1), are instead able to localize the canonical MAPK cascade to different cellular compartments. Furthermore, a growing number of inhibitors/modulators of selected members of the cascade have been described, including the Raf kinase inhibitor protein (RKIP) which blocks Raf-mediated MEK phosphorylation by preventing Raf-MEK physical interaction. Interestingly, RKIP levels were found reduced in metastatic cancer cells, thereby strengthening its possible tumor suppressor role [53]. However, a recent study suggested its role in the synergistic interaction of the Raf-inhibitor sorafenib with erlotinib in NSCLC cells [54].

Several members of the canonical MAPK cascade and upstream activators are frequently altered in human tumors, and different tumor-driving alterations can lead to a constitutively activated MAPK canonical pathway. The most prominent aberrations involve constitutive activation of Ras and Raf proteins. Mutations involving these players have been extensively described. Among the three Ras human genes, KRAS is the most commonly mutated (e.g., about 85 % KRAS mutations in pancreatic cancer). The large majority of somatic mutations occur on nucleotides belonging to codon 12 in exon 2. Wild-type codon 12 encodes a glycine residue that guarantees a minimal steric hindrance in-side the GTP-hydrolyzing pocket. Thus, a number of missense substitutions produce residues with side chains that impair GTP-hydrolyzing capability of the protein, constitutively activating the molecule. Ras mutations involving codon 61 (exon 3) and codon 146 (exon 4) occur with a reduced frequency [55]. Among the Raf family, the BRAF gene (encoding for B-Raf) bears the largest amount of clinically relevant mutations. Up to 90 % of B-Raf mutations consist in a glutamic acid substitution for valine at codon 600 (i.e., V600E). The valine residue is crucial to maintain B-Raf inactive. Thus, V600E-mutant B-Raf protein activates MEK in a Ras-independent fashion, a feature not apparent for A-Raf or C-Raf. This is due to the higher basal kinase activity of B-Raf than of C-Raf and A-Raf. In fact, B-Raf serine 445 is constitutively phosphorylated, whereas the homologous C-Raf residue needs to be phosphorylated to fully transduce a signal. B-Raf mutations are regarded as possible early tumor-initiating events in melanoma carcinogenesis [56]. Genes encoding MEK and ERK are far less subject to mutations. Exon 2 of the MAP2K1 gene (i.e., encoding the MEK1 protein) has been pointed out to harbor low-frequency mutations in melanoma, lung, and colorectal cancer [57, 58].

The aberrations of the ERK pathway frequently found in cancer cells have led to great efforts in developing compounds to strike components of the cascade. In particular, the Ras proteins were at first the most attractive targets, as their downstream activity is exerted through different survival pathways, and Ras inhibition approaches (i.e., inhibition of Ras post-translational modification), have been tested in the last decades. Additional targets in the ERK cascade are the Raf kinases. Sorafenib, the first inhibitor of B-Raf kinase activity to be approved for clinical use, is scarcely selective for B-Raf and is now regarded as a multi-kinase inhibitor, exerting its activity mainly by inhibiting pro-angiogenic receptor kinases like vascular endothelial growth factor receptor 2 and 3 (VEGFR2, 3), platelet-derived growth factor receptor beta (PDGFRB) and c-Kit [59]. Vemurafenib is a more selective B-Raf inhibitor, capable of efficiently inhibiting the V600E mutant B-Raf, and was approved in

2011 by the FDA for first-line treatment of metastatic and unresectable melanoma in patients carrying B-Raf mutations [60].

MEK inhibition seems another promising approach to target the pathway, because MEK have a unique activation loop, rendering MEK inhibitors particularly specific among kinase inhibitors [61]. Furthermore, as ERK1/2 are in close contact with MEK1/2, MEK inhibition represents a precious approach to target ERK, for which specific inhibitors have never been described. The first two described MEK inhibitors (i.e., PD98059 and U0126) displayed a great potency but a poor pharmacological profile. CI-1040 (PD184352) was then developed as an orally active drug that displayed promising activity in phase I evaluation. Anyway, due to low pharmacokinetic properties, its clinical development was stopped during phase II studies [62]. Two second-generation drugs were then developed, i.e., PD0325901 and selumetinib (AZD6244, ARRY-142886). Despite a 50-fold increased potency with respect to CI-1040, PD0325901 development was interrupted due to a high toxicity [63]. Phase II clinical trials for the use of selumetinib in melanoma, NSCLC and colorectal cancer have been completed. The drug displayed a clinical activity comparable (but not superior) to current standard therapies, although it has been suggested that its efficacy could be higher in B-Raf-mutated patients [64].

Interestingly, it was recently proposed that the clinical use of both B-Raf and MEK inhibitors may provide an additional therapeutic advantage as they may be able to control the dormancy of putative pro-metastatic disseminated tumor cells [65]. It has indeed been hypothesized that dormancy of tumor cells could be associated with ERKlow/p38 high activation pattern. In this view, the pharmacological treatment of patients with inhibitors of the ERK cascade during asymptomatic conditions may be advantageous to control the awakening of dormant cells, while inhibitors of p38 should be used cautiously, as they may accelerate the development of metastases [65]. However, strategies aiming to stimulate p38 and inhibit JNK may have benefit for tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-based therapies in NSCLC [66].

Agents Inhibiting Angiogenesis

Tumor angiogenesis is the multi-step process whereby new blood vessels are formed from the existing vasculature. The new blood vessels constitute an important route for the tumor cells to exit the primary tumor site, enter the circulation, and travel to distant organs. Therefore, angiogenesis, as determined by vascular density, represents a significant prognostic indicator of tumor growth and metastatic potential in several tumor entities [67]. Being involved in tumor progression and metastasis, angiogenesis represents an attractive therapeutic target for cancer treatment. One of the major players in tumor angiogenesis is the mammalian VEGF family which is composed by five glycoproteins known as VEGFA (commonly referred to as VEGF), VEGFB, VEGFC, VEGFD (also known as FIGF), and placenta growth factor (PIGF or PGF). These ligands are able to bind and activate three tyrosine kinase receptors known as VEGFR1 (or FLT1), VEGFR2 (or KDR) and VEGFR3 (or FLT4). VEGFR2 is mainly expressed in the vasculature and is the key mediator of VEGF-induced angiogenesis. The activation of the VEGF pathway and downstream signaling network promotes tumor angiogenesis by inducing a series of cellular processes, including proliferation, survival, migration and invasion of endothelial cells, enhanced permeability of existing blood vessels, and increased chemotaxis and homing of bone marrow-derived vascular precursor cells [68]. Additionally, VEGF can act as a direct growth factor on tumors by inducing immune suppression and displaying autocrine effects on tumor cells (survival, migration, invasion). Considering the key role of the VEGF pathway on tumor angiogenesis and the relevance of this process for tumor growth and metastasis, considerable efforts have been made to develop VEGF-targeted agents that can be used in cancer therapy. These agents include neutralizing antibodies to VEGF or VEGFRs, soluble VEGF receptors or receptor hybrids, and TKIs with selectivity for VEGFRs [68].

Bevacizumab is a humanized murine mAb that binds to VEGF, leading to its functional inactivation. This antibody has been approved by

the FDA as first-line treatment for patients with metastatic colorectal cancer. This approval was mainly based on the results of a randomized phase III trial with 813 patients with previously untreated metastatic colorectal cancers. About half of the patients received a regimen of irinotecan, bolus 5-FU, and leucovorin (IFL) plus bevacizumab and the other half received IFL plus placebo. The addition of bevacizumab significantly prolonged the median overall survival (OS) of the patients by almost 5 months (20.3 months vs. 15.6 months), which corresponded to a hazard ratio (HR) for death of 0.66 (P<0.001), or a reduction of 34 % in the risk of death in the bevacizumab group. Additionally, the median duration of PFS was 10.6 months in the group given IFL plus bevacizumab, as compared with 6.2 months in the group given IFL plus placebo. Furthermore, the addition of bevacizumab to IFL was also associated with an increased response rate (44.8 % vs. 34.8 %; $P=0.004$) and an increased median duration of response (10.4 months vs. 7.1 months) [69]. More recently, the efficacy and safety of bevacizumab added to first-line oxaliplatin-based chemotherapy (either capecitabine plus oxaliplatin (XELOX) or 5-FU/folinic acid plus oxaliplatin, i.e., FOLFOX-4) was evaluated. Also in this trial, the group receiving bevacizumab experienced a statistically significant improvement in median PFS. However, the OS differences did not reach statistical significance and the response rates were similar in both arms [70]. Bevacizumab was also evaluated in combination with FOLFOX-4 as second-line treatment for metastatic colorectal cancer (mCRC) in the Eastern Cooperative Oncology Group Study E3200. In this phase III trial, 829 patients with irinotecan-refractory metastatic colorectal cancers were randomly assigned to one of three treatment groups: FOLFOX-4 plus bevacizumab, FOLFOX-4 alone, or bevacizumab alone. The combination of FOLFOX-4 with bevacizumab was superior in all efficacy parameters when compared with FOLFOX-4 or bevacizumab alone. The median OS was 12.9 months for the group treated with FOLFOX-4 plus bevacizumab, 10.8 months for the group treated with FOLFOX-4 alone (HR for death=0.75; $P=0.0011$), and 10.2 months for the

group treated with bevacizumab alone. The median PFS was 7.3 months for the group receiving the FOLFOX-4 and bevacizumab combination therapy, compared with 4.7 months for the group receiving FOLFOX-4 alone (HR for progression=0.61; $P<0.0001$), and 2.7 months for the group treated with bevacizumab alone. Finally, the corresponding response rates were 22.7, 8.6, and 3.3 %, respectively [71]. Therefore, on June 20, 2006, the FDA granted approval for the use of bevacizumab in combination with intravenous 5-FU-based chemotherapy, as second-line treatment for metastatic colorectal cancers.

When added to standard chemotherapy, bevacizumab increased survival also in NSCLC patients [72]. In the pivotal phase III study ECOG 4599, nonsquamous NSCLC patients were randomized to receive either chemotherapy or the combination of chemotherapy with this mAb. The addition of bevacizumab prolonged the OS from 10.3 to 12.3 months, and increased the RR from 15 to 35 %. Based on this study, bevacizumab gained FDA and EMEA approval as first-line therapy for advanced nonsquamous NSCLC [73].

Several agents active against multiple tyrosine kinases including VEGFR have been investigated, and the TKIs sorafenib and sunitinib are currently used in the clinical setting. Monotherapy with sorafenib prolongs OS and delays the time to progression (TTP) in patients with advanced hepatocellular carcinoma who are not candidates for potentially curative treatment or transarterial chemoembolization. Therefore, sorafenib represents an important advance in the treatment of these tumors and is the new standard of care for this condition [74]. A phase III trial showed that, compared with placebo, treatment with sorafenib prolongs PFS also in patients with advanced clear cell RCC. The median PFS was 5.5 months in the sorafenib group and 2.8 months in the placebo group (HR for disease progression in the sorafenib group, 0.44; $P<0.01$) [75]. Similarly to sorafenib, the multi-kinase inhibitor sunitinib has been tested in a number of settings/tumor types and was approved by the FDA for the treatment of RCC [76]. Sunitinib inhibits cellular signaling by targeting platelet-derived growth factor (PDGF-Rs) and VEGFRs. The simultaneous

inhibition of these targets, therefore, leads to both reduced tumor vascularization and cancer cell death, and ultimately tumor shrinkage.Sunitinib also inhibits KIT, which is a receptor TK that drives the majority of gastrointestinal stromal cell tumors (GIST). It has been recommended as a second-line therapy for patients whose tumors develop mutations in KIT that make them resistant to imatinib, or who become intolerant to the drug [76].

Pharmacokinetics

Most of the available pharmacokinetics information on new targeted agents is based on data obtained from in vitro experiments, animal studies, drug–drug interaction studies, and mass-balance studies in healthy volunteers with a single dose. In general, these TKIs are substrates of several drug transporters and metabolizing enzymes. Some of them are also capable to inhibit drug transporters and enzymes making their disposition and metabolism at steady-state pharmacokinetics rather complex and unpredictable. However, it is difficult to translate the results of these studies to the clinical oncology practice where these drugs are commonly administered on a daily basis with possible auto-inhibiting mechanisms significantly altering the pharmacokinetics outcomes as well as the relevance of claimed drug interactions. Most information is available for the TKIs that are used for the longest time in clinical practice after their approval. Therefore, in the following sections, we reported the main information on the pharmacokinetics of the EGFR-TKIs gefitinib and erlotinib [77, 78].

Bioavailability

The solubility of both erlotinib and gefitinib is pH-dependent. Agents that alter gastric pH, such as H2-receptor antagonists and proton-pump inhibitors, can substantially reduce the plasma levels of EGFR-TKIs, and their concomitant use should be avoided.

Moreover, both the bioavailability and the AUC of erlotinib increase considerably when the drug is ingested with food. Most oncologists recommend the administration of erlotinib on an empty stomach, at least 1 h before or 2 h after a meal, when it has an oral bioavailability of 60%. Conversely, when taken with food, erlotinib has a bioavailability of nearly 100%, which potentiates side effects. After 7–8 days erlotinib concentrations reach steady-state, and its elimination half-life is 31 h. Erlotinib is evenly distributed in the plasma and tumor tissue (plasma: tumor ratio=1:1). Binding to plasma proteins is approximately 95% bound to serum albumin and alpha-1 acid glycoprotein (AAG) of the serum. For erlotinib a 30% dose reduction is allowed. This dose reduction regards 6–16% of patients because of side effects.

In contrast, food does not affect the absorption of gefitinib. The absorption after oral administration is moderately slow and peak plasma concentrations are obtained after 3–7 h from administration, with elimination half-life of 48 h, and mean bioavailability of 60%. This drug is distributed extensively in tissues, and plasma protein binding is approximately 90%.

Metabolism and Clearance

Erlotinib and gefitinib are metabolized primarily by CYP3A4 and to a lesser extent by CYP3A5 and CYP1A1 [79]. Erlotinib is metabolized primarily in the liver by different cytochrome enzymes (especially by CYP3A4), but intestinal and lung cancer cells could partly contribute to its catabolism. Moreover, cigarette smoking induces CYP1A1 and has been correlated with a reduction in erlotinib exposure after a therapeutic dose [80]. Erlotinib excretion is more than 90% by stools, while the remaining 10% is excreted through the kidney. Less than 2% of delivered dose is excreted as unchanged drug. Gefitinib is also excreted mainly as metabolites in stools, with renal elimination account for less than 4% of the administered dose.

Erlotinib is a lipophilic drug; however, its lipophilicity is about three times lower than that

of gefitinib. This could help to explain some of the differences in the pharmacokinetic and pharmacodynamic properties of the two compounds, since a greater lipophilicity also leads to a higher susceptibility to the action of catabolic mechanisms, an increase in biliary excretion and a decrease in plasma concentrations of free drug. In fact, erlotinib is less exposed to hepatic cytochrome enzyme action, resulting in a slower clearance.

Other clinical factors that affect the pharmacokinetics of erlotinib include serum total bilirubin, Alpha-1 Acid Glycoprotein (AAG) concentrations, and current smoking. Increased serum concentrations of total bilirubin and AAG concentrations were associated with a reduced erlotinib clearance. Similarly, a recent study collected interesting data on the pharmacokinetics of erlotinib and its interaction with smoke: drug exposure is reduced by 50–60% in smokers, and the maximum tolerated dose is increased to 300 mg [80].

Pharmacogenetics

Targeted therapies should not be given to all patients irrespectively of their characteristics. Indeed, their clinical activity has been related to different clinical and biological parameters, such as the EGFR-activating mutations for gefitinib and erlotinib. However, not all clinical outcomes, including tolerability, are explained by these characteristics, and the identification of novel biomarkers is a viable area of research.

Assessing germline genetic polymorphisms as either predictive or prognostic markers is very appealing, especially in the advanced cancer setting, when diagnosis is usually done from small needle biopsy samples and tumors are either not resected or resected after chemotherapy, so that the handling of tumor material can be problematic. Polymorphisms are inherited genetic variants harbored by all the cells of the body and, although a genotype represents a static value unable to change in response to a different situation, such as exposure to chemotherapy, and may not reflect changes in tumor DNA, such as loss of

heterozygosity, and previous studies showed no differences in SNPs analyzed in tumor and normal tissues [81]. 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 laser microdissection and subsequent sophisticated infrastructure.

Several germ-line DNA variations of EGFR and other genes have been associated with clinical outcome after TKIs treatment, and this section focuses on the relationship between these candidate germline polymorphisms (Fig. 3.2) and the response and toxicity to gefitinib. However, studies on polymorphisms affecting their outcome have the potential to be extended to cover TKIs of similar structure and activity such as erlotinib, sorafenib, sunitinib, imatinib, lapatinib, vandetanib, and canertinib, among a growing list of many structurally related compounds with increasing clinical application.

Several studies evaluated variants in the region which regulates the expression of the EGFR gene have been evaluated. The regulatory regions of EGFR are located within the 5'-flanking region and intron-1, and both the EGFR -191C/A and -216G/T polymorphisms lie in the transcriptional start site of the promoter region, wherein multiple nuclear regulatory affinity sites are located. The -191C/A polymorphism has been correlated with enhanced EGFR promoter gene expression and activity, while the A-G variant, which leads to the substitution of an arginine with a lysine at codon 497 (R497K), has been associated with the reduction of EGFR activity [82, 83]. Similarly the -216G/T genotype is located in the binding site for the transcription factor Sp1, and the T allele is associated with increased EGFR mRNA expression [84]. The -216G/T, -191C/A, and R497K EGFR polymorphisms were evaluated in a study conducted in 92 advanced NSCLC Caucasian patients treated with gefitinib, and the association of the -216G/T variant with longer PFS was reported. The T allele was also associated with significantly higher rates of stable disease/partial response ($P=0.01$) and a significantly higher risk of treatment-related rash/diarrhea
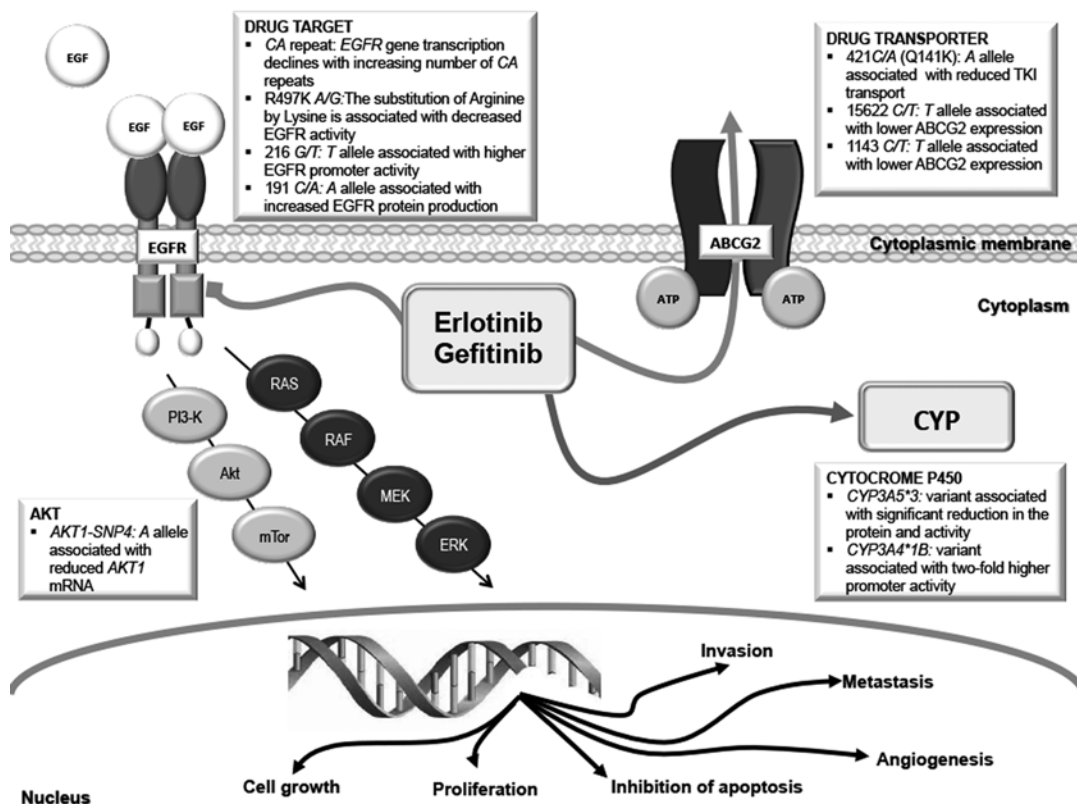
Fig. 3.2 Some of the most relevant polymorphisms in key genes involved in pharmacokinetics and pharmacodynamics of EGF receptor (*EGFR*) tyrosine kinase inhibitors (*TKI*) correlated with gefitinib and erlotinib response and toxicity in nonsmall-cell lung cancer patients. (Adapted from Galvani E, Peters GJ, and Giovannetti E. 2012 [109]

(*P*=0.004) [85]. A recent study in 98 NSCLC Japanese patients treated with gefitinib screened for EGFR mutations and polymorphisms -216G/T and -191C/A reported the mutations as predictive factors of sensitivity to gefitinib, OS, and PFS, but no correlation was found between polymorphisms and clinical outcome [86]. In another study, 175 NSCLC Caucasian patients treated with gefitinib were screened for the same EGFR polymorphisms, and a significantly lower response rate was observed in patients carrying the G-C haplotype [87].

A highly polymorphic region is also located in the EGFR intron-1 as 14–21 CA-repeats [88]. Shorter alleles of a CA-dinucleotide repeat polymorphism in intron-1, of lower frequency in Asian population, are associated with an increase in transcription of EGFR [89]. In particular, the longer allele 21 has been reported to induce an 80 % decrease in the gene expression compared with the shorter allele 16 [90, 91]. Most studies reported a better response to gefitinib treatment in NSCLC patients harboring the short EGFR-CA repeat genotype [92–94]. Ichihara and colleagues firstly studied in 98 NSCLC Japanese patients treated with gefitinib the relation between clinical outcome and several genetic factors, including the EGFR-CA repeat variant. In this analysis, among patients with EGFR activating mutations, individuals carrying the shorter CA alleles had a trend toward a significantly longer OS (*P*=0.13) compared with those with the long alleles (defining long CA repeats equal or greater than 19, or the sum of two alleles greater than 39, and short CA repeats as less than 19, or the sum of two alleles less than 39) [86]. Another study was fo-

cused on the correlation of clinical outcome after gefitinib treatment with EGFR mutations and CA-repeat genotype in 86 Korean patients with advanced NSCLC. In this investigation, short CA was defined as the sum of both alleles <or=37, while long CA was defined as sum >or=38. In agreement with the previous study, EGFR activating mutations were associated with sensitivity to gefitinib and OS, and short CA-repeat status was also correlated with better response and longer TTP [92]. In a following study performed by Nie et al. in 70 NSCLC Chinese patients treated with gefitinib, significantly higher response rate was associated with shorter CA-repeat status (defined as any allele less than or equal to 16). These patients had also higher EGFR expression and prolonged OS compared to those with long CA. No evidence of correlation was reported for clinical outcome with the R497K polymorphism or EGFR expression [95]. Shorter CA repeats (16 or less) was associated with significantly improved PFS and OS in another study in 92 Caucasians NSCLC patients treated with gefitinib [85]. However, in the largest pharmacogenetic analysis in NSCLC Caucasian patients (n=175) treated with gefitinib, no association of the EGFR intron-1 CA-repeat status with clinical outcome was observed, 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 others [87].

Other potentially predictive polymorphisms include variations in the EGFR downstream signaling pathways such as AKT1, as well as the DNA repair genes and those of the genes encoding for the drug transporter ABCG2, which has been shown to be active in removing gefitinib from the cell. Different studies reported the association of the haplotype including two functional polymorphisms (AKT1-SNP3 and SNP4) with lower Akt protein levels in tissues from Caucasians, and with the lowest apoptotic response of EBV-transformed lymphoblastoids to radiation [96, 97]. Furthermore, in 96 Caucasian patients, the AKT1-SNP4 A/A genotype was correlated with shorter OS. No other poor prognostic factors were found to potentially explain the short survival of patients carrying the AKT1-SNP4-A/A

variant (n=6) since their baseline demographic and biological characteristics resulted similar to the average of the studied population. Moreover, the AKT1-SNP4 polymorphism remained an independent predictive parameter of progression and death risk at multivariate analysis [81]. A recent trial in esophageal cancer patients treated with fluoropyrimidines, platinum compounds, and taxanes, but not with EGFR-TKIs, correlated other genetic polymorphisms in AKT1 with increased recurrence and significantly shorter survival. Similarly a recent study in Korean NSCLC patients showed that other AKT1 polymorphisms could be used as prognostic markers for patients with early-stage NSCLC. These studies suggested that genetic variations in the PI3K/AKT pathway may be prognostic and/or predictive factors of response to different drugs [98, 99]. 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.

A number of common SNPs in the ABCG2 gene that might affect ABCG2 protein expression, function, and localization have been described. ABCG2 is a member of the family of ATP-binding cassette (ABC) transporters and its overexpression is commonly associated with resistance to a wide range of anticancer agents, including camptothecins, anthracyclines, and antifolates. Interactions between EGFR-TKIs and ABCG2 have been recently suggested. Of note, gefitinib is an ABCG2 substrate at clinically achievable concentrations (≤1 μM), while at higher drug concentration (>1 μM) gefitinib leads to the inhibition of the same transporter [100]. Therefore, gefitinib resistance phenotypes both in vitro and in vivo might be affected by ABCG2 expression. Furthermore, since gefitinib is an orally active compound and ABCG2 is highly expressed in the gastrointestinal tract where it participates in the uptake of several xenobiotics, one might also expect an important role for ABCG2 in the absorption and elimination of this agent. In particular, the ABCG2 421C/A polymorphism resulting in a glutamine to lysine amino acid change at position 141 (Q141K) has been correlated with the reduction of ABCG2 protein expression and/or activity and with increase accumulation of both

gefitinib and erlotinib [101]. However, no correlation between ABCG2 421C/A polymorphism and protein expression, as well as with outcome after gefitinib treatment, was observed in a tissue microarray of 50 lung cancer tissues [102].

Several other studies evaluated the correlation between selected polymorphisms and toxicity induced by gefitinib. Indeed, even if the specificity of gefitinib for its target results in a more favorable safety profile than most standard chemotherapy agents, the treatment with this agent leads to the development of rash and diarrhoea as major adverse specific effects. At present, little is known about the etiology of these effects, and there is a high interpatient variability that might be explained by the pharmacogenetic heterogeneity of patients [103]. A study in 52 NSCLC patients treated with gefitinib performed by Huang and colleagues analyzed the correlation of genetic factors with skin rash. In particular, patients were screened for the EGFR intron-1 CA repeat status, the EGFR SNPs -216G/T, -191C/A, and R521K. Among these polymorphisms, only the intron-1 CA repeat variant was correlated with grade 2–3 skin rash, observed in 21 % of patients with L/L genotype (19–22 repeats), 31 % S/L genotype (15–18 repeats) and 71 % with S/S genotype (<15 repeats) [104]. Of note, the early grade-2/3 rash was associated with tumor response, but not the EGFR intron-1 CA-repeat genotype ($P=0.35$). No correlation was found with diarrhoea for any of these polymorphisms. Another study reported the association of the EGFR 216 G/T variant with a significantly higher risk of both rash and diarrhoea in 92 NSCLC patients treated with gefitinib [85]. Similar results were observed in our population of 96 NSCLC patients treated with gefitinib, in which grade >1 diarrhoea was significantly more frequent in patients harboring the EGFR 191C/A, A/A, EGFR 216G/G, and R497K A/A variants [81]. These results might be explained by the pathophysiology of anti-EGFR-induced diarrhoea, which is thought to result from excessive chloride secretion, inducing secretory diarrhoea. Therefore, diarrhoea might result from the higher EGFR expression in the intestinal lumen associated with the EGFR promoter polymorphism variants, as reported previ-

ously [105]. In contrast, EGFR ligand binding alterations were associated with the A allele in the R497K variant resulting in a reduced EGFR phosphorylation in colorectal cancer tissues. A strong correlation between the ABCG2 421C/A variant and diarrhoea was reported by Cusatis and colleagues in gefitinib-treated NSCLC patients [106]. In particular, they showed that only 13 (12 %) of 108 patients homozygous for the wild-type genotype of ABCG2 developed diarrhoea, while 7 (44 %) of 16 patients heterozygous for ABCG2 421C/A presented the adverse effect. The authors suggested that the altered ATPase activity of the polymorphic ABCG2 421C/A in the intestine, together with the reduced protein levels, might affect the oral absorption and/or elimination of gefitinib resulting in increased plasma concentrations in the steady-state and causing the diarrhoea. In contrast, no correlation between the ABCG2 421C/A variant and gefitinib-induced toxicity was found in a population of 94 Caucasian patients affected by NSCLC [102]. However, in the same population, we observed a correlation among moderate-severe diarrhoea with the ABCG2 15622C/T polymorphism and the ABCG2 (1143C/T, -15622C/T) haplotype. However, in the same population, moderate-severe diarrhoea was correlated with the ABCG2 15622C/T polymorphism and the ABCG2 (1143C/T, -15622C/T) haplotype.

Finally, both gefitinib and erlotinib are metabolized mainly by the CYP3A4, CYP3A5, and CYP1A isozymes, while CYP1A2 is involved in the metabolism of erlotinib, but not of gefitinib. Since all these CYPs are polymorphic, the distribution of specific variant CYP alleles might explain the different pharmacokinetics and activity of TKIs. However, the impact of several CYP polymorphisms to tailor in vivo treatment with TKIs remains largely to be elucidated. In the study by Rudin and collaborators [105], CYP3A4 polymorphisms were marginally associated with skin rash in erlotinib-treated patients. 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 CYP3A5*3 G polymorphism was also marginally associated with grade ≥2 rash

(P=0.094, dominant model) and any grade diarrhoea (P=0.062). These marginal associations warrant further studies on the role of CYP3A4 and CYP3A5 polymorphisms in determining activity levels of EGFR-TKIs, as well as other TKIs.

In conclusion, despite the intriguing findings of several studies, the small sample size together with the interethnic differences, and the retrospective nature of most studies, make it difficult to draw any clear conclusions regarding the role of these pharmacogenetic biomarkers in determining the clinical outcome or toxicity in gefitinib treatment. Hopefully, the accurate planning of new prospective trials, the increased knowledge of key mechanisms affecting drug distribution/activity, and the use of novel technologies, including genome-wide approaches, may provide critical and essential tools to improve the prospects of pharmacogenetic research for novel molecularly targeted agents.

Conclusions

New insights into the molecular biology of cancer and tumorigenesis have recently identified key biological processes and several potential molecular targets for anticancer treatment. Novel agents targeting these aberrant processes have revolutionized the management of certain molecular subsets of cancers, and have contributed to recent improvements in survival rates, as well as in defining novel subgroups of nosological entities. For example, for EGFR mutant and EML4-ALK fusion subgroups, which are detected in approximately 15 and 4% of lung adenocarcinomas, mutation status predicts response to targeted therapy with selective inhibitors. These results led to the approval of both the EGFR-TKIs erlotinib and gefitinib and the ALK inhibitor crizotinib as first-line treatments in molecularly selected NSCLC patients [107].

However, the oncologists are still facing relevant inter-individual variability in drug activity and the occurrence of several drug resistance mechanisms. In particular, resistance to targeted agents is a general phenomenon and can be caused by several mechanisms, which are partially overlapping with the main factors involved in resistance toward chemotherapy [108]. One commonly described mechanism of resistance involves additional genetic alterations within the target oncogene itself. Additional genetic mechanisms include downstream or "bypass" activation of other components of signaling pathways, or compensatory activation of other signaling pathways. Recent studies have also shed light on nongenetic mechanisms that may have a reversible, epigenetic component, such as EMT or cancer stem cells. Taken together, these observations highlight a pressing need to further elucidate the various mechanisms that drive disease progression during drug treatment as a key step toward developing therapeutic strategies to prevent or overcome such drug resistance in individual patients, according to the specific molecular characteristics of their tumor.

Therefore, to improve the rational use of this emerging arsenal of highly selective, targeted cancer therapeutics, the conventional histopathological assessment of tumors should be associated with a refined pharmacological evaluation, including the analysis of several signaling pathways that fuel deregulated cell proliferation. Since novel genetic technologies played a pivotal role in the emergence of the so-called "personalized medicine," the integration of classical pharmacodynamics, -kinetics, and -genetics analyses with the latest generation of whole-genome analyses will be essential to further improve the customization of treatment for individual patients.

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