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Small Molecules in Oncology

Third Edition

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Third Edition

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Preface

The past two decades have resulted in major breakthroughs in the treatment of cancer. Even though conventional chemotherapy currently remains the backbone of most treatment regimens, the paradigm of cancer therapy is shifting unambiguously toward more selective, mechanism-based strategies.

With the tremendous advances in our recent understanding of aberrant signaling pathways in various types of cancer—including leukemia, breast and lung cancer, and melanoma—plenty of crucial regulators of malignant behavior in cancer cells have emerged as promising candidates for molecular target-based cancer therapies.

Specific alterations in key signaling molecules driving the progression of individual cancers can now precisely be targeted by small low-molecular-weight compounds. This new class of rationally designed anticancer agents is able to induce striking regressions in molecularly defined subsets of patients.

One of the early pioneers has been imatinib mesylate (Glivec®) that showed remarkable efficacy for the treatment of patients with Philadelphia chromosome-positive CML, changing the course of this formerly deadly disease profoundly. A recent major breakthrough represents the discovery of synthetic lethality of PARP inhibitors in cancers defective in homologous recombination repair (HR), e.g., those associated with *BRCA1* and *BRCA2* mutations.

With the third edition of *Small Molecules in Oncology*, we aim to give you a comprehensive survey of both, already established drugs as well as promising new substances. All chapters have been contributed by renowned scientists and clinicians, offering first-hand insight into the exciting and rapidly evolving field of targeted cancer therapies. Due to the tremendous amount of available agents, the book has now been divided into two volumes, while "Small Molecules in Oncology" covers the treatment options in solid tumors and "Small Molecules in Hematology" focuses mainly on molecularly targeted drugs in hematologic malignancies.

Heilbronn, Germany — Uwe M. Martens

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Erlotinib

Martin Steins, Michael Thomas and Michael Geißler

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Abstract

The epidermal growth factor receptor (EGFR) has been implicated in a multiplicity of cancer-related signal transduction pathways like cellular proliferation, adhesion, migration, neoangiogenesis and apoptosis inhibition, all of which are important features of cancerogenesis and tumour progression. Its tyrosine kinase activity plays a central role in mediating these processes and has been intensely studied to exploit it as a therapeutic target. Inhibitors of this pathway have been developed and assessed in trials with significant efficacy in clinical applications. The current review focuses in particular on the clinical data of EGFR tyrosine kinase inhibition in different tumour entities, preferably non-small cell lung cancer and pancreatic cancer with emphasis to the approved small molecule *erlotinib*. Its clinical applications, evidence-based efficacy and toxicity as well as predictive markers of response are discussed.

Keywords

Epidermal growth factor receptor · Erlotinib · Tyrosine kinase inhibitor

1 Introduction

The development of small molecule inhibitors against various tyrosine kinases evoked a new era of antineoplastic agents in cancer therapy besides conventional cytotoxic drugs. The principle of this anticancer treatment is based on the inhibition of receptor tyrosine kinases which are essential components of the intracellular signalling apparatus. Several cellular receptors on the cell surface regulate their signalling via extracellular binding of ligands with consecutive activation of intracellular tyrosine kinase domains and tyrosine phosphorylation. One of these receptors, the epidermal growth factor receptor (EGFR), has gained considerable interest as a possible useful therapeutic target of tumour cells. EGFR is frequently overexpressed in solid tumours and plays a pivotal role in signal transduction pathways involved in cell proliferation, migration, adhesion, angiogenesis induction and apoptosis inhibition. Its overexpression correlates in some tumour entities with disease progression and poorer prognosis (Brabender et al. 2001).

In clinical practice, the uses of the EGFR tyrosine kinase inhibitors (EGFR-TKI) erlotinib (Fig. 1), gefitinib, afatinib and osimertinib have been approved so far for patients with non-small cell lung cancer (NSCLC) for selected indications. In addition, erlotinib combined with gemcitabine has also gained approval for systemic treatment in advanced, non-operable pancreatic carcinoma. The TKI benefit is mainly based on tumour control and overall survival (OS) rather than rapid tumour responses and complete remission rates. In contrast to cytotoxic agents, these responses have been achieved by a specific molecular mechanism disturbing enzyme-mediated signal pathways in cancerogenesis.

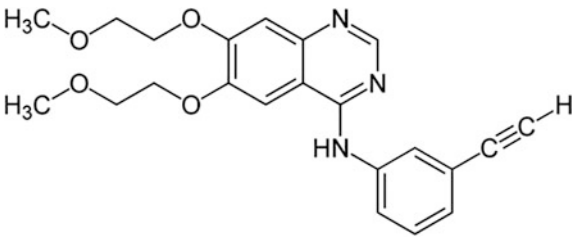

Fig. 1 *Erlotinib*. Chemical formula $C_{22}H_{23}N_3O_4$. Molecular mass 393.436 g/ml. Bioavailability 59%, protein binding 95%, half-life 36.2 h, excretion >90% via faeces, 9% via urine

2 Mechanism of Action

EGFR, the primary therapeutic target for erlotinib, belongs to the human epidermal growth factor receptor (HER) family 1, also known as erbB. The structure of this 170-kDa membrane-spanning glycoprotein consists of an extracellular cysteine-rich ligand-binding region, a transmembrane part and the cytoplasmatic tyrosine kinase domain, which is the binding site for kinase inhibitors like erlotinib. Extracellular binding of ligands like the epidermal growth factor (EGF) and transforming growth factor-alpha (TGF-α) renders the receptor from inactive monomers to active homo- or heterodimers through confirmational changes with subsequent phosphorylation of tyrosine residues (Fig. 2). These phosphorylated tyrosine residues serve as

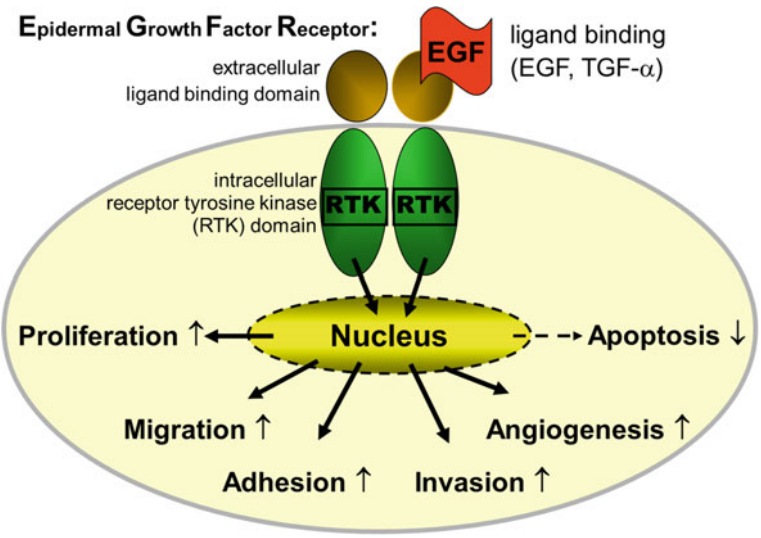

Fig. 2 Function of *epidermal growth factor receptor*: induction of signal transduction pathways by extracellular binding of ligands with consecutive activation of the receptor tyrosine kinase

binding sites for signal transducers with initiation of a cascade of signalling pathways resulting in tumour growth and progression (Salomon et al. 1995; Alroy and Yarden 1997). In contrast, the small molecule TKIs inhibit the intracellular tyrosine kinase of EGFR by competitive and reversible docking at the ATP binding site of the catalytic domain. Subsequently, the autophosphorylation of the receptor is prevented which results in weakening of the downstream signalling pathways (Hynes and Lane 2005). Therefore, signals induced by extracellular ligand binding cannot be conveyed to the tumour cell nucleus where genes involved in cellular differentiation, proliferation and apoptosis are regulated. Consequences are on the one hand reduced potency for tumour cell migration and invasiveness, on the other hand, induction of apoptosis (Fig. 3). This TKI mechanism differs from the active principle of anti-EGFR antibodies like cetuximab, panitumumab or necitumumab which function via a competitive binding to the extracellular domain. But it explains the striking efficacy of EGFR-TKIs in patients with somatic mutations of the EGFR kinase domain, as it targets a key protein in the tumorigenesis of these patients.

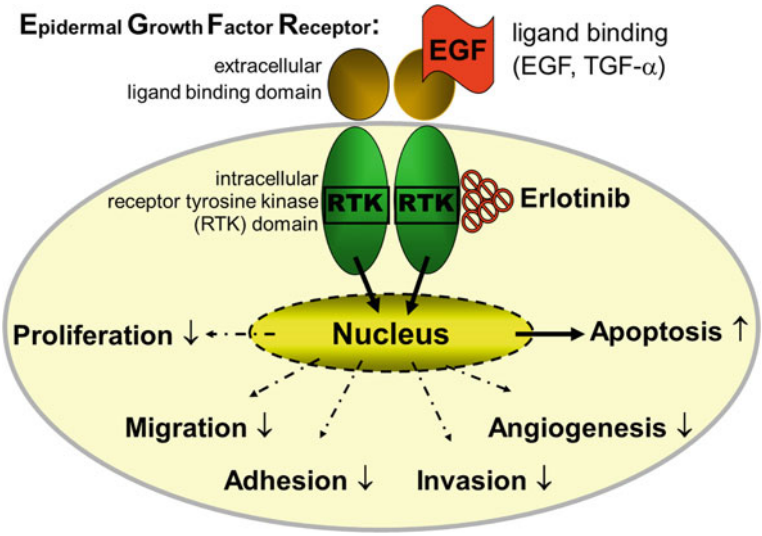

Fig. 3 Activity of *erlotinib*: binding to the intracellular tyrosine kinase domain of the epidermal growth factor receptor and blocking of its ATP binding site. Subsequent disturbance of signal transduction to downstream cascades

3 Non-small Cell Lung Cancer

Lung cancer does not only belong to the most frequent tumour entities in Western countries, it is also in cancer mortality statistics on the first range in men, and on the third (after breast and colorectal cancer) in women. This is the consequence of late detection due to delayed and unspecific symptoms in patients with locally advanced or metastasized disease at the time of first diagnosis. But also in earlier and locally limited tumour stages, the risks for relapse are quite high. Altogether, only 15% of all lung cancer patients survive 5 years after diagnosis despite multimodal therapeutic concepts and new chemotherapeutic agents. Prognosis of the disease still remains serious. Therefore, the development of new agents with different efficacy mechanisms compared to conventional chemotherapy has encouraged the pharmaceutical development. Since 10–15 years, these efforts have led to the emergence of group of TKIs with approvals of the EGFR inhibitors erlotinib, gefitinib, afatinib and osimertinib in advanced NSCLC under certain conditions. In unselected patients, these inhibitors have shown objective tumour responses in 8% up to 19% in particular study groups with prolongation of overall survival of 2 months (Fukuoka et al. 2003; Kris et al. 2003; Pérez-Soler et al. 2004; Shepherd et al. 2005). Especially, this last trial, the BR.21 study of Shepherd et al., has led to the approval of erlotinib in the United States and the European Community in the years 2004 and 2005, respectively, as a TKI for patients with advanced NSCLC who did not respond sufficiently to systemic chemotherapy or suffered a tumour relapse. Approval was based on the data of 731 patients in this randomised, placebo-controlled, multicenter phase III trial performed by the National Cancer Institute of Canada. Oral erlotinib was used as single agent in the second or third therapy line in patients with stage IIIb or IV according to UICC/AJCC. It demonstrated advantage in terms of overall survival and significant release of disease-related symptoms like dyspnoea, pain and cough (Bezjak et al. 2006). Whereas response rates in the erlotinib group comprise only 8.9% with 0.4-month difference in progression-free survival (PFS), the OS—previously defined as the study's primary end point—was 2 months longer compared with the placebo group (6.7 vs. 4.7 months, hazard ratio 0.70, $p < 0.001$). According to the prolongation of median survival, 31% of patients treated with erlotinib in this study were alive at 1 year versus 22% in the placebo group. After a head-to-head comparison of erlotinib against afatinib in patients with squamous NSCLC, afatinib additionally has been approved by the European Medicines Agency (EMA) since 2016 for the second-line therapy of this patient group after the failure of previously applicated, platinum-containing chemotherapy (Soria et al. 2015). As independent clinical predictors for survival non-smoking status, female gender, adenocarcinoma histology and Asian ethnicity have been identified in the BR.21 trial (Tsao et al. 2005), which are often related to the presence of activating EGFR gene mutations. EGFR mutations of the tyrosine kinase domain have been found in 10% up to 17% of NSCLC patients, preferably with adenocarcinoma and non-smoking status (Marchetti et al. 2005; Pao and Miller 2005; Zhu et al. 2008).

These mutations, mainly within the exons 19 and 21 (exon 19 deletion, L858R mutation), are the most relevant biologic factors associated with an improved response to first- and second-generation TKIs like erlotinib (Zhu et al. 2008). Various studies also with afatinib and gefitinib have demonstrated that the presence of EGFR gene mutations within the kinase domain of the receptor correlates with TKI sensitivity (Lynch et al. 2004; Paez et al. 2004; Pao et al. 2004). In addition, analyses of EGFR copy numbers by fluorescence in situ hybridization (FISH) in the BR.21 study revealed high EGFR gene copy as a predictive marker of survival benefit from erlotinib.

On the other hand, erlotinib´s efficacy for OS has also been described in patients not presenting the reported clinical characteristics which are associated with the greatest degree of benefit like non-smoking status, female gender or adenocarcinoma histology.

Gefitinib, another EGFR-TKI, was positively associated with clinical benefits, such as tumour response, health-related quality of life and increased survival, in two large randomised phase II studies (IRESSA Dose Evaluation in Advanced Lung Cancer IDEAL-1 and IDEAL-2) in pretreated NSCLC patients (Fukuoka et al. 2003; Natale 2004). However, it did not result in a statistically significant improvement in OS time in comparison with best supportive care in pretreated NSCLC patients of the ISEL (Iressa Survival Evaluation in Lung Cancer) trial, although in preplanned subgroup analyses a significant survival benefit was shown in never-smokers and Asian patients. In the past, the INTEREST trial (Iressa Non-small cell lung cancer Trial Evaluating Response and Survival against Taxotere) and the INVITE trial (open-label, parallel-group study compared gefitinib with vinorelbine in chemotherapy-naïve elderly patients) met their primary endpoints of demonstrating non-inferiority in terms of overall survival for gefitinib in comparison with docetaxel or vinorelbine (Kim et al. 2008; Crinó et al. 2008). Moreover, patients treated with gefitinib experienced a lower treatment-related toxicity and better improvement in quality of life. Nevertheless, a recently performed second-line trial comparing erlotinib and docetaxel showed significantly worse results in OS and PFS for the TKI applied in EGFR wild-type patients (Garassino et al. 2013).

On the other hand, small molecule EGFR-TKIs have class-specific adverse effects mainly including skin reactions like xerosis, acneiform eruption and eczema or mucosa-associated toxicity like diarrhoea. Rash has been reported in up to 75% of patients treated with these agents in phase II/III clinical trials. The rash that occurs with EGFR-targeted agents is generally mild to moderate; severe (grade 3/4) rash is rare (<10–15% in NSCLC trials). In a number of clinical trials, a positive correlation between severity of rash (grade ≥ 2) and clinical outcome with EGFR-targeted therapy has been demonstrated (Dudek et al. 2006; Pérez-Soler 2006; Cedrés et al. 2009) suggesting rash as a surrogate marker for response. Other side effects have been reported rarely like liver dysfunction or interstitial lung disease (Sandler 2006).

For the first-line treatment of metastatic NSCLC, several phase II and III trials have been conducted utilising EGFR-TKIs in this setting. Patients with advanced NSCLC who are lifelong never-smokers, those with EGFR mutations and/or with bronchioloalveolar cell carcinoma histology seem to have promising efficacy with EGFR-TKI first-line therapy compared with unselected patients receiving the same agents. In fact, based on the data of the I-PASS (Iressa PanASia Study, Mok et al. 2009) the EMA has recommended the approval of gefitinib for mutation-positive NSCLC patients in all treatment lines including upfront therapy. This study performed in never or light former smokers yielded a statistically significant PFS for the gefitinib-treated patient group compared to carboplatin/paclitaxel in first-line therapy of EGFR-mutated NSCLC (HR 0.48, $p < 0.0001$). Similar therapeutic efficacy could be shown for erlotinib in EGFR mutation-positive Chinese (HR 0.16, $p < 0.0001$) and Caucasian (HR 0.37, $p < 0.0001$) patients (Zhou et al. 2011; Rosell et al. 2012). However, in contrast to PFS, no significant differences could be detected in OS when mutation-positive patients were treated with conventional chemotherapy at first and received TKI treatment as second-line therapy (Fukuoka et al. 2011). Nevertheless, quality of life and improvement of symptoms favoured TKI treatment compared to conventional chemotherapy procedures during first-line therapy (Thongprasert et al. 2011; Chen et al. 2013).

Generally, no improvement in survival could be demonstrated in phase III trials when EGFR-TKIs were directly combined with conventional platinum-based doublets, with the exception of subset analysis in non-smokers (Giaccone et al. 2004; Herbst et al. 2005; Gatzemeier et al. 2007). Improvements in response rates and PFS could be shown rather in erlotinib combinations with other targeted agents. In this context, a meta-analysis of 24 phase II/III trials with various erlotinib combination therapies (mainly with targeted drugs) randomised against erlotinib as monotherapy has revealed increased overall response rates and disease control rates as well as longer PFS, but again so far no improvements in OS compared with erlotinib alone (Gao et al. 2017). However, one study, the JO25567 trial, leads to the approval of the anti-vascular endothelial growth factor (VEGF) antibody bevacizumab combined with erlotinib as first-line therapy for EGFR mutation-positive patients (Seto et al. 2014), though grade III or higher adverse events, especially hypertension, increased when using the bevacizumab combination instead of erlotinib alone. But these adverse reactions are known and manageable in anti-angiogenic treatment and did not provoke early drug discontinuation in this trial (Kato et al. 2017).

Future research and development activities try to reduce the risk of TKI failure and define the best sequence therapy in EGFR mutation-positive patients. An important step forward in this respect was the approval of osimertinib in EGFR mutation-positive patients with the acquired T790M resistance mutation (Yang et al. 2017). A subsequent study suggests osimertinib´s application already as first-line therapy in EGFR mutation-positive patients even independent of the presence of T790M mutation (Ramalingam et al. 2017). Further scientific efforts investigate erlotinib in combination with other anti-angiogenic agents like the

VEGF-receptor antibody ramucirumab (Garon et al. 2017). And last but not least, combination studies of EGFR-TKIs also with immune-oncologic agents are under investigation.

4 Pancreatic Adenocarcinoma

Pancreatic cancer is the thirteenth most common cancer and the eighth leading cause of cancer death worldwide (Parkin et al. 2005). Only few patients with pancreatic cancer (15–20%) present with resectable disease, where surgery offers a chance of cure. Following resection for operable pancreatic cancer, the median disease-free survival interval is 13.4 months for patients treated with adjuvant gemcitabine and 6.9 months for untreated patients. The longer median disease-free survival time associated with adjuvant gemcitabine has translated into a significant 5-year overall survival (OS) advantage (21 vs. 9%) (Neuhaus et al. 2008). A much higher percentage of patients, however, present with metastatic disease (40–45%) or unresectable locally advanced disease (40%). These disease stages are characterised by median survival times of 3–6 or 8–12 months, respectively. In locally advanced, unresectable disease, patients typically receive 5-fluorouracil (5-FU)-based chemoradiation or gemcitabine chemotherapy alone. The benefits of chemoradiation over chemotherapy alone in locally advanced disease have not been well established. Erlotinib has been evaluated in two phase I studies using a multimodal chemoradiation approach. One study examined erlotinib plus gemcitabine and paclitaxel plus radiation followed by maintenance with erlotinib and reported a partial response rate of 46% and median survival time of 14 months (Iannitti et al. 2005). These results are supported by the other trial of erlotinib plus gemcitabine and radiation for patients with locally advanced, unresectable pancreatic cancer (Duffy et al. 2008). Single-agent gemcitabine is the standard first-line agent for the treatment of advanced inoperable pancreatic cancer with a marginally superior clinical benefit and survival compared with fluorouracil (FU) approximately 10 years ago (Burris et al. 1997). A number of randomised controlled trials performed over the last decade have aimed to demonstrate superiority of alternative cytotoxic agents and cytotoxic combinations over gemcitabine alone with mostly disappointing results. A recent meta-analysis, however, suggested a survival benefit with a reduction of 9% in risk of death for gemcitabine-based combination chemotherapy (14 trials, 4.060 patients; HR = 0.91; 95% CI, 0.85–0.97) (Sultana et al. 2007). In parallel, our understanding of the underlying genetic and molecular abnormalities that drive the development of pancreatic cancer has expanded significantly over the last decade (Schneider et al. 2008). Alterations to oncogenes and tumour suppressor genes, such as *KRas*, *TP53* and *p16INK4,* are thought to play a critical role in the development of pancreatic cancer. In addition, expression of the human epidermal growth factor receptor (HER-1/EGFR) in pancreatic cancer cells is associated with the stimulation of tumour cell proliferation, poor disease outcomes and lower sensitivity to chemotherapy (Birk et al. 1999; Nicholson et al.

2001; Xiong and Abbruzzese 2002). These observations have allowed for the rational development of targeted therapies for this hard-to-treat disease. However, with the exception of erlotinib, the completed phase III trials have not confirmed an important clinical benefit (Van Cutsem et al. 2004; Moore et al. 2003; Bramhall et al. 2002; Moore et al. 2007; Kindler et al. 2010; Philip et al. 2007; Shapiro et al. 2005). Based on a phase III randomised, placebo-controlled trial (NCIC-CTG study), erlotinib in combination with gemcitabine received US Food and Drug Administration approval as treatment for chemotherapy-naïve locally advanced and metastatic pancreatic cancer in 2005 (Moore et al. 2007). The EMA subsequently licensed erlotinib in combination with gemcitabine restricted for the treatment of patients with metastatic pancreatic cancer only because there was no survival benefit in the locally advanced stage (HR 0.94; 0.63–1.39). In total, 569 patients were randomly assigned in a 1:1 ratio to receive standard gemcitabine plus erlotinib (100 mg/day orally) or gemcitabine plus placebo in this double-blind, international phase III trial. The primary endpoint of a longer OS time was achieved statistically with an HR of 0.82 (95% CI, 0.69–0.99; $p = 0.038$) and a median survival duration of 6.24 versus 5.91 months. Secondary endpoint results from this trial showed a 1-year survival rate of 23% in the erlotinib plus gemcitabine arm, versus 17% with gemcitabine monotherapy ($p = 0.023$). The progression-free survival (PFS) duration was also significantly longer with the combination regimen (3.75 vs. 3.55 months; HR, 0.77; $p = 0.004$). Objective response rates were not significantly different between the arms, although more patients on erlotinib had disease stabilisation. The clinical significance of these efficacy results has been questioned by several investigators and treating physicians. A review of toxicities may further discourage the use of gemcitabine plus erlotinib. Patients receiving erlotinib and gemcitabine experienced higher frequencies of rash (72%), diarrhoea (56%), infection (43%) and stomatitis (23%), generally grade 1 or 2. Grade 3 or 4 toxicities were similar, except for diarrhoea and cutaneous rash, which were more frequent with the two-drug combination (6% each). The six protocol-related deaths were all in the erlotinib–gemcitabine arm. Two were attributed to treatment complications (interstitial pneumonitis and sepsis), and four were attributed to a combination of cancer and protocol treatment complications (interstitial pneumonitis, sepsis, cerebrovascular accident and neutropenic sepsis). Interstitial lung disease was observed in seven patients receiving erlotinib plus gemcitabine and in one patient receiving placebo plus gemcitabine. In fact, there may be an interaction between gemcitabine and erlotinib contributing to increased pulmonary toxicity (Boeck et al. 2007).

An unplanned analysis of the NCIC-CTG study suggested the development of rash as a predictive marker for response to therapy with erlotinib. Patients with advanced pancreatic cancer who experienced grade 2 rash or higher ($n = 102$) had a reported median survival time of 10.5 months and a 1-year survival rate of 43%. Rash development was linked to overall and progression-free survival, and these correlations increased with grade (grade 1 vs. no rash: hazard ratio (HR) 0.47, $p < 0.001$; grade 2 or more vs. no rash: HR 0.29; $p < 0.001$). These data were supported by a combined analysis from two large phase III studies (National Cancer

Institute of Canada Clinical Trials Group Studies BR.21 in non-small cell lung cancer and NCIC-CTG PA.3 in pancreatic cancer). Presence of rash strongly correlated with overall survival in both studies. Similar results were observed for PFS (Wacker et al. 2007). In addition, a retrospective exploratory analysis of the phase III AVITA study (gemcitabine + erlotinib + placebo vs. gemcitabine + erlotinib + bevacizumab) confirmed the results of the NCIC-CTG study (Van Cutsem et al. 2009). In the placebo arm, overall survival was only 4.3 months in patients without rash compared to 7.1 and 8.3 months in patients with grade 1 and grade >1 rash, respectively ($p < 0.0001$). In the NCIC-CTG study, however, rash was also present in 18% of placebo-taking patients with median survival 8.2 months (Moore et al. 2007). Placebo-taking patients who did not develop rash had a median survival of 4.7 months. In the combined treatment arm (gemcitabine plus erlotinib), 81% of the patients developed a rash, compared with 30% of patients in the control group. Since no reliable molecular predictive biomarker exists for the medical treatment of pancreatic cancer physicians and patients should view rash development as a positive event indicative of greater likelihood of clinical benefit. It is important to understand that the development of rash following erlotinib treatment is not an intrinsic effect of erlotinib itself but more likely correlated to individual differences in drug exposure, the integrity of the immune system or EGFR polymorphisms (Saif et al. 2008; Lynch et al. 2007). Further studies are required to identify patients most likely to develop rash and to determine if dose escalation to induce rash can improve efficacy.

How shall we use rash in daily practice? It has been suggested that the rash clinically improves with continuation of treatment. Nevertheless, severe rash development may be a determining cause of treatment discontinuation by patients on erlotinib outside clinical trials. If rash development is in fact a surrogate marker for treatment success, then patients discontinuing treatment are potentially stopping a life-prolonging treatment. This is why it is crucial to exploit all means available in the treatment of the erlotinib-induced skin rash, in order to discourage patients from stopping it. Assessing the tumour response according to RECIST or WHO criteria remains the standard of care independent on the development of rash because there may exist responders without rash and, contrary, patients with a tumour progress despite the development of rash.

Since it is unclear if every patient with advanced pancreatic cancer has to be treated with a combination chemotherapy of gemcitabine and erlotinib, there may be a rationale for sequential therapeutic strategies. Several drugs have been examined as a second-line therapy (Kulke et al. 2007). The most promising chemotherapeutic regimen may be the OFF-protocol consisting of Oxaliplatin, 5-FU and FA. In a randomised phase III study, this combination chemotherapy resulted in a significant survival advantage compared to 5-FU/FA alone (Pelzer et al. 2008). Another option in gemcitabine-pretreated patients would be the combination of erlotinib and capecitabine. In one single-arm phase II study with 32 patients, the median progression-free survival time was 3.4 months, and the median overall survival time was 6.5 months (Kulke et al. 2007). One-year overall survival was 26%. In contrast, disappointing results were reported in a retrospective analysis

of 13 patients treated with single-agent erlotinib (Epelbaum et al. 2007). No responses and a median time to progression (TTP) of only 1 month were observed.

At the current time, gemcitabine, either alone or in combination with erlotinib, remains the only approved first-line treatment for advanced pancreatic carcinoma. Multiple trials are planned that will employ new and novel targeted and biological agents together with the search for predictive biomarkers.

5 Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC) is the third largest cause of cancer-related death third to lung and colon cancers (Parkin et al. 2005). The incidence has increased in the Western world over the past 20 years primarily as a result of the prevalence of hepatitis C virus infection (El-Serag and Mason 1999). Management of HCC is complex and is guided by the Barcelona Liver Clinic (BCLC) staging system, which has important prognostic value (Llovet et al. 1999). The BCLC system is conceptually useful, because it helps to integrate liver function and tumour features into a classification that is meaningful from a standpoint of treatment options. For example, BCLC C patients are those best suited for systemic therapies or clinical trials. Systemic chemotherapy, however, has largely been disappointing in terms of palliation or cure. Cytotoxic chemotherapy has been shown to provide no survival benefit. With that background in mind, the multitargeted tyrosine kinase inhibitor sorafenib was studied in HCC. Patients with advanced stage HCC who were not candidates for, or who had disease progression after locoregional therapy, were enrolled in the Sorafenib Hepatocellular Carcinoma Assessment Randomised Protocol (SHARP) trial (Llovet et al. 2008). The 1-year survival for the sorafenib group was 44 and 33% for the placebo group. The median survival for the sorafenib group was 10.7 months from enrollment compared to 7.9 months for those who received placebo. The survival benefit appeared to be correlated to a 2.7-month delay in radiologic progression (5.5 months for the sorafenib group vs. 2.8 months for the placebo group). A recent phase III study of sorafenib versus placebo in Asian patients reported a similar increase in survival (6.2 vs. 4.1 months) (Cheng et al. 2009). Sorafenib is now considered to be the standard medical treatment for patients with Child-Pugh stage A cirrhosis within the BCLC stage 3 group.

Epidermal growth factor receptor (EGFR) is frequently overexpressed in HCC (Buckley et al. 2008). In a phase II study, erlotinib was evaluated in 38 patients with unresectable or metastatic HCC (Philip et al. 2005). Most frequent grade 3 to 4 toxicities were skin rash (13%), diarrhoea (8%) and fatigue (8%). There was a correlation between the severity (grade 3 or higher) of toxicity and Child-Pugh classification: only 22% of the Child-Pugh A patients experienced severe toxicity compared to 70% of Child-Pugh B patients ($p = 0.02$). 32% of the patients were progression-free after 24 weeks. The overall confirmed response rate was only 9%. Seventeen patients (50%) achieved stabilisation of disease for a median of 3.8 months. There was no correlation between response and EGFR status.

The median overall survival time was 13 months, with a probability of 33% of patients alive at 18 months from entry into the study. In a second phase II study, 40 HCC patients were treated with erlotinib 150 mg daily for 16 weeks (Thomas et al. 2007). There were no complete or partial responses; however, 17 of 40 patients achieved stable disease at 16 weeks of continuous therapy. The PFS at 16 weeks was 43%, and the median overall survival was 43 weeks (10.75 months). No patients required dose reductions of erlotinib. Again, no correlation between EGFR expression and outcome was found.

In contrast to lung cancer, the gain of function in EGFR signalling in HCC seems mediated through increase in ligand–receptor interaction, rather than by point mutations or amplifications (Llovet and Bruix 2008). Erlotinib treatment of HCC might inhibit the mitogen-activated protein (MAP)-kinase pathway and signal transducer of activation and transcription (STAT)-mediated signalling resulting in an altered expression of apoptosis and cell cycle regulating genes (Huether et al. 2006). Overexpression of proapoptotic factors like caspases and gadds associated with a down-regulation of antiapoptotic factors like Bcl-2, Bcl(XL) or jun-D might account for erlotinib's potency to induce apoptosis. In addition, down-regulation of cell cycle regulators promoting the G_1/S-transition and overexpression of cyclin-dependent kinase inhibitors and gadds might contribute to the induction of a G_1/G_0 arrest of HCC cells in response to erlotinib. Together, erlotinib alone appears to have only modest activity against HCC and further randomised studies are needed to evaluate the potential benefit of erlotinib in HCC patients.

There is scientific rationale for combining bevacizumab and erlotinib in HCC (Llovet and Bruix 2008). Overexpression of proangiogenic factors, such as vascular endothelial growth factor (VEGF), platelet-derived growth factor and angiopoietin-2, has been demonstrated in HCC (Llovet and Bruix 2008; Villanueva et al. 2007; Chiang et al. 2008). As mentioned above, there is also a rationale to abrogate EGFR signalling in HCC. Thomas et al. (2009) reported a single-arm phase II study with 40 HCC patients treated with the combination of bevacizumab (10 mg/kg every 14 days) and erlotinib (150 mg daily). Regarding efficacy, objective response rate was 25%, and the median progression-free survival and overall survival times were 9 and 15.6 months, respectively. The results are encouraging but have to be interpreted with caution due to patient selection bias and the small sample size and short follow-up time.

Together, sorafenib is the standard of care in patients with advanced HCC as a result of robust data obtained in the setting of phase III investigations both in the West and Asia. The role of erlotinib and erlotinib combinations has to be explored in randomised phase II and III studies. In fact, a phase III study of erlotinib plus bevacizumab against sorafenib is under consideration within the North American GI Steering Committee Hepatobiliary Task Force.

6 Other Tumour Entities

Erlotinib has been examined in phase I and II studies in malignant glioma and colorectal, biliary, gastric, breast, ovarian, endometrial and renal cell cancer. Efficacy with respect to overall survival and response rates, however, was low. In contrast, single-agent erlotinib or erlotinib-based polychemotherapy may be promising in recurrent or metastatic squamous cell cancer of the head and neck. These studies are discussed in detail elsewhere (Tang et al. 2006).

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Lapatinib

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Abstract

The human epidermal growth factor receptor (HER) family of receptor tyrosine kinases plays an important role in the biology of many cancers. In breast and gastrointestinal cancer, and at lower rates also in additional tumor types, HER2 and its homo- or heterodimerization with HER1 or HER3 are essential for cancer cell growth and survival. Breast cancer patients overexpressing HER2 have a more aggressive course of their disease. The poor prognosis associated with HER2 overexpression can be substantially improved by adding HER2-targeted therapy to standard of care using the monoclonal antibody trastuzumab. Lapatinib, an oral dual tyrosine kinase inhibitor, blocks HER1 and HER2 tyrosine kinase activity by binding to the ATP-binding site of the receptor's intracellular domain, resulting in inhibition of tumor cell growth. Lapatinib is generally well tolerated with diarrhea being the most common adverse effect. However, although being mainly of mild to moderate severity, interruption or discontinuation of treatment has been reported in a substantial proportion of patients in clinical trials. In 2007, lapatinib has been approved in combination with capecitabine in patients with advanced HER2-positive breast cancer upon progressive disease following standard therapy with anthracyclines, taxanes, and trastuzumab. In 2013, the approval was extended to a chemotherapy-free combination with trastuzumab for patients with metastatic HER2-positive, hormone receptor-negative breast cancer progressing on prior trastuzumab and chemotherapy. Since 2010, lapatinib is approved in combination with letrozole in the treatment of postmenopausal women with advanced HER2- and hormone receptor-positive breast cancer. In contrast, in first-line cytotoxic-based therapy of both early and advanced HER2-positive breast cancer, data from clinical trials did not provide evidence of additional benefit of lapatinib compared to trastuzumab. Moreover, over the past few years, novel HER2-targeted drugs, either alone or as a combined anti-HER2 approach, have been extensively evaluated, demonstrating a more favorable outcome. Also, neither in first- nor second-line treatment of advanced gastric cancer, lapatinib has been proven to be superior compared to trastuzumab as hitherto standard of care HER2 blockade. Therefore, lapatinib has become somewhat less important in patients with HER2-positive breast cancer during the past 10 years since its first introduction. Nevertheless, consideration of treatment with lapatinib appears to be reasonable in selected patients not only in the approved applications but also beyond, and further indications such as HER2-positive refractory metastatic colorectal cancer may arise in future. Also, lapatinib may have distinct advantages over antibodies in targeting truncated HER2 and crossing the blood–brain barrier. Finally, the favorable cardiac toxicity profile of lapatinib makes it an attractive alternative to trastuzumab-based regimens in patients at risk for cardiac events.

Keywords

Lapatinib · HER2 · Tyrosine kinase inhibitor · Breast cancer · Gastric cancer

1 Introduction

1.1 The Epidermal Growth Factor Receptor Family of Tyrosine Kinases

The human epidermal growth factor receptor family (HER, EGFR, ErbB) comprises four receptor tyrosine kinases (RTKs): HER1 (=EGFR1 or ErbB1), HER2 (=HER2/c-neu or ErbB2), HER3 (=ErbB3), and HER4 (=ErbB4) (Citri and Yarden 2006; Lemmon and Schlessinger 2010). RTKs consist of an extracellular ligand-binding domain with specific docking sites for various adapter proteins and ligands, a transmembrane domain and an intracellular cytoplasmic domain containing the tyrosine kinase catalytic site. Upon ligand binding, various downstream signaling pathways which are linked to cell proliferation, survival, and apoptosis are activated (Wee and Wang 2017). The receptors are not fixed in the lipid bilayer of the plasma membrane. Therefore, dimerization can and does occur upon ligand binding to the extracellular domain. Such dimers can be homodimers or heterodimers comprised of two different members of the same RTK family (Fig. 1) (Mendelsohn and Baselga 2003). While a large number of ligands for HER1, 3, and 4 have been discovered in the past, no direct ligand for HER2 has been identified so

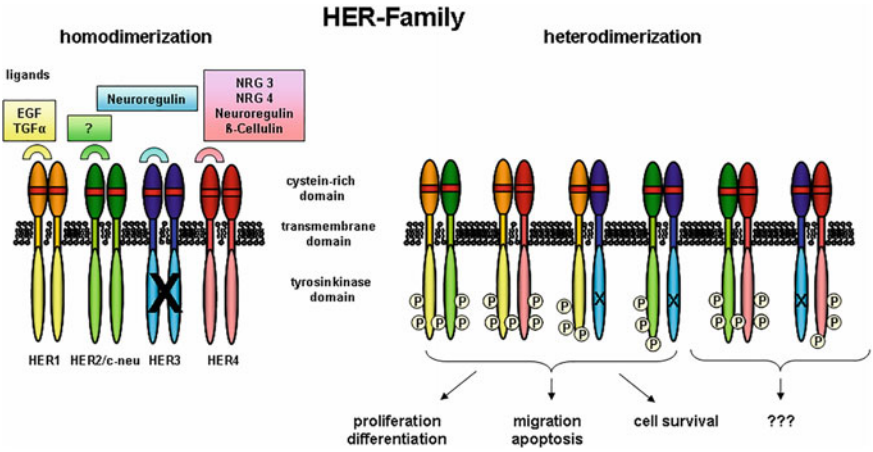

Fig. 1 Organizational principle of the epidermal growth factor receptor family and some dimerization possibilities with corresponding downstream biological events. The left half panel shows the names of the HER family members, depicted as homodimers. The right panel shows heterodimers and downstream effects upon dimerization. P symbolizes phosphorylation. Ligands are shown as semicircles (names in rectangles) and in the color corresponding to the suitable receptor. Note that HER2 does not have a known ligand, it presumably acts mostly as a combination partner for heterodimers. Also, note that HER3 homodimers lack tyrosine kinase activity (indicated by X), but upon ligand binding, the receptor can initiate signal transduction as heterodimer (mainly with the preferred dimerization partner HER2) through the other HER family member's intracellular domain, resulting in multiple downstream effects influencing cell growth and survival

far. However, with its dimerization arm constitutively exposed, HER2 primarily functions as a co-receptor for each of the other ligand-activated EGF receptors (Maruyama 2014). In fact, HER2 is the preferred dimerization partner for all members of the HER family (Graus-Porta et al. 1997), and in contrast to homodimers which are either inactive (like HER3 homodimers) or provide only weak signaling, HER2-containing heterodimers have attributes that prolong and enhance downstream signaling (Tomas et al. 2014).

1.2 Human Epidermal Growth Factor Receptors and Their Inhibition in Cancer

Numerous in vitro and in vivo studies have indicated the functional importance of the HER family in a wide range of cancers as they are often overexpressed and constitutively activated in tumor cells resulting in promotion of their cell proliferation (Hynes and Lane 2005). Hence, development of agents that target these receptors, including monoclonal antibodies like cetuximab, or small molecule inhibitors of the receptor tyrosine kinase (TKIs) such as erlotinib and gefitinib was prompted (Rivera et al. 2008; Kohler and Schuler 2013).

In breast cancer, overexpression of HER1 and HER2, each present in up to 30% of patients is clearly associated with poor prognosis (Ross and Fletcher 1998; Witton et al. 2003, Ansquer et al. 2005). However, the outcome of early and advanced HER2-positive breast cancer patients has been substantially improved upon the addition of trastuzumab, a monoclonal antibody binding to the extracellular domain of HER2, thus inhibiting heterodimerization of HER2 with subsequent activation signals in cancer cells (Slamon et al. 2001; Piccart-Gebhart et al. 2005).

HER2-overexpression also plays a substantial role in gastroesophageal cancer in which about 20% of patients can be identified as HER2-positive (Abrahao-Machado and Scapulatempo-Neto 2016). While previous studies yielded inconsistent findings regarding its prognostic relevance in this entity, a recent meta-analysis demonstrated a significant relation between high HER2 expression and poor prognosis (Zhang et al. 2017). Like in breast cancer, a survival benefit has been achieved by adding trastuzumab to standard first-line chemotherapy in HER2-positive advanced gastroesophageal cancer (Bang et al. 2010).

2 Structure and Mechanism of Action

Lapatinib ditosylate (Fig. 2) is an orally applicable, dual receptor TKI targeting two members of the HER family receptors: HER1 (EGFR1/ErbB1) and HER2/c-neu (ErbB2) (Nelson and Dolder 2006; Medina and Goodin 2008).

Lapatinib interacts intracellularly by reversibly binding to the cytoplasmic ATP-binding site of the tyrosine kinase domain (Fig. 3). Subsequently, phosphorylation and, therefore, activation of the receptor is blocked, resulting in the

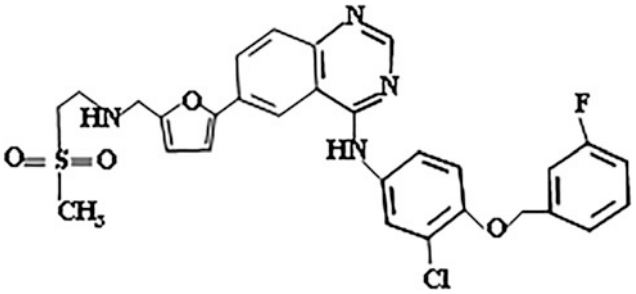

Fig. 2 Chemical structure of lapatinib. Lapatinib is a 4-anilinoquinazoline derivative, distinguishing it from the small head group quinazolines tyrosine kinase inhibitors such as erlotinib and gefitinib

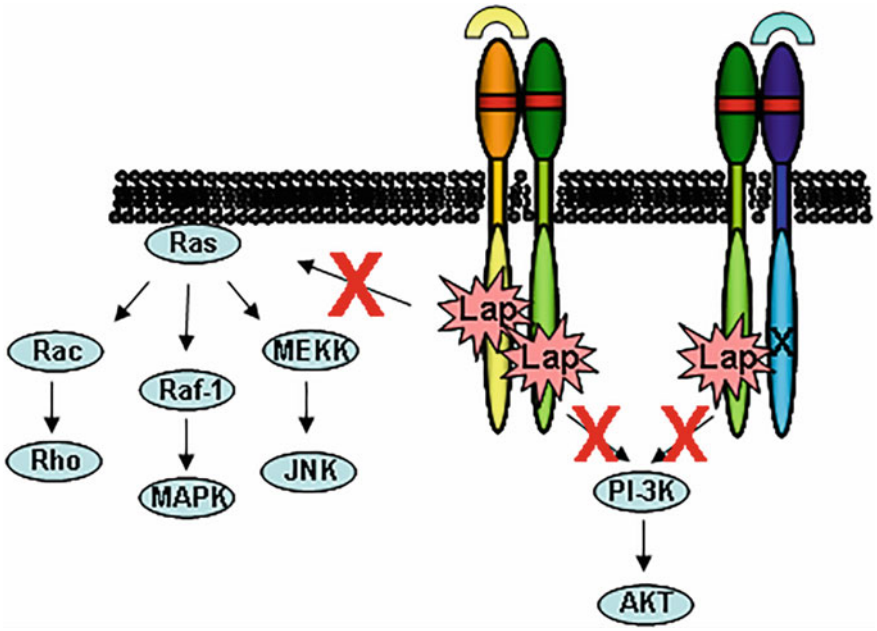

Fig. 3 Intracellular action of lapatinib. Lapatinib binds to the tyrosine kinase domain of HER1 and HER2, blocking the ATP-binding site, and thus preventing (symbolized by "X") the activation of downstream cascades. HER1 is depicted in yellow, HER2 in green, HER3 in blue. Ligands are shown as semicircles in corresponding color. Abbreviations: *Lap* lapatinib; *JNK* Jun-N-terminal kinase; *MAPK* mitogen-activated protein kinase; *MEKK* MAPK/extracellular signal-related kinase (ERK) kinase; *PI3K* phosphatidyl-inositol-3-kinase

inhibition of various downstream signaling cascades such as the extracellular signal-related kinase1/2 (ERK1/2) and the phosphatidylinositol 3′-kinase (PI3K)/AKT pathway, both involved in cell proliferation and apoptosis (Lackey 2006).

By binding the inactive conformation of EGFR, lapatinib differs from other EGFR TKIs such as erlotinib or gefitinib. Furthermore, lapatinib has a slower dissociation rate from HER1 and HER2 than other TKIs. Both could contribute to a greater duration of effect at the target site (Wood et al. 2004).

There are several theoretical advantages of small molecules, inhibiting the tyrosine kinase activity of HER1 and HER2 over monoclonal antibodies such as cetuximab and trastuzumab, targeting the extracellular domain of HER1 or HER2, respectively. In cancer, HER1 and HER2 receptors can be truncated. While still exhibiting tyrosine kinase activity, these truncated forms lack the extracellular domain of the receptors. They are necessarily resistant to the treatment with antibodies binding the extracellular HER domain. Yet, truncated HER2 is still sensitive to the TKI lapatinib (Xia et al. 2004). Another distinctive feature of lapatinib compared to antibody-based anti-HER strategies is its biodistribution. Lapatinib is the first approved small molecule inhibitor with the ability to cross the blood–brain barrier making it suitable for targeting brain metastases (Gril et al. 2008).

In view of the downstream signaling characteristics within the HER family, it is reasonable to assume that agents affecting more than one member of the HER family may suppress cancer cell growth and survival more effectively. First, simultaneous inhibition of HER1 and HER2 may overcome escape mechanisms mediated by redundancy in cell signaling pathways, a form of resistance observed in single tyrosine kinase inhibition, in which upregulation of other members of the HER family occurs (Lin and Winer 2004; Stern 2012). Second, synergistic inhibition of cancer cell growth has been demonstrated upon simultaneous targeting of HER1 and HER2, resulting in a more potent repression of cell growth or greater apoptotic effect compared with inhibiting either HER1 or HER2 alone (Burris 2004). Third, a dual HER1/HER2 TKI may be a useful substrate in a wider range of patients, with regard to the impact of heterodimerization in the progression of a variety of cancer types (Olayioye et al. 2000).

Therefore, the dual HER TKI lapatinib was expected to overcome resistance to monoclonal anti-HER2 antibodies and have superior activity compared to mono-target TKIs. Furthermore, albeit primarily developed for and evaluated in breast cancer, the potential of lapatinib was assumed to reach beyond this disease.

3 Clinical Application

3.1 Pharmacology

Since lapatinib is administered orally, intestinal resorption rates may vary. Intake together with food, particularly high-fat meals, greatly increases its bioavailability (Ratain and Cohen 2007; Devriese et al. 2014). To minimize variability in plasma concentrations, lapatinib intake is recommended under fasting conditions, i.e., no less than 1 h before or at least 1 h after a meal. Following resorption, lapatinib is largely bound to proteins, mainly albumin and acidic alpha1 glycoprotein with peak

plasma levels achieved 3–6 h after administration (Medina and Goodin 2008). With a half-life of approximately 17–24 h when given repeatedly, the drug is administered at a once-daily schedule. Lapatinib is eliminated by hepatic metabolism, primarily through cytochrome P450 isoenzyme, CYP3A4, and biliary excretion. Therefore, inducers or inhibitors of CYP3A4 may alter the metabolism of lapatinib and, vice versa, lapatinib may increase the level of other CYP3A4 substrates (e.g., benzodiazepines and calcium channel blockers) as well as CYP2C8 substrates (e.g., amiodarone and pioglitazone) (GlaxoSmithKline 2007; Medina and Goodin 2008). Furthermore, administration of the drug in patients with impaired liver function, e.g., due to diffuse hepatic metastases, has to be done—if at all—with particular care in a dose-reduced schedule, even though it has not been systemically investigated in this setting so far. The recommended single daily dose of lapatinib is 1250 mg in combination with capecitabine in patients with advanced HER2-positive breast cancer progressing upon therapy with anthracyclines, taxanes, and trastuzumab, 1000 mg in combination with trastuzumab in patients with metastatic HER2-positive, hormone receptor-negative breast cancer upon progression on trastuzumab- and chemotherapy-containing regimens and 1500 mg in combination with hormone therapy for postmenopausal patients with advanced hormone receptor- and HER2-positive breast cancer, respectively (Geyer et al. 2006; Johnston et al. 2009; Blackwell et al. 2010).

4 Results from Clinical Trials

4.1 Efficacy in Breast Cancer

Several preclinical data provided the biological rationale to evaluate lapatinib in patients with HER2-positive breast cancer (Konecny et al. 2006; Nelson and Dolder 2006). A number of phase I–III clinical trials have been conducted in breast cancer at different stages, evaluating lapatinib as a single agent or in combination with other therapeutics including chemotherapy, hormone therapy, or monoclonal anti-HER2 antibodies (Table 1). Phase I clinical trials suggested a favorable side effect profile of lapatinib, revealing good tolerability for the majority of trial participants (Bence et al. 2005; Burris et al. 2005; Chu et al. 2007, 2008). Phase II and III studies demonstrated substantial clinical activity of lapatinib in HER2-positive breast cancer patients, as discussed below.

4.1.1 Second-Line Treatment and Beyond in Advanced Breast Cancer

Based on the results of the pivotal EGF100151 trial, lapatinib was first approved in 2007 by the FDA and in 2008 by the EMA for its combined use with capecitabine in patients with advanced HER2-positive breast cancer after progression upon therapy with anthracyclines, taxanes, and trastuzumab (Geyer et al. 2006). In this open-label phase III trial, patients were randomized to either receive capecitabine

Table 1 Lapatinib in phase I–III clinical trials

Indication	Treatment	Patients (*n*)	Phase	Response (%)			Reference
				CR	PR	SD	
Healthy volunteers	L[1a]	47	I	NA	NA	NA	Bence et al. (2005)
Various solid tumors	L[2b]	59	I	0	7	41◇	Burris et al. (2005)
	X + L[3b]	45	I	2	7	49	Chu et al. (2007)
Breast, ovarian, endometrial cancer	Let + L[3b]	39	I	0	6	59	Chu et al. (2008)
Metastatic breast cancer (second line)	L[4b]	36	II	0	8	14	Blackwell et al. (2004)
	a: X + L[5b]	163	III	<1	21	5◇	Geyer et al. (2006)
	b: X	161		0	14	4◇	
	L[6c]	237	II	0*	6*	37*	Lin et al. (2009)
	a: X + L[5b]	13	II	0*	38*	46*	Lin et al. (2011)
	b: Top + L[5b]	9		0*	0*	33*	
	a: L[4b]	145	III	2	5	28◇	Blackwell et al. (2010)
	b: T + L[7b]	146		1	9	39◇	
	a: X + L[5b]	389	III	0.5	30	d.n.a.	Verma et al. (2012)
	b: T-DM1	397		1	43	d.n.a.	
	a: X + L[5b]	116	II	4	36	23◇	Martin et al. (2013)
	b: N	117		2	27	15◇	
	X + L[5b]	44	II	0*	66*	18*	Bachelot et al. (2013)
	a: X + L[5b]	271	III	3	24	14◇	Pivot et al. (2015)
	b: X + T	269		4	27	12◇	
Metastatic breast cancer (first-line)	*a*: L[4b]	69	II	0	22	58◇	Gomez et al. (2008)
	b: L[8c]	69		0	26	45◇	
	a: P + L[4b]	291	III	5	30	33◇	Di Leo et al. (2008)
	a: P + T + L[7b]	288		2	23	43◇	
	a: Let + Plac	642	III	4	27	25◇	Johnston et al. (2009)
	b: Let + L[4b]	644		5	28	26◇	

(continued)

Table 1 (continued)

Indication	Treatment	Patients (*n*)	Phase	Response (%)			Reference
				CR	PR	SD	
	a: P + T + L^{7b}	29	III	3	76	10◇	Esteva et al. (2013)
	b: P + T + L^{7b}	14		7	64	7◇	
	c: P + T + L^{6b}	20		5	65	10◇	
	a: P + L^{4b}	222	III	7	62	5◇	Guan et al. (2013)
	a: P + Plac	222		3	46	6◇	
	a: Tax + L^{5b}	312	III	54¥	54¥	22◇	Gelmon et al. (2015)
	b: Tax + T	317		55¥	55¥	21◇	
Breast cancer (adjuvant)	*a*: L^{4b}	1,571	III	NA	NA	NA	TEACH (Goss et al. 2012)
	b: Plac	1,576					
	a: T	2,097	III	NA	NA	NA	ALTTO (Piccart-Gebhart et al. 2016)
	b: $L^{4b‡}$	2,100		NA	NA	NA	
	c: T followed by L^{4b}	2,091		NA	NA	NA	
	d: T + $L^{7b‡}$	2,093		NA	NA	NA	
Breast cancer (neoadjuvant)	*a:* P + L^{4b}	154	III	25**	NA	NA	NeoALTTO (Baselga et al. 2012)
	b: P + T	149		30**	NA	NA	
	c: P + T + L^{7b}	152		51**	NA	NA	
	a: EC + D + T	307	III	30**	NA	NA	GeparQuinto (Untch et al. 2012)
	b: EC + D + L^{5b}	308		21**	NA	NA	
	a: P + FEC + T	36	IIb	25**	NA	NA	CHER-LOB (Guarneri et al. 2012)
	b: P + FEC + L^{4b}	38		26**	NA	NA	
	c: P + FEC + T + L^{7b}	45		47**	NA	NA	
	a: Let + L^{4b}	43	IIb	12***	58	23◇	LETLOB (Guarneri et al. 2014)
	b: Let + Plac	49		2***	61	29◇	
	a: P + T	177	III	53**	NA	NA	NSABP (B-41) (Robidoux et al. 2013)
	b: P + L^{5b}	171		53**	NA	NA	

(continued)

Table 1 (continued)

Indication	Treatment	Patients (*n*)	Phase	Response (%)			Reference
				CR	PR	SD	
	c: P + T + L[6b]	171		62**	NA	NA	
	a: P + T	118	III	46**	NA	NA	CALGB 40601 (Carey et al. 2016)
	b: P + L[4b]	64		32**	NA	NA	
	c: P + T + L[7b†]	117		56**	NA	NA	
Advanced gastroesophageal cancer	*a:* P + L[4b]	132	III	27[¥]	27[¥]	d.n.a.	TyTAN (Satoh et al. 2014)
(second-line)	*b:* P	129		9[¥]	9[¥]	d.n.a.	
Advanced gastroesophageal cancer	*a:* XelOX + L[5b]	249	III	2	50	27	LOGiC (Hecht et al. 2016)
(first-line)	*b:* XelOX + Plac	238		2	37	40	
Advanced colorectal cancer	T + L[7b]	27	II	4	26	30	HERACLES (Sartore-Bianchi et al. 2016)
Advanced pancreatic cancer	Gem + L[4b]	29	II	0	10	NA	Safran et al. (2011)
	X + L[5b]	17	II	0	0	35	Wu et al. (2015)

CR complete response; *PR* partial response; *SD* stable disease; *NA* not applicable; *d.n.a* data not available; *a* cohort A; *b* cohort B; *c* cohort C; *d* cohort D
A Doxorubicin; *C* Cyclophosphamide; *D* Docetaxel; *E* Epirubicin; *F* Fluoruracil; *FOLFOX* Oxaliplatin, Folinic acid, Fluoruracil; Gem Gemcitabine; *L* Lapatinib; *Let* Letrozole; *N* Neratinib; *P* Paclitaxel; *Plac* Placebo; *T* Trastuzumab; *Tax* Taxane; *Top* Topotecan; *X* Capecitabine; *XelOX* Capecitabine and oxaliplatin
[a]single and multiple doses, [b]once daily, [c]twice a day
[1]10–175 mg; [2]500–600 mg; [3]1250–1500 mg; [4]1500 mg; [5]1250 mg; [6]750 mg; [7]1000 mg; [8]500 mg
*Data refer to the CNS objective response rate
**Data refer to pathological complete response
***No patient achieved pathological complete response. Data refer to clinical response assessed by ultrasonography, mammography and/or palpation
◇Stable disease for at least 6 months
¥Data refer to overall response rate including patients with complete and partial response
‡During concomitant chemotherapy, lapatinib dosage was reduced to 750 mg once daily
†Due to emerging data regarding exceeding diarrhea, lapatinib was reduced to 750 mg once daily after accrual of 34 patients

alone (201 patients) or a reduced dose of capecitabine and lapatinib (198 patients). Time to disease progression (TTP) was the primary endpoint of this study. A planned interims analysis (Geyer et al. 2006a) revealed 49 disease-progression events in the lapatinib group versus 72 events in the control group, resulting in a 51% risk reduction in time to progression. Based on these data, randomization within this trial was stopped and patients in the control arm could also receive lapatinib in addition to capecitabine. An updated analysis of the trial confirmed the positive results of the interims analysis with TTP improvement from 4.3 to 6.2 months upon addition of lapatinib (hazard ratio (HR) = 0.57, 95% confidence interval (CI) 0.43–0.77, $P < 0.01$) (Cameron et al. 2008). Since premature enrollment termination with subsequent crossover resulted in an insufficient statistical power, exploratory analyses demonstrated only a trend toward a survival advantage with combination therapy (Cameron et al. 2010).

A few years later, the randomized phase III EMILIA trial compared the combination of lapatinib and capecitabine with the novel antibody-drug conjugate trastuzumab emtansine (T-DM1) in patients with HER2-positive advanced breast cancer previously treated with trastuzumab and a taxane (Verma et al. 2012; Dieras et al. 2017). With a significantly prolonged progression-free survival (PFS) as well as overall survival (OS) and less toxicity in the experimental arm, T-DM1 was subsequently approved in 2013 for its use in HER2-positive advanced breast cancer, progressing following treatment with trastuzumab and a taxane. Therefore, in patients with uncontrolled HER2-positive advanced breast cancer upon therapy with trastuzumab regimens, current guidelines recommend lapatinib in combination with capecitabine as a therapeutic option in third- and further-line rather than second-line treatment in trastuzumab-resistant HER2-positive breast cancer (Giordano et al. 2014; Cardoso et al. 2017; Thill et al. 2017).

Combination therapy of lapatinib and capecitabine was also compared to neratinib, an irreversible pan-TKI of HER1, HER2, and HER4, in patients with HER2-positive advanced breast cancer following prior trastuzumab-containing regimens (Martin et al. 2013). In this randomized phase II trial, patients receiving lapatinib with capecitabine showed a nonsignificant prolongation of PFS (6.8 months) as well as OS (23.6 months) compared to patients treated with neratinib (4.5 months, HR 1.19, 95% CI 0.89–1.60 and 19.7 months, HR 1.25, 95% CI 0.83–1.86, respectively). Currently, the phase III NALA trial investigates the efficacy and safety of lapatinib and neratinib each in combination with capecitabine in the metastatic setting of HER2-positive breast cancer following at least two prior HER2-directed regimens (NCT01808573).

A pertinent question concerns the benefit of combining anti-HER2 targeted drugs, whether different monoclonal anti-HER2 antibodies with each other or in addition to a HER TKI. Based on preclinical models of dual anti-HER2 therapy with trastuzumab and lapatinib, an enhanced blockade of HER2 signaling by synergistic interaction and their partly nonoverlapping mechanisms of action was proposed (Konecny et al. 2006; Scaltriti et al. 2009). In the pivotal EGF104900 phase III trial, 291 patients with HER2-positive metastatic breast cancer progressing on a trastuzumab-containing regimen were randomly assigned to receive treatment

with either lapatinib alone or in combination with trastuzumab (Blackwell et al. 2010). With prior anthracycline- and taxane-based chemotherapy as further inclusion criteria and a median of three preceding trastuzumab-containing regimens, patients were heavily pretreated and the majority of patients (73%) had visceral disease. In the final analysis, PFS as the primary endpoint of this study was modestly, yet significantly longer in patients treated with the combination therapy (11.1 weeks) compared to those receiving single-agent lapatinib (8.1 weeks, HR 0.74, 95% CI 0.58—0.94, $P = 0.01$). Further, OS was significantly improved with 14 versus 9.5 months (HR 0.74, 95% CI 0.57–0.96, $P = 0.03$) (Blackwell et al. 2012). Patients with estrogen receptor-positive breast cancer did not have an OS benefit from the combination therapy. In contrast, within the cohort of 163 patients with HER2-positive and hormone receptor-negative breast cancer, an even increased magnitude of effect of the combination therapy was demonstrated compared with lapatinib alone (median OS 16.5 vs. 8.9 months, respectively, HR 0.68; 95% CI 0.47–0.98, $P = 0.12$). Based on these results, in 2013, the EMA extended the approved indication for lapatinib to include its use in the chemotherapy-free combination with trastuzumab in patients with HER2-positive metastatic breast cancer that has recently progressed on trastuzumab-containing regimens. However, due to the lack of comparative data with endocrine-based therapy, thus questioning the benefit in patients with hormone receptor-positive breast cancer, the indication is restricted to patients with hormone receptor-negative tumors.

HER2-overexpression itself is a predictive factor for the development of brain metastases in patients with breast cancer. Yet, trastuzumab does not cross the blood–brain barrier. Therefore, cerebral metastases represent a major problem among patients treated with trastuzumab for metastatic HER2-positive breast cancer, with incidence rates of up to 43% in this group (Clayton et al. 2004; Leyland-Jones 2009). The efficacy of lapatinib in combination with capecitabine in HER2-positive breast cancer patients with previously untreated brain metastases (i.e., not having received lapatinib, capecitabine or whole brain radiotherapy) was first demonstrated in the phase II LANDSCAPE study (Bachelot et al. 2013). Twenty-nine of 44 patients (65.9%) showed an objective CNS response and median overall survival was 17.0 months. In contrast, in patients with prior whole brain radiation therapy and trastuzumab, treatment with single-agent lapatinib resulted in an only moderate clinical benefit with 15 of 237 (6%) patients achieving an objective response and 88 patients (37%) showing stable disease (Lin et al. 2009). However, these results have to be seen in view of a group of patients with very little treatment options and an extremely high risk of disease progression. Moreover, in these extensively pretreated patients, lapatinib given in combination with capecitabine may further increase the objective response rate of brain metastases to about 30%, as shown in a recent meta-analysis (Petrelli et al. 2017). Therefore, lapatinib in combination with capecitabine is a reasonable approach in HER2-positive breast cancer patients with brain metastases not suitable for (re)irradiation therapy (Ramakrishna et al. 2014). In terms of prevention of brain metastases, the randomized phase III CEREBEL study was conducted, comparing the incidence of brain metastases as the first site of relapse in a total of 540 patients with

HER2-positive metastatic breast cancer receiving capecitabine in combination with either lapatinib or trastuzumab (Pivot et al. 2015). Albeit underpowered for its primary endpoint, incidence of new brain metastases did not differ between the lapatinib and trastuzumab arm (3 vs. 5%, $P = 0.36$). However, patients receiving the trastuzumab-regimen showed a significant prolongation of PFS (HR 1.30; 95% CI 1.04–1.64) and a trend toward longer OS time (HR 1.34, 95% CI 0.95–1.90).

4.1.2 First-Line Treatment in Advanced Breast Cancer

In 2010, Lapatinib was approved for first-line treatment of postmenopausal women with hormone receptor-positive metastatic breast cancer that overexpresses the HER2 receptor and for whom hormonal therapy is indicated. This approval was based on the EGF30008 phase III trial, in which 1286 postmenopausal women with hormone receptor-positive metastatic breast cancer, irrespective of HER2 expression status, were randomized to receive either the aromatase inhibitor letrozole alone or in combination with lapatinib (Johnston et al. 2009). In HER2-positive patients ($n = 219$), the addition of lapatinib increased PFS from 3.0 to 8.2 months (HR 0.71, 95% CI 0.53–0.96, $P = 0.02$), while HER2-negative patients ($n = 952$) showed no improvement in PFS. Similar results, albeit slightly less improvement in PFS for HER2-/hormone receptor-copositive metastatic breast cancer patients, could be achieved with the combination of trastuzumab and the aromatase inhibitor anastrozole compared to endocrine therapy alone (Kaufman et al. 2009). Since, however, no direct comparison between the two anti-HER2 drugs in combination with hormone therapy has been performed, it remains unclear, which HER-inhibiting combination partner for endocrine therapy is better. Toward this end, it is also important to note the recently published results of the phase II PERTAIN trial, evaluating a dual anti-HER2 approach with trastuzumab and pertuzumab, a monoclonal antibody inhibiting the dimerization of HER2 with other HER receptors, in addition to hormone therapy (Arpino et al. 2016). Yet, results of an ongoing phase III trial (NCT02344472), further evaluating this therapeutic regimen have to be awaited.

In patients with HER2-positive advanced breast cancer for whom chemotherapy is indicated, lapatinib in combination with taxane-based treatment has been proven to be effective as first-line treatment in several placebo-controlled phase III studies (Di Leo et al. 2008, Guan et al. 2013). Subsequently, a direct comparison of lapatinib and trastuzumab as the hitherto standard combination partner for first-line chemotherapy has been performed in a phase III trial (Gelmon et al. 2015). Therapy-naïve patients ($n = 636$) with HER2-positive metastatic breast cancer were randomized to receive a taxane-based chemotherapy with either lapatinib or trastuzumab, each for 24 weeks, following anti-HER2 monotherapy for 4 years or until progressive disease. After a median follow-up of 21.5 months, patients receiving trastuzumab had a significantly longer PFS (11.3 months) than patients receiving lapatinib (9.0 months; HR 1.37, 95% CI 1.13–1.65, $P < 0.01$). Hence, in patients with HER2-positive metastatic breast cancer, priority as anti-HER2 combination partner to first-line chemotherapy is clearly given to trastuzumab over lapatinib, at least if HER2-targeted treatment is confined to one agent and if there is no

trastuzumab contraindication such as severe cardiac disease. In first-line therapy of HER2-positive breast cancer, the recent randomized phase III CLEOPATRA trial demonstrated a clear improvement in PFS and OS by adding pertuzumab to trastuzumab and docetaxel without further increase of toxicity (Swain et al. 2015). As a result, international guidelines currently recommend this triple therapy regimen as first-line treatment in patients with HER2-positive metastatic breast cancer (Giordano et al. 2014; Gradishar and Salerno 2016; Cardoso et al. 2017). Still, the efficacy and safety of a dual anti-HER2 inhibition with lapatinib and trastuzumab in addition to a taxane-based chemotherapy is evaluated as first-line treatment in HER2-positive metastatic breast cancer in an ongoing randomized, double-blind, placebo-controlled phase III study (NCT00272987). Before initiation of this trial, a safety study with three different dose regimens of paclitaxel, trastuzumab, and lapatinib was conducted in 63 patients with HER2-positive advanced breast cancer, revealing higher rates of severe diarrhea in patients receiving standard doses of lapatinib (Esteva et al. 2013). Therefore, it remains uncertain whether this triple combination offers a manageable safety profile with superior efficacy compared to trastuzumab in combination with pertuzumab and docetaxel in the first-line treatment of HER2-positive advanced breast cancer.

4.1.3 Neoadjuvant Treatment in Early Breast Cancer

Following encouraging first data of lapatinib in the metastatic therapy of breast cancer, a number of trials investigated its role in the neoadjuvant setting.

The randomized phase III GeparQuinto trial evaluated potential benefits of either lapatinib or trastuzumab, each combined with epirubicin and cyclophosphamide followed by docetaxel as chemotherapy backbone prior to surgical removal of the primary tumor in the breast (Untch et al. 2012). Of 309 patients assigned to chemotherapy plus trastuzumab, 30.3% showed pathological complete response (pCR), defined as absence of invasive tumor cells in the breast at the time of surgery. In contrast, only 22.7% of 311 patients receiving chemotherapy with lapatinib showed pCR (OR 0.68, 95% CI 0.47–0.97, $P = 0.04$).

The combination of the two drugs in the neoadjuvant setting was assessed in the open-label, multicenter phase III NeoALTTO study (Baselga et al. 2012). 455 women with HER2-positive early breast cancer were randomly assigned to receive either trastuzumab and lapatinib or each drug individually, both in combination with paclitaxel chemotherapy. The rate of pCR was significantly higher in the cohort with combined anti-HER2 therapy (51.3%) than in the group given trastuzumab alone (29.5%; difference 21.1%, $P < 0.01$) with no significant difference between the trastuzumab and lapatinib group (24.7%, $P = 0.34$). However, although not powered to detect significant differences in terms of survival outcomes, neither 3-year event-free survival nor 3-year OS significantly differed between patients treated with combination therapy (84% and 95%, respectively) and those assigned to trastuzumab mono (76% and 90%, respectively) (de Azambuja et al. 2014).

Further, evidence of superior efficacy of a neoadjuvant dual anti-HER2 treatment approach with trastuzumab and lapatinib was provided by the randomized phase IIb

CHER-LOB (Guarneri et al. 2012) as well as phase III NSABP B-41 (Robidoux et al. 2013) and the CALGB 40601 trials (Carey et al. 2016).

Finally, in a recent meta-analysis, including 1155 patients with HER2-positive breast cancer from a total of six randomized trials, neoadjuvant chemotherapy combined with trastuzumab and lapatinib as dual HER2 blockade was associated with a significant 13% increase in pCR rate compared to chemotherapy with trastuzumab alone (Clavarezza et al. 2016). Interestingly, similar to the pivotal EGF104900 trial, a greater benefit was seen in patients with hormone receptor-negative breast cancer compared to those with hormone receptor-positive status, indicating an inhibitory cross talk between endocrine and HER2 pathways (Li et al. 2015).

In conclusion, dual inhibition of HER2 seems to be a valid approach to neoadjuvant treatment of HER2-positive breast cancer. However, concurrently to lapatinib, efficacy and safety of pertuzumab as further HER2-targeting combination partner to trastuzumab was investigated in the neoadjuvant treatment of HER2-positive breast cancer too. Largely based on the phase II NeoSphere trial (Gianni et al. 2012) demonstrating a significant increase in complete response rate, current guidelines recommend consideration of dual HER2 blockade with trastuzumab and pertuzumab together with chemotherapy for neoadjuvant treatment of HER2-positive breast in patients with locally advanced breast cancer (Senkus et al. 2015; Gradishar and Salerno 2016; Liedtke et al. 2017). Dual anti-HER2 therapy with lapatinib in the preoperative setting of HER2-positive breast cancer should be restricted to highly selected cases only (e.g., contraindication to trastuzumab).

4.1.4 Adjuvant Treatment in Early Breast Cancer

To address the role of lapatinib in the adjuvant setting, the TEACH trial, a large randomized phase III study, has been performed (Goss et al. 2012). A total of 3147 women with HER2-positive early breast cancer who had completed trastuzumab-free adjuvant chemotherapy and had no evidence of disease were randomly assigned to receive daily lapatinib or placebo for up to 12 months. After a median follow-up of 4 years, disease-free survival (DFS) events occurred in 13% in the lapatinib group and 17% in the placebo group (HR 0.83, 95% CI 0.70–1.00, $P = 0.053$), thus very closely not meeting the prespecified criteria for statistical significance. Exploratory analyses restricted to patients with centrally confirmed HER2-positive status (78% in the lapatinib group and 80% in the placebo group) indicated a significant, though marginal benefit for patients receiving lapatinib (HR 0.82, 95% CI 0.67–1.00, $P = 0.04$). In terms of therapy onset, subgroup analyses showed a slight improvement in DFS in patients starting lapatinib treatment within 1 year of initial diagnosis (HR 0.70, 95% CI 0.50–0.99, $P = 0.04$).

Efficacy and safety of adjuvant treatment with lapatinib has also been evaluated as part of a dual anti-HER2 blockade with trastuzumab in the phase III ALTTO-study (Tomasello et al. 2008). A total of 8381 patients with HER2-positive early breast cancer were randomly assigned to either receive one year of adjuvant trastuzumab alone, lapatinib alone or the combination of both drugs, simultaneously as well as in sequential order. Based on a preplanned interim analysis in 2011

failing to demonstrate non-inferiority of single-agent therapy with lapatinib in terms of DFS as primary end point, lapatinib monotherapy was discontinued early. In 2015, final results with a median follow-up of 4.5 years were published (Piccart-Gebhart et al. 2016). Compared to standard therapy with trastuzumab alone, dual anti-HER2 treatment, either concurrently or sequentially given, did not result in a significant improvement of DFS (HR 0.84, 95% CI 0.70–1.02, $P = 0.05$ and HR 0.96, 95% CI 0.80–1.15, $P = 0.61$, respectively).

As a result, lapatinib, neither as single-agent nor in combination with trastuzumab can be recommended in the adjuvant treatment of HER2-positive early breast cancer (Senkus et al. 2015; Liedtke et al. 2017).

4.2 Efficacy in Gastrointestinal Cancer

Preclinical and early clinical evidence showed promising activity of lapatinib not only in breast cancer but also in HER 2-overexpressing gastroesophageal cancer cell lines (Kim et al. 2008; Wainberg et al. 2010). In contrast, clinical trials revealed only limited efficacy of lapatinib in patients with gastric cancer (Table 1). The Asian randomized phase III clinical trial TyTAN evaluated the benefit of adding lapatinib to paclitaxel as second-line therapy in patients with advanced gastric cancer who were HER2-positive by fluorescence in situ hybridization (FISH) (Satoh et al. 2014). In the intent-to-treat population ($n = 261$), median overall survival was superior upon combined treatment compared to paclitaxel alone (11 vs. 8.9 months) but this was only significant in the subgroup of patients with HER2 immunohistochemistry (IHC) 3+ (14 vs. 7.6 months, HR 0.59, 95% CI 0.37–0.93, $P = 0.02$). Moreover, lapatinib was also evaluated in the first-line treatment of HER2-positive advanced esophagogastric cancer. In the phase III LOGiC trial, a total of 545 patients were randomized to receive capecitabine and oxaliplatin in combination with lapatinib or chemotherapy alone (Hecht et al. 2016). With a median overall survival of 12.2 months in the lapatinib arm compared to 10.5 months in patients treated with chemotherapy only, there was no significant improvement in overall survival (HR 0.91, 95% CI 0.73–1.12, $P = 0.35$). However, overall response rate was significantly higher in the lapatinib cohort (53 vs. 39%, $P < 0.01$). In conclusion, in HER2-positive metastatic gastric cancer, lapatinib does not seem to generally provide additional value over current standard of care with chemotherapy and trastuzumab. Yet, a small subset of patients may benefit from lapatinib, albeit valid biomarkers are mandatorily needed for further identification of these patients.

Besides its clinical impact in esophagogastric cancer, there are interesting data from preclinical investigations on dual HER2 inhibition in colorectal cancer. The combination of lapatinib with trastuzumab led to sustained inhibition of tumor growth in patient-derived cetuximab-resistant xenografts of HER2-positive metastatic colorectal cancers (Bertotti et al. 2011). Consequently, clinical investigation of this dual anti-HER2 blockade was performed within the phase II HERACLES study, recruiting patients with KRAS exon 2 wild-type, HER2-positive metastatic

colorectal cancer refractory to standard of care including cetuximab or panitumumab (Sartore-Bianchi et al. 2016). Forty-eight of 914 patients (5%) were found to be HER2-positive with 27 of them finally enrolled. At a median follow-up of 94 weeks, 8 patients (30%) achieved an overall response rate and 12 patients (44%) showed stable disease. Combination therapy was generally well tolerated with maximum grade 3 adverse events in six patients (22%). Thus, although eligible for only a minor subgroup of metastatic colorectal cancer patients, combination therapy of lapatinib and trastuzumab may be an effective therapeutic option in these heavily pretreated patients.

HER2 overexpression can also be demonstrated in about 20% of patients with pancreatic adenocarcinoma. However, in a phase II trial evaluating lapatinib in combination with gemcitabine in metastatic pancreatic cancer, a planned six months analysis showed only minor clinical benefits with solely three of 29 patients (10%) achieving partial response (Safran et al. 2011). Furthermore, in a cohort of 17 patients with gemcitabine-refractory metastatic pancreatic cancer, receiving second-line treatment with capecitabine and lapatinib within a phase II trial, none of the patients attained a response and only six patients (35%) showed stable disease (Wu et al. 2015). In terms of the minor clinical benefits and the limited number of study participants so far, there is currently no indication of lapatinib in the treatment of metastatic pancreatic cancer.

4.3 Tolerability

In healthy volunteers, oral administration of lapatinib revealed good tolerability (Bence et al. 2005). Commonly reported side effects included diarrhea, skin rash, and headache. In a phase I, dose escalation study of 67 heavily pretreated HER2-positive cancer patients, the main toxicity was grade 1 and 2 diarrhea (Burris et al. 2005). With linear relation to the dosage of lapatinib over the 500–1600 mg range, but not to serum concentration, diarrhea may rather evolve from direct toxic effects on the intestinal epithelium.

In the pivotal EGF100151 trial, diarrhea, dyspepsia, and skin rash, the most common adverse events occurred significantly more frequently in patients receiving capecitabine plus lapatinib than capecitabine alone (Geyer et al. 2006). However, these differences were largely due to an increase in grade 1 events and adverse event-related discontinuation of therapy was similar in both treatment arms (13% in the combination arm vs. 12% in the monotherapy group).

In the approval trial for postmenopausal women with hormone receptor-positive metastatic breast cancer, the combination of lapatinib and letrozole caused grade 3 and 4 diarrhea in 10% of patients compared to 1% of patients receiving letrozole alone, resulting in discontinuation (15%) or interruption of therapy (36%), dose reduction (19%), or supportive treatment without dose adjustments (31%) (Johnston et al. 2009).

In the EGF104900 trial, 41% of patients receiving single-agent lapatinib and 60% of those treated with the combination of lapatinib and trastuzumab

experienced grade 1 or 2 diarrhea, while the incidence of diarrhea grade 3 or higher was similar with 7% in both groups. Besides, all other adverse events including those reported in $\geq 10\%$ of patients such as rash, nausea, and fatigue did not differ between single- and combined HER2-therapy. Adverse events led to permanent discontinuation of treatment in 11% of patients receiving the combination regimen compared to 6% treated with single-agent lapatinib.

In all phase III trials evaluating lapatinib in the neoadjuvant setting of breast cancer, grade 3 adverse events, mainly diarrhea, with subsequent discontinuation of therapy were more common in patients receiving lapatinib with or without trastuzumab than in patients receiving trastuzumab alone (Baselga et al. 2012; Guarneri et al. 2012; Untch et al. 2012; Robidoux et al. 2013; Carey et al. 2016). Further, in the phase III ALTTO trial, all lapatinib-containing adjuvant treatment arms were associated with more adverse events compared to trastuzumab monotherapy. The most common side effects of lapatinib resulting in dose modification or interruption were diarrhea, rash, and neutropenia.

Taken together, diarrhea is a frequent problem upon therapy with lapatinib, although symptoms appear to be mostly manageable. However, particularly in combination with chemotherapy, more severe diarrhea may result in treatment limitations.

Therapy with lapatinib is less frequently associated with cardiac failure than treatment with trastuzumab, in which reduction in left ventricular output has been a significant concern, prevents simultaneous treatment with anthracyclines, and excludes patients with coexisting cardiac failure (Xin et al. 2016; Choi and Chang 2017). Still, because cardiac events were slightly more common in patients receiving lapatinib than in patients in control arms, routine evaluation of cardiac function is usually recommended before initiating treatment with lapatinib (Dias et al. 2016).

Additionally, quite infrequently reported adverse events upon treatment with lapatinib were hepatotoxicity and interstitial pneumonitis (GlaxoSmithKline 2007; Guarneri et al. 2012). Therefore, routine laboratory evaluation of liver function and clinical observation of pulmonary function are recommended before and during treatment with lapatinib.

However, altogether, life-threatening events (grade 4) or death (grade 5) attributable to lapatinib treatment seem to be very rare (Moy and Goss 2007).

5 Biomarkers

Accurate assessment of HER2 status is mandatory to predict a potential response to anti-HER2 treatment. To define HER2 positivity, either HER2 protein overexpression or gene amplification has to be determined by IHC or (F)ISH, respectively.

In terms of IHC, results of HER2 testing are categorized in a four scale score (range, 0 through 3+), based on the percentage of positive tumor cells and staining intensity. According to the ASCO/CAPs guidelines, a HER2 IHC score of 0 to 1+ is

considered as HER2-negative, while all patients with a score of 3+ should be determined as HER2-positive. Patients with a IHC score of 2+ are regarded equivocal, demanding further assessment by (F)ISH (Wolff et al. 2014). Since HER2 expression essentially differs between breast and gastric cancer with regard to membranous distribution of the antibody and intratumoral heterogeneity, different IHC scoring systems have been proposed (Hofmann et al. 2008).

Generally, high concordance exists between both methods (Bahreini et al. 2015). However, in breast cancer patients with dissenting HER2 test results, HER2 amplification by FISH seems to characterize HER2 status more accurately compared to at least some frequently used IHC assays and may predict benefit from lapatinib treatment more precisely than IHC-defined HER2 protein overexpression (Press et al. 2002, 2008). Conversely, in gastric cancer, patients not only showing HER2 amplification by FISH but also high HER2 protein expression (i.e., IHC3+) seem to benefit most from anti-HER2 treatment (Bang et al. 2010; Satoh et al. 2014).

However, even in precise assessment of HER2 status by (F)ISH and IHC, HER2 amplification and overexpression appear to be necessary, yet not always sufficient for response to anti-HER2 drugs. Therefore, additional biomarkers are urgently needed to increase the positive predictive value of HER2. In this regard, continuous quantitative measurement of HER2 protein expression may be more useful for accurate stratification of patients with respect to response to (dual) HER2-targeted treatment approaches than semiquantitative protein measurements by IHC (Scaltriti et al. 2015). In addition, assessment of serum HER2 extracellular domain levels (ECD) may not only be useful as an overall prognosis tool at diagnosis of HER2-positive breast cancer but also in monitoring the efficacy of anti-HER2 treatment, as previously shown in the neoadjuvant setting with trastuzumab (Reix et al. 2016). Moreover, somatic HER2 mutations should be further explored as predictors of response or resistance to specific HER2-targeted drugs (Bose et al. 2013).

6 Conclusion and Future Perspectives

Patients with HER2-positive breast cancer have a high risk of disease progression upon treatment with conventional chemotherapeutic drugs but benefit enormously of the additional HER2-targeted therapy with trastuzumab. In the past, failure to respond to trastuzumab-containing regimens has posed a therapeutic dilemma to patients and clinicians. Lapatinib in combination with capecitabine was the first alternate anti-HER2 treatment approach to demonstrate further efficacy in patients with advanced HER2-positive but trastuzumab-resistant breast cancer. Moreover, dual anti-HER2 blockade with continuation of trastuzumab and lapatinib offers a significant survival benefit in trastuzumab-pretreated patients with uncontrolled metastatic HER2-positive, hormone receptor-negative breast cancer. In addition, lapatinib is an effective combination partner with hormonal therapy in postmenopausal women with advanced, triple-positive breast cancer.

However, there also have been major drawbacks in the evaluation of the efficacy of lapatinib; In first-line therapy of breast cancer, single-agent lapatinib in combination with standard chemotherapy did not show superiority over trastuzumab-containing regimens in both, early and advanced HER2-positive breast cancer, but resulted in higher toxicity with subsequent treatment interruption in, an albeit, small proportion of patients. Furthermore, favorable data with novel anti-HER2 antibodies, antibody-drug conjugates, and second-generation irreversible HER TKIs such as pertuzumab, T-DM1, and neratinib, respectively, have recently emerged, postponing lapatinib to third line and beyond rather than first or second line in the treatment of HER2-positive breast cancer. In the treatment of advanced HER2-positive gastric cancer, lapatinib showed only minor benefit compared to trastuzumab.

Despite some restrictions, one may speculate about the future role of lapatinib in the treatment of HER2-positive cancer as some open questions remain. These include: (i) with ongoing evaluation of modern anti-HER2 drugs in the treatment of early and advanced HER2-positive breast cancer, which position will lapatinib adopt in the sequence of therapeutic options?, (ii) is lapatinib plus capecitabine an equal or even more efficient alternative to trastuzumab in the special setting of patients with HER2-positive breast cancer and brain metastases?, (iii) in advanced triple-positive breast cancer, is lapatinib in combination with letrozole inferior to dual anti-HER2 therapy with trastuzumab and pertuzumab together with endocrine therapy?, (iv) does the benefit outweigh the risks of dual anti-HER2 therapy with trastuzumab and lapatinib in combination with chemotherapy in the treatment of advanced HER2-positive breast cancer?, (v) what is the role of a potential triple HER2-targeted therapy using lapatinib, trastuzumab, and pertuzumab with or without concomitant chemotherapy?, (vi) will lapatinib become an effective treatment approach in HER2-positive advanced colorectal cancer progressing upon standard of care including anti-EGFR antibodies?, and (vii) which biomarkers can accurately predict response to HER2-targeted therapies?

Many of these topics will be addressed in ongoing or future clinical trials. The results are eagerly awaited, but may not be available for quite some time. Until then, the combination of lapatinib with capecitabine or trastuzumab in HER2-positive (and hormone receptor-negative, respectively) advanced breast cancer, progressing upon treatment with trastuzumab, as well as the combination of lapatinib with letrozole as first-line therapy in triple-positive breast cancer are the only approved, but probably not the only effective applications of this agent.

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Regorafenib

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Abstract

Regorafenib (BAY 73-4506, Stivarga®) is an oral diphenylurea multi-kinase inhibitor that targets angiogenic (VEGFR1-3, TIE2), stromal (PDGFR-β, FGFR), and oncogenic receptor tyrosine kinases (KIT, RET, and RAF). Regorafenib is the first small-molecule multi-kinase inhibitor to achieve survival benefits in metastatic colorectal cancer that has progressed after all standard therapies. Consequently, Regorafenib was FDA approved for this indication in 2012. In addition, Regorafenib treatment resulted in a significant improvement in progression-free survival (PFS) compared to placebo in patients with metastatic gastrointestinal stromal tumors (GIST) after progression on standard treatments and is also FDA-approved in this indication since 2013. In 2017, Regorafenib has been FDA approved for the treatment of patients with advanced hepatocellular carcinoma (HCC) previously treated with Sorafenib. In this situation, Regorafenib significantly improved PFS and overall survival (OS) compared to placebo. Regorafenib has also been examined in several clinical trials (mostly phase II) in different tumor entities, including renal cell carcinoma (RCC), soft-tissue sarcoma (STS), and additional phase II trials ongoing (e.g., second- and third-line treatment for medullary thyroid cancer, NCT02657551).

Keywords

Regorafenib · CRC · HCC · GIST · TKI

1 Structure, Mechanism of Action, and Pharmacokinetics

1.1 Mechanism of Action

Regorafenib (see Fig. 1) is a small-molecule inhibitor of various membrane-bound and intracellular kinases involved in normal cellular functions as well as pathologic processes, such as oncogenesis, tumor angiogenesis, and maintenance of the tumor microenvironment. In biochemical in vitro or cell-based assays, Regorafenib or its major human active metabolites M-2 and M-5 inhibited the activity of RET, VEGFR 1-3, KIT, PDGFR-alpha, PDGFR-beta, FGFR1, FGFR2, TIE2, DDR2, TrkA, Eph2A, RAF-1, BRAF, BRAFV600E, SAPK2, PTK5, and Abl at concentrations that can be achieved clinically.

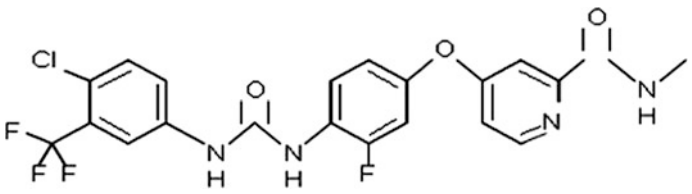

Fig. 1 Chemical structure of Regorafenib

1.2 Pharmacokinetics and Elimination

The standard dose of Regorafenib is 160 mg taken orally once daily as tablets. The mean relative bioavailability of orally taken Regorafenib is 69%. Regorafenib undergoes enterohepatic circulation with multiple plasma concentration peaks observed across the 24-h dosing interval. Regorafenib is highly bound (99.5%) to human plasma proteins and metabolized by CYP3A4 and UGT1A9. The main circulating metabolites of Regorafenib in human plasma are M-2 (*N*-oxide) and M-5 (*N*-oxide and *N*-desmethyl). Both metabolites have similar in vitro pharmacological activity and are highly protein bound (>99.0%). The elimination half-life for Regorafenib and its M-2 metabolite in plasma are 28 and 25 h, respectively. M-5 has a longer elimination half-life of 51 h. Approximately, 71% of a radiolabeled dose of Regorafenib is excreted via feces and 19% of the dose is excreted via urine. Based on a population pharmacokinetic analysis, there is no clinically relevant effect of age, gender, or weight on the pharmacokinetics of Regorafenib.

1.3 Regorafenib in Renal or Hepatic Impairment

Because of its major elimination via feces, no differences in the mean steady-state exposure to Regorafenib, its M-2 or M-5 metabolites were observed in patients with mild renal impairment compared to patients with normal renal function. There are no clinical data for patients with severe renal impairment or end-stage renal disease. There were no clinically important differences observed in the mean exposure to Regorafenib, M-2, or M-5 in patients with mild or moderate hepatic impairment (Child-Pugh A and B) compared to the patients with normal hepatic function. There are no clinical data for patients with severe hepatic impairment (Child-Pugh C).

2 Preclinical Data

Regorafenib is a multitarget small-molecule compound with potent activity against a number of angiogenic and stromal TKs (tyrosine kinases), including VEGFR-2, TIE2, FGFR-1, and the PDGFR. Regorafenib also blocks KIT, RET, wild-type, and V600 mutant BRAF. An antiangiogenic effect of Regorafenib was demonstrated

in vivo by dynamic contrast-enhanced magnetic resonance imaging. Regorafenib administered once orally at 10 mg/kg significantly decreased the extravasation of Gadomer, an intravascular macromolecular MRI contrast agent, in the vasculature of rat GS9L glioblastoma tumor xenografts (Wilhelm et al. 2011). In a daily dosing study, the pharmacodynamic effects persisted for 48 h after the last dosing and correlated with tumor growth inhibition (TGI). A significant reduction in the tumor microvessel area was observed in a human colorectal xenograft after daily dosing at 10 and 30 mg/kg.

Regorafenib exhibited potent dose-dependent TGI in various preclinical human xenograft models in mice with tumor shrinkage observed in breast MDA-MB-231 and renal 786-O carcinoma models (Wilhelm et al. 2011). Pharmacodynamic analyses of the breast cancer model revealed a strong reduction in Ki-67 immunoreactivity (a proliferation marker) and phosphorylation/activation of ERK 1/2.

Various low concentrations of Regorafenib were examined in vitro in two human HCC cell lines with respect to its effects on alpha-fetoprotein (AFP) levels, cell growth, migration, and invasion (Carr et al. 2013). AFP secretion was inhibited at 0.1–1 μM Regorafenib. Cell migration and invasion were inhibited at similar drug concentrations. Interestingly, a 10-fold higher drug concentration was required to inhibit cell growth in both AFP-positive and AFP-negative cell lines (Carr et al. 2013). These data demonstrate that Regorafenib is an active multi-kinase inhibitor with a distinct target profile.

3 Clinical Data

3.1 Regorafenib in Metastatic Colorectal Cancer (mCRC)

There are limited treatment options available for patients with metastatic colorectal cancer progressing after all approved standard therapies. However, many patients maintain a good performance status and are eligible for further therapy. An international phase III trial, the CORRECT trial (Grothey et al. 2013), was performed to assess Regorafenib in patients with mCRC progressing after all approved standard therapies. 760 patients with a good performance status (ECOG 0 and 1) were randomized in a 2:1 ratio to receive Regorafenib (n = 505) or placebo (n = 255), respectively. The primary endpoint of overall survival was met at a preplanned interim analysis. The median overall survival was 6.4 months in the Regorafenib group versus 5.0 months in the placebo group (hazard ratio 0.77; 95% CI 0.64–0.94; one-sided p = 0.0052). Treatment-related adverse events occurred in 465 (93%) patients assigned to Regorafenib and in 154 (61%) of those assigned to placebo. The most common adverse events of grade 3 or higher related to Regorafenib were hand–foot skin reaction (83 patients, 17%), fatigue (48, 10%), diarrhea (36, 7%), hypertension (36, 7%), and rash or desquamation (29, 6%). Thus, Regorafenib is the first small-molecule multi-kinase inhibitor with survival benefits

Table 1 Results from the CORRECT trial (Grothey et al. 2013) in patients with colorectal cancer

	Regorafenib (n = 500)	Placebo (n = 253)	
mOS (month)	6.4	5.0	HR 0.77; 95% CI 0.64–0.94; p = 0.0052
mPFS (month)	1.9	1.7	HR 0.49, 95% CI 0.42–0.58, p < 0.0001
CR	0	0	
PR	4%	1%	(p = 0.19)
DCR	41%	15%	(p < 0.0001), 6 weeks after randomization

mOS Median overall survival, *mPFS* progression-free survival, *HR* hazard ratio, *CI* confidence interval, *CR* complete remission, *PR* partial remission, *DCR* disease control rate

in mCRC progressing after all standard therapies. The FDA approved the use of Regorafenib for this indication in September 2012 (Table 1).

The combination of Regorafenib with FOLFOX or FOLFIRI as first- or second-line treatment of mCRC was tested in a multicenter, phase Ib study (Schultheis et al. 2013) with 45 patients. Safety and pharmacokinetics were the primary objectives, and tumor response was the secondary objective. Patients were treated every 2 weeks with mFOLFOX 6 or FOLFIRI. On days 4–10, patients received Regorafenib 160 mg orally once daily. The median duration of treatment was 108 days (range 2–345 days). Treatment was stopped for adverse events or death (17 patients), disease progression (11 patients), and withdrawal of consent or by investigators decision (11 patients). Drug-related adverse events occurred in 44 patients ($\geq$ grade 3 in 32 patients: mostly neutropenia and leukopenia, hand–foot skin reaction, and hypophosphatemia). Thirty-three patients achieved disease control (partial response or stable disease) for a median of 126 days (range 42–281 days). With 71% $\geq$ grade 3 toxicity in this small study, Regorafenib exhibited a not ideal tolerability in this setting. Another phase II trial examining mFOLFOX6 in combination with Regorafenib as first-line treatment of mCRC failed its primary endpoint and showed no difference in the response rate compared to historical controls (Argiles et al. 2015).

In the second-line setting, a phase II trial examined Regorafenib (R) in combination with FOLFIRI (F) versus Placebo (P) + FOLFIRI (O'Neil et al. 2016). 181 patients were enrolled, and 62.5% had prior anti-VEGF treatment. Median PFS was 6.14 months for the combination of F plus R versus 5.29 months for F plus P (HR 0.69, log-rank p = 0.02). Median OS was 13.2 months and 12 months, and RR was 32% versus 19% for F plus R versus F plus P, respectively (HR 1.06, p = 0.76). Thus, Regorafenib in combination with FOLFIRI could improve PFS compared to FOLFIRI alone in this setting. The higher efficacy has to be balanced against a higher toxicity: There was more grade $\geq$ 3 neutropenia, diarrhea, hypophosphatemia, fatigue, HTN, and hand–foot syndrome in the F + R group (Table 2).

Table 2 Toxicity data (adverse events: CTC Grades 3 and 4) taken from the CORRECT trial (phase III) (Grothey et al. 2013)

Event	Regorafenib (n = 500)		Placebo (n = 253)	
	Grade 3	Grade 4	Grade 3	Grade 4
Any event	51%	3%	12%	2%
Fatique	9%	<1%	5%	<1%
Hand–foot skin reaction	17%	0	<1%	0
Diarrhea	7%	<1%	1%	0
Anorexia	3%	0	3%	0
Voice changes	<1%	0	0	0
Hypertension	7%	0	1%	0
Oral mucositis	3%	0	0	0
Rash or desquamation	6%	0	0	0
Nausea	<1%	0	0	0
Fever	1%	0	0	0
Vomiting	1%	0	0	0
Sensory neuropathy	<1%	0	0	0
Muscle pain	<1%	0	<1%	0
Headache	1%	0	0	0
Pain, abdomen	<1%	0	0	0
Laboratory event				
Thrombocytopenia	3%	<1%	<1%	0
Hyperbilirubinaemia	2%	0	1%	0
Proteinuria	1%	0	<1%	0
Anemia	2%	<1%	0	0
Hypophosphatemia	4%	0	<1%	0

3.2 Regorafenib in Metastatic Gastrointestinal Stromal Tumors (mGIST)

Metastatic GIST is a life-threatening disease with no therapy of proven efficacy after failure of imatinib and sunitinib. Mutant KIT and PDGFR-alpha, both Regorafenib targets, remain dominant oncogenic drivers in GIST refractory to imatinib and sunitinib. Efficacy and safety of Regorafenib were evaluated in a multicenter, single-arm phase II trial (n = 34) of Regorafenib in patients with advanced GIST after failure of imatinib and sunitinib (George et al. 2012). This trial revealed positive results for Regorafenib with respect to tumor control. Consequently, the GRID trial, an international, multicenter, randomized, double-blind, placebo-controlled phase III trial in unresectable, locally advanced, or metastatic GIST, who had been previously treated with imatinib and sunitinib, was initiated (Demetri et al. 2013).

The primary outcome measure in this trial was progression-free survival (PFS) based on disease assessment by independent radiological review using the

modified RECIST 1.1 criteria. In modified RECIST, lymph nodes and bone lesions are not counted as target lesions and a progressively growing new tumor nodule within a pre-existing tumor mass is classified as progression. The key secondary outcome measure was overall survival.

199 patients were randomized 2:1 to receive Regorafenib (n = 133) or placebo (n = 66), respectively. Median PFS per independent blinded central review was 4.8 months (IQR 1.4–9.2) for Regorafenib and 0.9 months (0.9–1.8) for placebo (hazard ratio [HR] 0.27, 95% CI 0.19–0.39; $p < 0.0001$). Upon progression, 56 patients (85%) assigned to placebo crossed over to Regorafenib. Drug-related adverse events were reported in 130 (98%) patients assigned to Regorafenib and 45 (68%) patients assigned to placebo. The most common Regorafenib-related adverse events of grade 3 or higher were hypertension (31 of 132, 23%), hand–foot skin reaction (26 of 132, 20%) and diarrhea (seven of 132, 5%).

Thus, oral Regorafenib significantly improves PFS compared to placebo in patients with metastatic GIST after progression on standard treatments. These results led to the approval of Regorafenib for this indication by the FDA in February 2013.

3.3 Regorafenib in Hepatocellular Carcinoma (HCC)

In 2017, Regorafenib has been approved by the FDA for the treatment of patients with advanced HCC who progressed on Sorafenib. This decision was based on the final data of the RESOURCE-trial (Bruix et al. 2017), a randomized, double-blind, placebo-controlled, multicenter phase III study of Regorafenib in patients with Child-Pugh A liver cirrhosis (maximum) and Barcelona Clinic Liver Cancer staging system (BCLC) Stage B or C HCC with documented disease progression following Sorafenib. Primary endpoint of the study was mOS. 573 patients were randomly assigned in a 2:1 ratio to Regorafenib or placebo (Regorafenib 160 mg orally once daily plus best supportive care (BSC) or matching placebo plus BSC for the first 21 days of each 28-day cycle). Treatment was continued until disease progression or unacceptable toxicity. The trial demonstrated a statistically significant improvement in OS (10.6 versus 7.8 months, HR = 0.63, $p < 0.0001$) and PFS (3.1 and 1.5 months, HR = 0.46, $p < 0.0001$) in favor for the Regorafenib arm. The overall response rate, based on modified RECIST, was 11% versus 4% in favor of regorafenib. Toxicity and safety were similar to other phase III trials with Regorafenib. The data showed that Regorafenib has acceptable tolerability and evidence of antitumor activity as single agent in patients with intermediate or advanced HCC that progressed following first-line Sorafenib.

3.4 Regorafenib in Metastatic Renal Cell Carcinoma (RCC)

Regorafenib inhibits VEGF receptors 1, 2, and 3 and PDGF receptors like other antiangiogenic tyrosine kinase inhibitors approved for treatment of advanced renal

cell cancer. Regorafenib also inhibits other potentially important angiogenic kinases like TIE2, activation of which is thought to be important in tumor escape mechanisms.

A phase II, open-label, non-randomized study assessed the safety and efficacy of the multi-kinase inhibitor Regorafenib for the treatment of renal cell carcinoma (Eisen et al. 2012). Patients with previously untreated metastatic or unresectable clear cell renal cell carcinoma received oral Regorafenib (160 mg per day) in cycles of 3 weeks on and 1 week off until disease progression. The primary efficacy endpoint was the proportion of patients who achieved an objective overall response. 49 patients received Regorafenib. The median duration of treatment was 7.1 months (range 0.7–34.4), and at the time of data cutoff, six patients (12%) were still on treatment. 48 patients were assessable for tumor response. 19 patients (39.6, 90% CI 27.7–52.5) had an objective response, all of which were partial responses. Grade 3 drug-related adverse events were common, most frequently hand and foot skin reaction (16 patients, 33%), diarrhea (5 patients, 10%), renal failure (5 patients, 10%), fatigue (4 patients, 8%), and hypertension (3 patients, 6%). Two patients had grade four treatment-related adverse events: two cardiac ischemia or infarction, one hypomagnesaemia, and one chest or thorax pain. Four patients died during study treatment or within 30 days of the last dose. Two of these deaths were regarded likely to be related to the study drug. In summary, based on this phase II trial, the efficacy of Regorafenib in the first-line setting of unresectable RCC appears comparable to that of other targeted first-line drugs. However, testing Regorafenib in standard phase III trials seems inappropriate in view of its toxic effects.

3.5 Regorafenib in Soft-Tissue Sarcoma (STS)

The randomized phase II REGOSARC trial examined safety and efficacy of regorafenib in patients with advanced soft-tissue sarcoma (Mir et al. 2016). 182 patients who had previously received doxorubicin or other anthracyclines for soft-tissue sarcoma were randomized into four cohorts—liposarcoma, leiomyosarcoma, synovial sarcoma, and other sarcomas. Participants received either Regorafenib (R) or placebo (P). In the latter group, there was an optional crossover in case of centrally confirmed disease progression. Progression-free survival (PFS) was the primary endpoint. PFS for R versus P, respectively, was in the liposarcoma cohort 1.1 months versus 1.7 months, in the leiomyosarcoma cohort 3.7 months versus 1.8 months (HR 0.46, $p = 0.0045$), in the synovial sarcoma cohort 5.6 months versus 1.0 months (HR 0.10, $p < 0.0001$), and in the other sarcoma cohort 2.9 months versus 1.0 months (HR 0.46, $p = 0.0061$). Grade ≥ 3 side effects more frequent in the R group before crossover were arterial hypertension, foot skin reaction, and asthenia. There was one treatment-related death in the R group due to liver failure. Taken together, Regorafenib has a relevant antitumor effect in non-adipocytic soft-tissue sarcomas by improving PFS (Mir et al. 2016).

4 Detailed Analysis of Toxicity

4.1 Dermatological Toxicity

In the CORRECT trial (Grothey et al. 2013) (mCRC, 760 patients), Regorafenib caused adverse reactions involving the skin and subcutaneous tissues (72% vs. 24%) including hand–foot skin reaction (HFSR) and severe rash requiring dose modification. Serious adverse skin reactions including erythema multiforme (0.2% vs. 0%) and Stevens–Johnson syndrome (0.2% vs. 0%) were more frequent in Regorafenib-treated patients. Toxic epidermal necrolysis occurred in 0.17% of 1200 Regorafenib-treated patients across all clinical trials.

In a meta-analysis (Belum et al. 2013), 1078 patients treated with Regorafenib for mCRC, GIST, renal cell carcinoma (RCC), and hepatocellular carcinoma (HCC) were included. The overall incidence of all-grade and high-grade HFSR were 60.5 and 20.4%, respectively. The relative risk (RR) of all-grade and high-grade HFSR with Regorafenib compared to controls was increased for all-grade (RR = 5.4) and high-grade (RR = 41.99) HFSR. Interestingly, the incidence of HFSR varied significantly with tumor type ($p = 0.007$), and was 71.4% in RCC, 60.2% in GIST, 50.0% in HCC, and 46.6% in mCRC, respectively.

4.2 Hypertension, Cardiac Ischemia, and Infarction

In the CORRECT trial (Grothey et al. 2013) (mCRC, 760 patients), Regorafenib increased the incidence of hypertension (30% vs. 8%), myocardial ischemia, and infarction (1.2% vs. 0.4%).

4.3 Hepatotoxicity

In the CORRECT trial (Grothey et al. 2013) (mCRC, 760 patients), fatal hepatic failure occurred in 1.6% of patients in the Regorafenib arm and in 0.4% of patients in the placebo arm; all of the patients with hepatic failure had metastatic liver disease.

4.4 Gastrointestinal Perforation or Fistula

In the GRID trial (George et al. 2012) (GIST, 199 patients), 2.1% of Regorafenib-treated patients who were treated during the blinded or open-label portion of the study developed gastrointestinal fistula or perforation; two cases were fatal.

4.5 Hemorrhage

Regorafenib increased the incidence of hemorrhage in the CORRECT trial (Grothey et al. 2013) (mCRC, 760 patients). The overall incidence (CTC Grades 1–5) was 21% in Regorafenib-treated group compared to 8% in placebo group. Fatal hemorrhage occurred in 0.6% of Regorafenib-treated patients and involved the respiratory, gastrointestinal, or genitourinary tract.

4.6 Embryo–Fetal Toxicity

Regorafenib was found to be embryonic lethal and teratogenic in rats and rabbits at concentrations lower than those achieved in man at the recommended dose. Thus, the drug is likely to cause harm when administered during pregnancy.

5 Drug Interactions

5.1 Effect of Strong CYP3A4 Inducers

Co-administration of a strong CYP3A4 inducer (e.g., rifampicin, phenytoin, carbamazepine, phenobarbital, and St. John's Wort) together with Regorafenib decreased the mean exposure of Regorafenib, increased the mean exposure of the active metabolite M-5, and resulted in no change in the mean exposure of the active metabolite M-2. Thus, concomitant use should be avoided.

5.2 Effect of Strong CYP3A4 Inhibitors

Co-administration of a strong CYP3A4 inhibitor (e.g., clarithromycin, grapefruit juice, itraconazole, ketoconazole, posaconazole, telithromycin, and voriconazole) with Regorafenib increased the mean exposure to Regorafenib and decreased the mean exposure to the active metabolites M-2 and M-5. Thus, concomitant use should be avoided.

5.3 Effect of Regorafenib on UGT1A1 Substrates

Regorafenib and its metabolites (M-2 and M-5) competitively inhibit UGT1A9 and UGT1A1 at therapeutically relevant concentrations. Eleven patients received irinotecan-containing combination chemotherapy together with Regorafenib at a dose of 160 mg (Schultheis et al. 2013). The mean AUC of irinotecan is increased by 28% when irinotecan was administered 5 days after the final seven doses of Regorafenib.

6 Biomarkers

An analysis of plasma biomarkers and KRAS mutations in patients with mCRC treated with Regorafenib (Strumberg et al. 2012) (phase I study, 38 patients) revealed that during Regorafenib treatment VEGF plasma levels increased by 62.4% (change in arithmetic mean) and 95.6% at days 21 and 49, respectively. sVEGFR decreased by 35.8% (d 21) and 42.8% (d 49), respectively. KRAS mutations were detected in 53% of patients. Changes in VEGF and sVEGFR-2 did not correlate with PFS. Patients with mutated or wild-type KRAS were equally distributed among those who benefitted clinically (PFS $\geq$ 100 d). The observed changes in angiogenic plasma cytokines are supportive of the antiangiogenic activity of Regorafenib in patients with advanced CRC. The KRAS status was not predictive for a clinical benefit as determined by PFS. Recently, it has been suggested that in patients with Ras mutant CRC, a decay in Ras mutant clones as assessed by ctDNA after 8 weeks of treatment with Regorafenib and/or a decrease in the product of the median values of volume transfer constant and the enhancing fraction (KREF) as determined by dynamic contrast-enhanced (DCE) MRI prior to and at day 15, post-treatment can predict the duration of a response to regorafenib (Khan et al. 2017).

7 Summary and Perspectives

Regorafenib is an orally active multi-kinase inhibitor that is fairly well tolerated as a single agent in the clinical setting as judged by the available data from phase I, II, and III trials. The toxicity profile is comparable to other oral multi-kinase inhibitors with similar targets. Regorafenib has promising antineoplastic activity in several tumor entities. Three large, randomized phase III studies in patients with "difficult to treat" clinical settings, advanced mGIST, advanced mCRC, and advanced HCC, have shown a benefit for Regorafenib treatment regarding overall survival (CRC, HCC) and progression-free survival (GIST, CRC, and HCC). Consequently, Regorafenib as single-agent treatment has been approved by the FDA for the palliative last-line situation in mCRC, mGIST, and HCC. Further, clinical development of Regorafenib as a single agent or in combination with standard chemotherapeutic agents in various malignant tumors is ongoing.

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Crizotinib

David F. Heigener and Martin Reck

Contents

Abstract

Crizotinib is an ATP-competitive small-molecule inhibitor of the receptor tyrosine kinases (RTK) C-Met, ALK and ROS1. There is a robust effectiveness in non-small-cell lung cancer (NSCLC) harbouring *EML4-ALK*-rearrangements resulting in constitutional activation of the ALK-RTK. The drug is approved for this entity, which represents no more than 3–5% of all NSCLC. However, in this population, impressive response rates are generated. The same is true for ROS-1 rearrangements; however, these only occur in approximately 1% of all NSCLC. In small series, efficacy is also reported in patients, whose tumours harbour a *MET* Exon 4 skipping mutation (approx. 3% of all NSCLC). Toxicities include

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visual impairment, nausea, peripheral edema, QT-prolongation and liver-enzyme elevation. Also, the occurrence of renal cysts is reported. The detection of ALK-protein by immunohistochemistry is a predictor of efficacy for crizotinib. In cases of doubt, fluorescence in situ hybridisation (FISH) detecting the ALK-rearrangement has to be performed on tumour tissue. FISH is also the method of choice to detect *ROS1*-rearrangement, whereas *MET*-mutations are detected by sequencing methods. The high efficacy of crizotinib in *ALK*- and *ROS*-rearranged as well as *MET* mutated lung cancer as new molecular targets beside the epidermal growth factor receptor (EGFR) underscores the importance of molecular typing in NSCLC.

Keywords
Crizotinib · ALK-Rearrangement · C-MET · ROS-1

1 Structure and Mechanism of Action

Crizotinib, (R)-3-[1-(2,6-dichloro-3-flourophenyl)ethoxyl]-5-(1-piperidin-4-yl-1H-pyrazol-4-yl)pyridine-2-ylamine (Fig. 1) was initially developed as a second generation, selective C-Met (Mesenchymal to epidermal transition)—inhibitor developed from a compound named PHA-665752 by Pfizer. This first-generation compound was modulated to become a potent small-molecule inhibitor of c-Met (Cui et al. 2011). It is an adenosine triphosphate (ATP) inhibitor of receptor tyrosine kinases. Besides c-met, it inhibits *ALK* (anaplastic lymphoma kinase), *ROS-1* and possibly other targets (Table 1) (Curran 2012).

Crizotinib in complex with its target, i.e. *ALK*, creates an inactive conformation of this oncogenic protein by inhibiting its phosphorylation as shown by crystalline structure analysis (Sasaki et al. 2010).

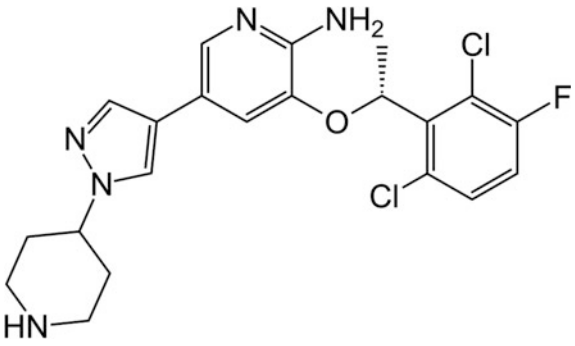

Fig. 1 Molecular structure of crizotinib

Table 1 Targets of crizotinib, reported entities harbouring this target and relative frequency in NSCLC

Targets of crizotinib	Types of cancer	Frequency of alteration in NSCLC
ALK-rearrangement	NSCLC, Lymphoma	4%
ROS-1-rearrangement	NSCLC, Chronic myelomonocytic leukaemia, gastric carcinoma	1%
MET Exon 14 skipping mutation	NSCLC, colon carcinoma, gastric carcinoma, cancer of unknown primary, glioma	Approx. 3% of NSCLC

For sources and abbreviations see text

2 Preclinical Data

Much preclinical data was obtained on its property of inhibiting *c-Met*. The IC_{50} of inhibiting the phosphorylation of wild-type c-Met in vitro in several human tumour cell lines has a mean of 4–8 nM. It inhibited cell growth and induced apoptosis in human GTL16 gastric carcinoma cell lines and was capable in suppression and migration of tumour cells in vitro (Rodig and Shapiro 2010).

In *ALK*-translocated cell lines, crizotinib inhibited downstream effector functions and induced apoptosis (Christensen et al. 2007). Moreover, the compound has antiangiogenic properties in preclinical studies (Zou et al. 2007).

3 Clinical Data

3.1 NSCLC

In a phase 1 trial, patients with any solid tumour and no further approved treatment option were treated with increasing doses of crizotinib. 250 mg bid was the maximum tolerated dose. In this cohort, two patients with NSCLC had improvements in tumour symptoms. Thus, an expansion cohort was created consisting of patients with NSCLC harbouring an *EML4*/*ALK*-rearrangement. They received the maximum tolerated dose (250 mg bid) in continuous 28-day cycles (Kwak et al. 2010). Median progression-free survival was 9.7 months (95% CI 7.7–12.8). Estimated overall survival (OS) at 6 and 12 months was 87.9% (95% CI 81.3–92.3) and 74.8% (66.4–81.5), respectively; however, the median was not reached by time of the publication. 39 patients continued to receive crizotinib for more than 2 weeks after progression because of perceived ongoing clinical benefit from the drug (12 for at least 6 months from the time of their initial investigator-defined disease progression) (Camidge et al. 2012). PROFILE 1007 compared crizotinib to either pemetrexed or docetaxel (by investigators' decision) in *ALK*-positive patients as

second-line therapy. 318 patients were randomized to either crizotinib or chemotherapy. Primary endpoint was PFS. OS was not feasible, because crossover of chemotherapy patients into a single-arm crizotinib trial (PROFILE 1005) was pre-planned. A median of 11 cycles of crizotinib and 4 cycles of chemotherapy were administered, respectively. Chemotherapy consisted of docetaxel in 41% of patients and pemetrexed in 57% of patients, respectively. Median PFS was 7.7 versus 3.0 months favouring crizotinib (HR 0.49; Confidence Interval [CI] 0.37–0.64; $p < 0.0001$). Interestingly, there was also a different PFS regarding to chemotherapy: patients receiving pemetrexed had a median of 4.3 months compared to docetaxel with 2.6 months ($p < 0.0001$) with the difference between pemetrexed and crizotinib remaining significant ($p = 0.0004$, Table 2). Response rates to crizotinib, pemetrexed and docetaxel were 65.7, 29.3 and 6.9% respectively. The preliminary data on OS showed no significant difference between crizotinib (20.3 months) and chemotherapy (22.8 months, $p = 0.5394$) because 111 out of 174 patients in the chemotherapy arm subsequently received crizotinib (Shaw et al. 2013) and thus benefitted from the drug as well.

First-line crizotinib was tested against standard chemotherapy with either cis- or carboplatin plus pemetrexed in the PROFILE 1014-trial in patients with *ALK*-rearranged tumours. 343 patients were randomized. Primary endpoint was again PFS, which was significantly longer in the crizotinib arm with 10.9 months versus 7.0 months under chemotherapy (HR 0.45; $p < 0.001$). Moreover, crizotinib was associated with better symptom control and greater improvement in quality of life (Solomon et al. 2014). Meanwhile, the OS-data are updated, showing that the median is still not reached in the crizotinib arm (CI 45.8—not reached), whereas the median is 47.5 months (32.2—not reached) with chemotherapy. Four-year OS is 56.6% versus 49.1% with crizotinib and chemotherapy, respectively. The crossover rate to crizotinib after chemotherapy was 84% (Mok et al. 2017).

There is further indirect evidence that crizotinib prolongs OS in patients harbouring an *ALK*-rearrangement: In a retrospective comparison of 82 patients with *ALK*-rearrangement receiving crizotinib, 36 patients with ALK-rearrangement not receiving crizotinib, 67 patients with an activating EGFR-mutation and 253 patients with wild-type *EGFR* and *ALK* survival were compared. *ALK*-positive patients treated second- or third-line with crizotinib had a 1-year survival of 70% (95%-CI 50–83%). ALK-positive patients treated with any other second- or third-line therapy had a 1-year survival of 44% (95-CI 23–64%; HR 0.36; 95% CI 0.17–0.75; $p = 0.004$). Survival of ALK-positive patients receiving crizotinib was comparable to those who harbour an activating *EGFR*-mutation receiving an *EGFR*-TKI (1-year survival% [95% CI 58–81] vs. 74% [61–83]). ALK-positive patients not treated with crizotinib had similar

Table 2 Efficacy of crizotinib compared to chemotherapies. The hazard ratios are for the comparison to crizotinib in all instances

Arm	PFS (month)	Hazard ratio	*p*-value
Crizotinib	7.7	0.49	<0.001
Chemotherapy	3.0		
Pemetrexed	4.2	0.59	<0.001
Docetaxel	2.6	0.3	<0.001

survival as 'double-wild-type' patients (median OS 20 months [95% CI 13–26] vs. 15 months (Costa et al. 2011; Tang et al. 2014; Peters et al. 2017; Choi et al. 2010; Gainor et al. 2016); $p = 0.244$) (Shaw et al. 2011).

A frequent site of treatment failure is the central nervous system, due to the fact, that crizotinib concentration in cerebrospinal fluid is much lower than in blood plasma (cerebrospinal fluid to plasma ratio 0.0026 (Costa et al. 2011)) and many patients develop brain metastases in their relatively long course of crizotinib treatment. Crizotinib is a substrate to the p-glycoprotein transporter (ABCB1), a transmembrane protein delivering the drug to the extracellular space (Tang et al. 2014). Newer ALK-inhibitors like alectinib, which are not a substrate to the p-glycoprotein transporter, show better disease control in the central nervous system (Peters et al. 2017).

Some of the molecular resistance mechanisms leading to crizotinib failure are already discovered. In fact, the first report on crizotinib resistance was published 'back to back' with the first clinical efficacy results described above (Choi et al. 2010). Meanwhile, a number of resistance mutations, most of them point mutations, have been identified. Drugs developed as ALK-inhibitors like ceritinib, alectinib, lorlatinib and brigatinib show effectiveness against several of these resistance mutations (Gainor et al. 2016).

In a direct comparison of crizotinib with the newer compound alectinib, it could be shown that the latter substance is superior in terms of the 12-month-PFS (68.4% vs. 48.7%, $p < 0.001$), a trend in the immature OS (HR 0.76, $p = 0.24$) and a slightly better toxicity profile (41% vs. 50% severe adverse events). The rate of CNS progression was significantly lower under alectinib compared to crizotinib (9.4% vs. 41.4%) (Peters et al. 2017).

ROS-1-rearrangements occur in about 1% of patients with NSCLC. *ROS1* and *ALK* are 'kissing cousins', i.e. closely akin within the human kinome. In vitro essays with crizotinib showed that the drug was capable of inhibiting growth of ROS-1 positive NSCLC cell lines (Yasuda et al. 2012). So, it was straightforward to test crizotinib in patients with a *ROS-1*-rearrangement. Patients were recruited in the dose escalation trial mentioned above (Kwak et al. 2010) as an own cohort. 50 patients harbouring such a rearrangement could be identified. Overall response rate was 72% with 3 complete and 33 partial responses. Duration of response was 17.6 months and median PFS was 19.2 months (Shaw et al. 2014). Crizotinib is approved for the treatment of ROS1-translocated NSCLC in the United States and Europe.

The driving event beyond crizotinib efficacy as a MET-inhibitor is the *MET* Exon 14 skipping mutation, which occurs in approximately 3% of patients with NSCLC and is also described in gastric and colon carcinomas as well as in gliomas and solid cancers of unknown primary site (Lee et al. 2015; Frampton et al. 2015). In a small retrospective series of 61 patients with metastatic NSCLC, 27 were treated with a MET-inhibitor (20 of these with crizotinib) and 34 were not. Median OS was 24.6 months for patients treated with a MET-inhibitor compared to 8.1 months in those not receiving such a drug (Awad et al. 2017). These data underscore, that crizotinib could be an effective drug in patients with this driver mutation.

3.2 Other Entities

In gastric carcinomas, *ROS-1* rearrangements and *c-MET* amplifications are described (Lee et al. 2013; Okamoto et al. 2012) suggesting a possible benefit of crizotinib in these subsets.

Targets drugable with crizotinib are also found in Ewing Sarcomas (Fleuren et al. 2013), anaplastic large cell lymphoma (Ordemann et al. 2013; Mosse et al. 2017), inflammatory myofibroblastic tumours (Mosse et al. 2017; Tothova and Wagner 2012), chronic myelomonocytic leukaemia (Cilloni et al. 2013) and neuroblastoma (Matthay et al. 2012) (Table 1). However, the clinical evidence is still sparse.

4 Toxicity

Crizotinib is comparably well tolerable. According to an analysis of phase I/II trials, most adverse events were mild to moderate with only 3–6% of treatment interruption due to adverse events. In order of frequency, the adverse events are

- Visual disturbances (in 62% of patients) including light flashes or perception of overlying shadows or after images. However, the disturbances were all short in duration and had minimal influence on the quality of life or activities of daily living.
- Nausea occurred in approximately 50% of patients, diarrhoea and vomiting occurred too. Again, these disturbances were mild and of short duration (Inc. P. Xalkori 2012).
- Further frequent side effects were peripheral edema, constipation, fatigue, decreased appetite and dizziness (Inc. P. Xalkori (2012).
- In 1.3% of cases, administration of crizotinib can result in prolongation of the QT-interval in the electrocardiogram (ECG) (Tothova and Wagner 2012). Patients already showing a prolonged QT-interval or taking drugs known to prolong it should be monitored periodically with an ECG (Curran 2012).

5 Drug Interactions

Crizotinib is primarily metabolized in the liver by CYP3A, which can result in drug interactions with CYP3A-inducers like rifampicin, resulting in decreased plasma levels of crizotinib. CYP3A-inhibitors like ketoconazole can lead to increased plasma levels of crizotinib. Finally, crizotinib itself may act as a CYP3A inhibitor, raising plasma levels of other substrates like midazolam (Curran 2012).

6 Biomarkers

The efficacy of crizotinib was tested in small subsets of patients with NSCLC, who are clearly defined on a molecular basis.

By now, three targets for crizotinib are identified: Rearrangements in *ALK* and *ROS* as well as Met Exon 14 skipping mutations. The gold-standard for detection of *ALK*- or *ROS*-rearrangements is fluorescence in situ hybridization (FISH). For ALK, 15% of cells must show this rearrangement for being classified as '*ALK*-positive'. This number is not chosen arbitrary but represents the double standard deviation from the number of ALK-rearrangements found in normal tissue. FISH is a time-consuming procedure and can only be done by specially trained personnel. It can partially be replaced by immunohistochemistry (IHC). Overexpression of *ALK* measured by IHC is a sufficient predictor of crizotinib efficacy, whereas negativity on ALK-IHC correlates strongly with a negative FISH test and less effectivity of the compound. Only in samples with low or moderate expression of ALK, FISH is needed as a confirmatory test (von Laffert et al. 2016).

ROS1 rearrangements have still to be determined by FISH, whereas *MET*-mutations are detected by sequencing techniques.

7 Summary and Perspectives

Crizotinib was the first-in-class drug to treat patients, whose tumours harbour the above-mentioned genetic alterations. Developed as a MET-inhibitor, it reveals to be prone to several pharmacodynamic and genetic resistance mechanisms in ALK-positive tumours which can be overcome by second and third generation ALK-tyrosinkinase-inhibitors. Especially because of the frequent failure in the central nervous system, its use might be challenged by these drugs in the treatment of *ALK*-rearranged lung cancer. However, it still has its place in the treatment of *ROS1*-rearranged tumours and probably in the future in those with *MET* Exon 14 skipping mutations.

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Cabozantinib: Multi-kinase Inhibitor of MET, AXL, RET, and VEGFR2

Carsten Grüllich

Contents

Abstract

Cabozantinib is a receptor tyrosine kinase inhibitor (TKI) with activity against a broad range of targets, including MET, RET, AXL, VEGFR2, FLT3, and c-KIT. Activity of cabozantinib towards a broad range of tumor models could be detected in several preclinical studies. Of note, cabozantinib decreases metastasis potential and tumor invasiveness when compared with placebo or agents that target VEGFR and have no activity against MET. Cabozantinib is clinically approved for the treatment of medullary thyroid cancer (MTC) and for renal cell cancer (RCC) in the second line. In MTC gain of function mutations, mutations of RET are central for

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tumorigenesis. Hereditary forms of MTC (MEN II) are caused by germline mutations of RET, in sporadic MTC up to 50% of cases RET mutations occur. Both MET and AXL have been described as mechanisms facilitating resistance against VEGFR-targeted tyrosine kinase therapy in clear cell RCC. Accordingly, cabozantinib has shown activity in RCC patients progressing after first-line VEGFR-TKI therapy in the pivotal METEOR trial. This phase III trial reported a benefit of 4.9 months in survival and an increase in response rate compared to standard everolimus over all patient subgroups. Of particular interest are the effects on patients with bone metastasis, which have a worse prognosis. In these patients, the beneficial effects of cabozantinib over everolimus were even more pronounced. Side effects of interest include diarrhea, hypertension, fatigue, and hand–foot syndrome.

Keywords
Multikinase-Inhibitor · Renal cell cancer · Medullary thyroid cancer · MET · AXL

1 Introduction

The tyrosine kinase MET is the receptor for hepatocyte growth factor (HGF), a cytokine with anti-apoptotic, pro-migratory, and mitogenic activity. Multiple signaling cascades can be recruited to the intracellular domain of MET (MEK, PI3K, and Jak/Stat) leading to their activation. Activation of MET can disrupt cell–cell contacts and facilitates cell migration (Trusolino and Comoglio 2002). These pro-migratory effects mediated by MET play an important role during embryogenesis and are also active in adults during tissue damage repair (Takayama et al. 1996). It has also been demonstrated that the HGF/MET pathway has protective activity in several degenerative diseases, including liver cirrhosis, nephropathies, and lung fibrosis (Matsumoto and Nakamura 2001; Michalopoulos and DeFrances 1997; Mizuno et al. 2001). Further, MET is expressed in endothelial cells and plays a role as in pro-angiogenic signaling (Bussolino et al. 1992) (Fig. 1).

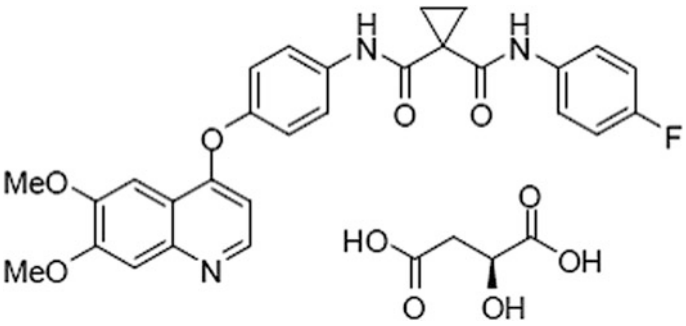

Fig. 1 Chemical structure of cabozantinib

Table 1 Types of molecular lesion of MET and AXL found in human cancers

Molecular lesion	Tumor entity
TPR-MET translocation	Gastric cancer
MET gene amplification	EGFR-targeting therapy resistant non-small-cell lung cancer Esophageal cancer Gastric cancer Liver metastasis from colon cancer Medulloblastoma
Germline MET mutations	Papillary renal cell cancer Gastric cancer
Somatic MET mutations	Papillary renal cell carcinoma Childhood hepatocellular carcinoma Lymph node metastases of head and neck squamous cell carcinomas
MET transcriptional activation	Thyroid carcinoma Ovarian carcinoma Pancreatic carcinoma Prostatic carcinoma Renal cell carcinoma Hepatocellular carcinoma Breast carcinoma Colorectal carcinoma Oral squamous cell carcinoma
AXL overexpression	Acute myeloid leukemia Breast Colorectal Lung adenocarcinoma Melanoma Osteosarcoma Ovarian adenocarcinoma Pancreatic ductal adenocarcinoma Pleural mesothelioma Renal cell carcinoma Urothelial carcinoma

Taken together, these properties make MET a typical candidate for oncogenic aberration and cancerous transformation by MET has been demonstrated as an early event. Since its discovery, activating changes in MET have been described in numerous cancers consisting either of activating mutations, gene duplications, or transcriptional activation that result in overexpression of wild-type MET (Table 1). The activating events in MET can either occur during primary tumorigenesis or as secondary events that drive further progression of the malignant phenotype (Trusolino and Comoglio 2002). Activating mutations have been identified in hereditary and sporadic papillary renal carcinoma (Soman et al. 1991; Schmidt et al. 1997), and overexpression of MET has been detected in many tumors, including renal cell

carcinoma, pancreatic cancer, prostate cancer, NSCLC, and gastric cancer (Soman et al. 1991; Schmidt et al. 1997; Houldsworth et al. 1990; Direnzo et al. 1995). In NSCLC, MET overexpression has been shown to be a mechanism of secondary resistance during treatment with EGFR antagonists (Bean et al. 2007), and in prostate cancer, MET is upregulated during hormonal ablation and higher expression levels are also associated with progression of bone and lymph node metastasis (Sirotnak et al. 2004; Verras et al. 2007). In clear cell RCC, overexpression of MET has been implicated in resistance development against VEGFR-TKI treatment (Macher-Goeppinger et al. 2017).

Different strategies to target MET for anticancer therapy are being followed, blockade of ligand binding by HGF antagonists or monoclonal antibodies against MET and HGF, and cell-permeable tyrosine kinase Inhibitors. The receptor tyrosine kinase AXL has recently also been implicated with tumor growth and poor prognosis. Its activation mediates proliferation, cell survival, and stem cell-like phenotype (Rankin and Giaccia 2016). Its overexpression can mediate treatment resistance against VEGFR-TKIs in renal cell carcinoma.

Cabozantinib developed by Exelixis is a broad-spectrum tyrosine kinase inhibitor with activity not only against MET but also against AXL, VEGFR2, FLT3, c-KIT, and RET. It was the first orally available MET inhibitor to enter clinical trials in 2005 (Macher-Goeppinger et al. 2017). A successful phase III trial in medullary thyroid cancer (MTC) met its primary endpoint showing a median PFS of 11.2 months for the cabozantinib arm over 4.0 months for the placebo arm (Schoffski 2012). Besides activating changes in MET, in MTC activating mutations of the proto-oncogene RET is found in hereditary and in sporadic cases. Dual kinase inhibition by cabozantinib may contribute to its clinical activity in MTC. Based on this positive trial cabozantinib was granted FDA approval for the first-line treatment of metastatic MTC in 2012. The approval for cabozantinib in clear cell RCC after failure of a previous anti-VEGF-directed therapy was obtained in 2015 after the successful METEOR trial (Choueiri et al. 2015) showed an improvement of PFS by 3.6 months and an OS advantage of almost 5 months over everolimus (Choueiri et al. 2016). At the end of 2017, cabozantinib was further approved for first-line treatment of intermediate and poor-risk clear cell RCC following the positive CABOSUN trial (Choueiri et al. 2017).

The positive phase II trials in prostate cancer, however, were not confirmed by two phase III trials, and consequently, the development for cabozantinib in prostate cancer was terminated.

Further, cabozantinib has shown clinical activity in various ongoing clinical trials in different tumor entities. The importance of MET and Axl activation for tumor progression has been confirmed in numerous cancers. Multiple tyrosine kinases might be especially beneficial in certain tumors. This review will summarize the preclinical and clinical data for cabozantinib in cancer treatment.

2 Preclinical Properties and Pharmacokinetics

Cabozantinib, initially coded XL 184, was developed by Exelixis (South San Francisco, CA). It was shown to have inhibitory activity at pharmacological doses against MET, VEGFR2, RET, KIT, FLT-3, AXL, and TIE-2 (tunica interna endothelial cell kinase-2), kinases that all play a role in the development and progression of different tumor diseases. Preclinically, MET phosphorylation was shown to be reduced in peripheral nerve sheath tumor cells by cabozantinib at low concentrations. Studies with xenografts in nude mice demonstrated reduced cell proliferation and reduced vascular density and increased apoptosis. Tumors size was decreasing in a dose-dependent manner upon treatment with cabozantinib (Yakes et al. 2011). Penetration of the blood–brain barrier was shown to be at 20% of plasma levels. In the phase I trial, the pharmacokinetics of cabozantinib were established. Oral bioavailability was demonstrated and the maximum tolerated dose was determined at 175 mg once daily (equals 140 mg free base). Peak plasma concentrations were reached after 5 h following oral administration. The half-life was shown to be 91 $\pm$ 33 h (Kurzrock et al. 2011).

3 Clinical Trials

3.1 Phase I

The phase I trial of cabozantinib was carried out to determine the maximum tolerated dose (MTD). Included were various solid tumor entities (Kurzrock et al. 2011). The MTD was determined at 175 mg per day. Following early reports of activity in medullary thyroid cancer (MTC), an expansion cohort for patients with MTC was added to the trial. A total of 85 patients were treated within the trial, 37 of these had MTC. Thirty-five of 37 MTC patients had measurable disease by RECIST. Of these, in 10 a partial response was confirmed and 25 had tumor shrinkage less than 30% or disease stabilization for at least 6 months. Interestingly, three patients with a confirmed response had been pretreated with vandetanib or sorafenib that also targets RET and VEGFR. This supports the hypothesis of MET being an escape mechanism to VEGFR inhibition. Tumor genotyping was performed in 31 patients with MTC an activating mutation of RET was detected in 25 of 31 patients. However, there was no correlation between mutations and clinical response. The tumor of one patient that was rapidly progressing harbored no RET mutation but an activating B-RAF mutation, which is downstream of RET and MET. In a subset of MTC patients (n = 15) analyzed for MET mutations in the tumor DNA, no mutations were detected, copy number gain was only assessed in a few samples, and three patients found to be increased. Toxicity was similar to other VEGFR tyrosine kinase inhibitors. Treatment-related adverse events (AE) were reported by 77 of 85 patients (90%). Of these, 43% reported grade 1 or 2 AEs.

The most frequent AEs were diarrhea, rash, hand–foot syndrome, liver enzyme elevation, fatigue, hypertension, nausea, and mucositis. One grade 4 AE was pulmonary embolism attributed to cabozantinib. Further, dose-limiting toxicities (DLT) were hand–foot syndrome and liver enzyme elevations.

3.2 Phase III EXAM Trial

A large phase III trial in medullary thyroid cancer was directly initiated following the responses seen in phase I. The EXAM (Efficacy of XL184 in Advanced Medullary Thyroid Cancer) trial was a randomized, double-blind placebo-controlled trial (Schoffski 2012). A total of 330 patients with MTC were randomized in a 2:1 ratio to cabozantinib versus placebo. The primary endpoint was progression-free survival (PFS) and crossing over was not allowed. Secondary endpoints included overall survival (OS) and response rate. Interim results were presented at the 2012 American Society of Clinical Oncology Annual Meeting. The primary endpoint had been met with a median PFS of 7.2 months in the treatment arm versus 4.0 months in the placebo arm. The difference reached statistical significance with a hazard ratio of 0.28. One-year progression-free survival was reported as 47.3% in the treatment arm versus 7.2% in the placebo arm. All subgroups showed an increased PFS in the treatment arm, including prior treatment with TKI and RET mutational status. Overall response rate was 28% in the cabozantinib group versus 0% in the placebo group ($p \leq 0.0001$), and duration of response was 14.6 months that was similar in both RET mutation-positive and RET mutation-negative patients. Overall survival data had not yet reached the required number of events for analysis, but there was no difference between the two arms at this early stage. At interim analysis, 45% of patients in the cabozantinib arm remained on treatment versus 13% in the placebo group. The primary reason for treatment discontinuation was progression of disease (20% in the treatment arm versus 60% in the placebo arm). Adverse events were the reason for discontinuation in 16 and 8% of cases, respectively. Adverse events were more common in the treatment arm with diarrhea and hand–foot syndrome of all grades in over 50% of patients. Further, fatigue, hypertension, and mucositis were also reported more often in the treatment arm. Assessment of calcitonin after 12 weeks of treatment showed a strong correlation with response. Calcitonin fell by a mean of 45% in the treatment arm and increased by a mean of 57% in the placebo arm.

3.3 Phase III Trials in Renal Cell Cancer (METEOR and CABOSUN)

The METEOR trial investigated the activity of cabozantinib in patients with clear cell renal cell carcinoma, who had received at least one line of VEGFR directed therapy. An intent to treat (ITT) population of 658 patients was randomized 1:1 between cabozantinib and everolimus. Primary endpoint was progression-free

survival (PFS), and secondary endpoints were overall survival (OS) and response rate (RR). Median PFS with cabozantinib was 7.4 months and 3.8 months with everolimus leading to 42% reduction of progression in the cabozantinib arm (HR 0.58; $p < 0.001$). Objective response rates were 21% with cabozantinib and 5% with everolimus ($p < 0.001$) (Choueiri NEJM). Overall survival was also improved with 21.4 months for cabozantinib and 16.5 months for everolimus (HR 0.66 $p = 0.00026$) (Choueiri Lancet) (Choueiri et al. 2016). In the first line phase II trial CABOSUN, 157 patients with intermediate and poor-risk features according to IMDC criteria were randomly assigned to cabozantinib ($n = 79$) or sunitinib ($n = 78$). Cabozantinib treatment significantly increased median PFS (8.2) compared with sunitinib (5.6 months), and was associated with a 34% reduction in the rate of progression or death (hazard ratio, 0.66). The ORR was 33% for cabozantinib versus 12% for sunitinib. Grade 3 or 4 adverse events were similar for cabozantinib (67%) and for sunitinib (68%) (Choueiri et al. 2017).

4 Cabozantinib Combination with Immuno-oncology

Cabozantinib is currently investigated for its activity in combination with Immune checkpoint inhibitors. The phase I study of cabozantinib and nivolumab and cabozantinib, nivolumab, and ipilimumab combinations in patients with urothelial cancer and other genitourinary tumors has already reported a good safety profile in combination with evidence for a strong synergistic activity (Nadal et al. 2017).

5 Discussion

Cabozantinib is a multi-kinase inhibitor with significant activity against MET, RET, AXL, and VEGFR2 among others. This combination of target specificity might prove especially useful in tumors where multiple target inhibitions might prevent tumor escape mechanisms to one target from getting activated. In MTC, the dual kinase inhibition of RET and MET seems to be particularly active. Both kinases have been implicated in tumorigenesis in this cancer. RET mutations are found as many as 50% of MTC tumor samples, while MET seems to be active mainly by overexpression. The influence of RET and MET was supported in the phase I trial, where the activity of cabozantinib against MTC was observed in RET-mutated and RET-unmutated tumors, alike (Kurzrock et al. 2011). The phase III exam trial confirmed a clinical benefit for the treatment of MTC patients with cabozantinib. Progression-free survival was improved to 7.2 months over 4.0 months with placebo, and cabozantinib was FDA approved in 2012 (Schoffski 2012). Cabozantinib is also active against AXL, a receptor tyrosine kinase involved in tumor promotion and therapy resistance in many cancers (Table 1) among those renal cell carcinoma a disease that also frequently displays MET over-activation as a mechanism of resistance to TKIs.

MET inhibition or multi-kinase inhibition as offered by substances like cabozantinib is a promising approach for various other tumors, where MET, AXL, and other kinases have been shown to play a role, including renal cell cancer, gastric cancer, and pancreatic cancer. Recently, MET activation has been shown to act as an escape mechanism in EGFR-mutated non-small-cell lung cancer during EGFR-targeting therapy (Bean et al. 2007). Therefore, combinations of EGFR and MET inhibitors, which have been shown to be active in vitro (Sennino et al. 2012; Nakagawa et al. 2012), are attractive approaches for clinical trials. However, excessive toxicities have shown to be a limiting factor in clinical approaches. Further, molecular mechanisms developing under the selection pressure of an existing targeted therapy have to be elucidated to further improve tailored strategies against treatment resistance (Dietz et al. 2017).

Cabozantinib is clinically effective in renal cell carcinoma and approved in the second line for clear cell renal cell carcinoma after failure of a previous anti-VEGFR therapy and for poor- and intermediate-risk patients in first line. The toxicities of cabozantinib are similar to those seen with other TKIs. Diarrhea, hand–foot syndrome, liver enzyme elevation, fatigue, and hypertension have been reported as the most common side effects. In general, side effects are mostly mild to moderate with the MTD determined by phase I and should be manageable by supportive means in clinical practice.

In conclusion, cabozantinib is a tyrosine kinase that has shown clinical activity in a variety of cancers and is approved for treatment of advanced medullary thyroid cancer and renal cell cancer. Cabozantinib was the first clinically approved MET and AXL inhibitor. Further indications remain to be elucidated, especially combinatorial approaches, e.g., with immune checkpoint inhibitors appear to be very promising. The exploratory biomarker analyses within the trials show the need for a better understanding of the pathways involved, especially of resistance and escape mechanism. And finally, for certain tumor entities, tyrosine kinase inhibitors with activity against multiple targets appear to be superior over substances that are specific only to a single target.

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Vemurafenib

Claus Garbe and Thomas K. Eigentler

Contents

Abstract

The activating BRAF mutation V600E and related mutations in this codon are most important for the activation of the RAS/RAF/MEK/ERK mitogen-activated protein kinase (MAPK) signalling pathway in melanoma. BRAF V600E mutations have been detected in ~40% of melanoma patients and BRAF

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V600K mutations in ~5% of melanoma patients. Activation of the MAPK pathway results in continuous stimulation of cell proliferation and inhibits programmed cell death. Vemurafenib (PLX4032) was developed as a low-molecular-weight molecule for the inhibition of the mutated serine–threonine kinase BRAF, and it selectively binds to the ATP-binding site of BRAF V600E kinase and inhibits its activity. The biochemical affinity of vemurafenib for mutated BRAF translates to potent inhibition of ERK phosphorylation and of cell proliferation exclusively in BRAF-mutant cell lines. In animal model experiments, it was demonstrated that vemurafenib achieved tumour regressions in cells harbouring the BRAF V600E mutation. The clinical trials with vemurafenib in unresectable metastatic melanoma in phases I, II and III for patients harbouring BRAF V600E mutations demonstrated all unexpected high objective response rates ranging between 50 and 80%. Median progression-free survival was prolonged from 2 months with dacarbazine to 7 months with vemurafenib, and median overall survival was, respectively, prolonged from 9 to 14 months. A major problem remains in the development of resistance to vemurafenib treatment after several months in the majority of patients, and multiple resistance mechanisms have already been described. Under vemurafenib treatment, about 25% of patients developed cutaneous squamous cell carcinomas of the keratoacanthoma type with low invasive potential and without the occurrence of metastasis. The overall tolerability of the drug was quite good, and many patients remained on treatment for long times. As other solid tumours like papillary thyroid cancer, colorectal cancer, non-small-cell lung cancer and ovarian cancer likewise harbour BRAF mutation, vemurafenib is also tested in these entities. In future, combinations of vemurafenib with other kinase inhibitors and with immunotherapies will improve its therapeutic potential.

Keywords
Vemurafenib · BRAF mutation · Melanoma · BRAF inhibitor

1 Introduction

A couple of years ago, the prognosis for patient with distant melanoma metastasis (AJCC stage IV melanoma) was generally poor with a median survival ranging from 8 to 10 months, and after diagnosis depending on the number and the sites of metastatic spread and serum LDH (Balch et al. 2009). Treatment with single-agent chemotherapy or with combined schedules can produce palliative clinical response in a minority of patients (Eigentler et al. 2003). The discovery of activating BRAF mutations in approximately 50% of patients with melanoma led to the development of a first targeted therapy of an activated oncogene in cutaneous melanoma, and many clinical trials in other tumour entities are underway (Eigentler et al. 2016; Davies et al. 2002).

Mutations in the BRAF gene which substitute the valine at amino acid position 600 with glutamic acid (V600E) represents over 80% of the BRAF mutations (Eigentler et al. 2016; Davies et al. 2002). Other variants of BRAF mutation are V600K with ~10%, and less common V600R and V600D. Vemurafenib is also active in these less common BRAF V600 mutations, probably to a lower degree. BRAF mutations were mainly found in melanoma, colorectal cancer, papillary thyroid cancer, non-small-cell lung cancer and ovarian cancer (Davies et al. 2002; Ikenoue et al. 2003; Kimura et al. 2003; Brose et al. 2002; Singer et al. 2003; Xing 2007). Additionally, nearly all patients with hairy cell leukaemia carry the BRAF V600E mutation (Tiacci et al. 2011).

Targeted therapy represents, nowadays, a promising therapy for metastatic melanoma harbouring a drug-sensitive mutation. Vemurafenib was licensed for the treatment of non-resectable metastasized melanoma by the Food and Drug Administration Agency in the USA in August 2011 (https://www.cancer.gov/about-cancer/treatment/drugs/fda-vemurafenib) and by the European Medicines Agency in Europe in February 2012 (http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/002409/human_med_001544.jsp&mid=WC0b01ac058001d124) on the basis of a phase III study for the treatment of patients carrying a BRAF V600 mutation (Chapman et al. 2011; Chapman et al. 2017; McArthur et al. 2014). With evaluated response rates ranging between 60 and 88%, vemurafenib represented a therapeutic milestone in melanoma patients since decades (Chapman et al. 2011, 2017; McArthur et al. 2014). Recently, data of the combination of vemurafenib and cobimetinib in comparison to vemurafenib and placebo were published indicating an additional therapeutic benefit for those melanoma patients who received the combination arm (Ascierto et al. 2016; Larkin et al. 2014). Before treating patients with vemurafenib, patients must have a positive result from a BRAF mutational testing.

2 Structure and Mechanism of Action

BRAF is a member of the RAF family of serine–threonine kinases (ARAF, BRAF and CRAF) which are part of the RAS/RAF/MEK/ERK mitogen-activated protein kinase (MAPK) signalling pathway (Schreck and Rapp 2006).

Therapy targeting the mitogen-activated protein kinase (MAPK) pathway through inhibition of oncogenic mutations in the BRAF kinase has become a standard treatment for patients who have metastatic melanoma with activating BRAF mutations. Mitogen-activated protein kinase cascades are key signalling pathways involved in the regulation of normal cell proliferation, survival and differentiation. The RAF/MEK/ERK signalling has implications in a wide variety of cellular functions. This pathway is central for cell proliferation, cell cycle arrest, terminal differentiation and cell death. RAF activates the MAPK kinase MEK1/2 which subsequently phosphorylates ERK1/2 (Peyssonnaux and Eychene 2001). Mutated BRAF V600E has a critical role for the proliferation and survival of

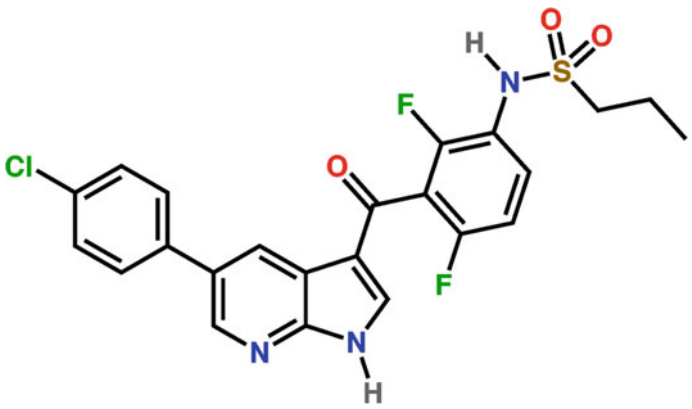

Fig. 1 Structure of vemurafenib: propane-1-sulfonic acid {3-[5-(4-chlorophenyl)-1H-pyrolo [2,3-b]pyridine-3-carbonyl]-2,4-difluoro-phenyl}-amide

melanoma cells through activation of the mitogen-activated protein kinase pathway. The mutation in the V600 codon changes the molecular confirmation of BRAF to the activated (phosphorylated) status. In June 2002, Davies and colleagues reported mutations of the BRAF gene in human cancers (Davies et al. 2002). BRAF mutation-induced oncogenes are present in approximately 5–10% of all human malignancies (Davies et al. 2002). BRAF is the most frequently mutated protein kinase in melanoma (Eigentler et al. 2016; Brose et al. 2002), and was identified in ~50% of malignant melanomas (Eigentler et al. 2016; Davies et al. 2002), in 15% of thyroid tumours (Kimura et al. 2003), in 8–10% of colon carcinomas (Ikenoue et al. 2003; Tanaka et al. 2006), in 4% of all solid tumours (Davies et al. 2002; Greenman et al. 2007) and up to 100% in hairy cell leukaemia (Tiacci et al. 2011).

Vemurafenib is a low-molecular-weight molecule, an orally available, selective inhibitor of BRAF with the V600E mutation but does not inhibit BRAF wild type; it selectively binds to the ATP-binding site of BRAF V600E kinase and inhibits its activity (Fig. 1).

3 Preclinical Data

The biochemical affinity of vemurafenib for mutated BRAF translates to cellular potent inhibition of ERK phosphorylation and of cell proliferation exclusively in BRAF-mutant cell lines. In preclinical cell line experiments, it was demonstrated that vemurafenib inhibited proliferation in cells harbouring the BRAF V600E mutation. Vemurafenib likewise caused tumour regressions of BRAF-mutant xenografts (Bollag et al. 2010).

3.1 Pharmacokinetics and Drug Interactions

After oral administration of a single 960 mg dose of vemurafenib, the substance was absorbed with a time needed to reach maximum concentration (tmax) of approximately four hours. Mean maximum concentration achieved in the blood (Cmax) at the 960 mg dose level was approximately 4.8 ± 3.3 μg/ml. Clearance is approximately 30 L/day. The mean half-life time (t1/2) is 54 h, resulting in six–nine-fold accumulation between day 1 and day 15. Vemurafenib is excreted via faeces (94%) and urine (1%) (Shah et al. 2013; Goldinger et al. 2015; Zhang et al. 2017; Sosman et al. 2012).

Vemurafenib is metabolized by CYP3A4 and the metabolites make up 5% of the components in plasma (Zhang et al. 2017). The parent compound makes up for the remaining 95%. Results from an in vivo drug–drug interaction study in patients with cancer demonstrated that vemurafenib is a moderate CYP1A2 inhibitor, a weak CYP2D6 inhibitor and a CYP3A4 inducer. Ketoconazole, itraconazole, clarithromycin, atazanavir, nefazodone, saquinavir, telithromycin, ritonavir, indinavir, nelfinavir and voriconazole are potent CYP3A4 inhibitors, concomitant administration of strong CYP3A4 inhibitors increases plasma concentration of vemurafenib. For further information, refer to the vemurafenib EPAR (http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/002409/WC500124400.pdf).

Phenytoin, carbamazepine, rifampicin, rifabutin, rifapentine and phenobarbital are CYP3A4 inhibitors, concomitant administration of strong CYP3A4 inhibitors decreases plasma concentration of vemurafenib. Co-administration of vemurafenib increased the AUC of caffeine (CYP1A2 substrate) 2.6-fold and increased the AUC of dextromethorphan (CYP2D6 substrate) by 47%, while it decreased the AUC of midazolam (CYP3A4 substrate) by 39%. Co-administration of vemurafenib resulted in an 18% increase in AUC of S-warfarin (CYP2C9 substrate) (Shah et al. 2013).

3.2 Vemurafenib in Melanoma

The clinical trials with vemurafenib in phases I, II and III for patients harbouring BRAF V600E mutations demonstrated all unexpected high objective response rates and improvement in progression-free and overall survival. The tolerability of the drug was quite good, and many patients remained on treatment for long times (Chapman et al. 2011, 2017; McArthur et al. 2014; Flaherty et al. 2010).

The results from a phase I study for the first time reported a high objective response rate in patients with metastatic melanoma harbouring BRAF mutations. Data of the phase I trial (BRIM1) were published in August 2010 (Flaherty et al. 2010). This trial had a two phases of design (dose-escalation phase and an extension phase). Complete or partial tumour responses occurred in 26 of 32 patients within the extension phase (81%). The response duration for the patients in dose-escalation phase ranged from 2 to more than 18 months. The median progression-free survival

among all patients was more than 7 months. In the dose-escalation phase, 11 of 16 patients (69%) with BRAF V600E mutant melanoma had a complete or partial response who were treated with doses of 240 mg twice daily or higher. 40% of the extension cohort had dose reduction from 960 mg twice daily to 720, 600 or 480 mg twice daily due to the side effects which were demonstrated to be proportional to the dose of the drug. Predominantly, cutaneous side effects have been observed as rash, photosensitivity, cutaneous squamous cell carcinoma and palmar-plantar dysaesthesia. Furthermore, fatigue and arthralgia were reported in nearly one-third of patient in the extension phase. Well-differentiated cutaneous squamous cell carcinoma was diagnosed in more than 20% of patients with low invasive potential and no metastatic course. The maximum tolerated dose was found to be 960 mg orally twice daily.

A multicentre, open-label phase II trial was conducted in patients with metastatic melanoma who had previously been treated with one or more prior systemic therapy (BRIM2) (Sosman et al. 2012). In this trial, 132 patients with melanoma harbouring a BRAF V600 mutation were treated with vemurafenib at a dose of 960 mg until the development of unacceptable toxic effects or disease progression. The median duration of response was 6.8 months (95% CI: 5.6—not reached). The confirmed overall response rate was 53%. Adverse events (AEs) were generally reversible (with dose modification or interruption). The most common adverse events (all grades) were arthralgia (seen in 59% of patients), rash (52%) and photosensitivity reactions (52%). The most common grade 3 adverse event was cutaneous squamous cell carcinoma (seen in 26% of patients), the majority of which were centrally reviewed as keratoacanthoma type. 45% of patients required dose reductions, most commonly for rash, arthralgia and liver function test abnormalities.

In June 2011, first results from an open-label phase III study with a total of 672 patients with previously untreated melanoma with the BRAF V600E mutation were reported (Chapman et al. 2011, 2017; McArthur et al. 2014). This study has been performed to evaluate the efficacy of vemurafenib as a monotherapy in comparison to dacarbazine chemotherapy. Vemurafenib treatment showed remarkable tumour responses in approximately 48% of patients with vemurafenib treatment compared with 55% for those on dacarbazine chemotherapy. Vemurafenib was associated with a relative reduction of 63% in the risk of death and of 74% in the risk of tumour progression (Chapman et al. 2011). In the final analyses, median overall survival (censored at crossover) was 13.6 months for vemurafenib in comparison to 9.7 months for dacarbazine (Chapman et al. 2017).

3.3 Toxicity

Vemurafenib is generally well tolerated with manageable side effects. The adverse events reported in vemurafenib clinical studies were demonstrated to be proportional to the dose and exposure to the drug. The toxic effects were largely related to the skin, joints, liver and CNS (McArthur et al. 2014; Sosman et al. 2012; Flaherty et al. 2010).

Only few patients needed to discontinue treatment permanently in the clinical studies due to adverse events. The frequency of adverse events leading to permanent discontinuation of treatment in phase I and phase II trials were 7 and 3%. Most common adverse reactions (in $\geq$30% of treated patients) were the following: arthralgia, rash, alopecia, fatigue, photosensitivity reactions, nausea, pruritus and skin papillomas including squamous cell cancer. Prolongation of the QT interval was also reported. 31% of patients in the extension phase developed well-differentiated SCC with low invasive potential and without the development of metastases (Flaherty et al. 2010).

In the phase II study, the most common adverse events reported were arthralgia, rash, mild-to-moderate photosensitivity reactions, fatigue and alopecia (Sosman et al. 2012). Transient elevations of liver enzyme levels were likewise reported. Three patients had transient palsies of the seventh cranial nerve, one patient had retinal vein occlusion and another patient had acute renal failure. 26% of patients developed SCC or keratoacanthoma; the median time to development of the first cutaneous squamous cell carcinoma or keratoacanthoma lesion was 8 weeks. The most common grade 3 adverse reactions were cutaneous SCC and rash. The possible mechanisms of developing SCC appears to be paradoxically increasing signalling of the MAPK pathway in cancer cells with wild-type BRAF that carry upstream RAS mutations, through signalling via CRAF (Oberholzer et al. 2012).

3.4 Vemurafenib in Colorectal Cancer

Approximately, 10% of all patients with colorectal cancer have BRAF V600E mutation (Tanaka et al. 2006). Patients with metastatic colorectal BRAF V600E mutation had poorer survival as compared with BRAF wild-type patients (Tie et al. 2011). The clinical responses to the vemurafenib in clinical trials were only 5% in BRAF-mutant colorectal cancer (Kopetz et al. 2015). The low response rate for vemurafenib treatment in patients harbouring BRAF mutation is possibly explained by resistance to the kinase therapy. Therefore, the parallel blockade of the epidermal growth factor receptor (EGFR) may be a successful strategy in colon cancers, as this showed a strong synergy with BRAF V600E blockade. Inhibition of the activity of EGFR with cetuximab, erlotinib or gefitinib, and combination with BRAF inhibitor may be more effective in those patients (Prahallad et al. 2012). Another resistance mechanism to BRAF inhibitors has been reported in BRAF-mutant colon cancer, which is the activation of the PI3K/AKT pathway. Therefore, inhibiting the PI3K pathway in combination with vemurafenib in BRAF-mutant CRC cell lines provided an improved anti-tumour action (Mao et al. 2013).

4 Vemurafenib in Papillary Thyroid Cancer

45–50% of patients with papillary thyroid cancers were reported to have activating BRAF mutations (Kimura et al. 2003; Xing 2007). The incidence rate of BRAF mutation in recurrent or metastatic PTCs was approximately 80%. A phase I study with vemurafenib showed a partial response and prolonged stabilization of disease in all patients with PTC treated with vemurafenib (Kim et al. 2013). In a non-randomized phase II study in kinase inhibitor-naïve patients, 10 of 26 patients experienced a partial response (best overall response 38.5%) (Brose et al. 2016).

4.1 Vemurafenib in Non-small-Cell Lung Cancer (NSCLC)

BRAF mutations are reported in approximately 1–5% of NSCLCs. The majority of the mutations were non-V600E (Naoki et al. 2002). Gautschi et al. reported one case with V600E mutation in NSCLC that responded to vemurafenib (Gautschi et al. 2012).

4.2 Vemurafenib in Hairy Cell Leukaemia

In 2011, Tiacci and colleagues reported of a 100% detection rate of the BRAF V600E mutation in patients suffering from hairy cell leukaemia (Tiacci et al. 2011). Recently, combined results of two phase II trials were published indicating overall response rates of 96 and 100% (Tiacci et al. 2015). Complete responses were observed in 35 and 42% in the two trials, respectively.

4.3 Vemurafenib in Other Cancers

Besides the above-mentioned tumours, BRAF mutations can be present in a wide variety of other neoplasms such as diffuse gliomas (Brennan et al. 2013), cholangiocarcinoma (Goeppert et al. 2014), multiple myeloma (Lohr et al. 2014), Langerhans cell histiocytosis (Badalian-Very et al. 2010) and especially in Erdheim–Chester disease (Haroche et al. 2012). In phase II, "basket" study of vemurafenib in BRAF V600 mutation-positive nonmelanoma cancer patients with Erdheim–Chester disease or Langerhans cell histiocytosis showed a response rate of 43% with a median treatment duration of 5.9 months; no patient had disease progression during therapy (Hyman et al. 2015). Also, some responses in patients with salivary-duct cancer, clear-cell sarcoma, low-grade serous ovarian cancer, glioblastoma, anaplastic ependymoma, pancreatic cancer and carcinoma of the unknown primary type were reported.

4.4 Biomarkers and Monitoring of Vemurafenib Treatment

Biomarkers in metastatic melanoma are used to assess the progression of disease, predict the response of treatment, and are part of staging examinations. In 2009, the American Joint Committee on Cancer (AJCC) included the serum lactate dehydrogenase (LDH) to classify stage IV into the M categories, M1a (soft tissue metastasis), M1b (pulmonary involvement) and M1c (involvement of other visceral organs or elevated LDH) (Balch et al. 2009). LDH is expressed ubiquitously in different healthy tissues. Elevated serum concentrations of the intracellular enzyme are mainly a result of cell lysis. Moreover, increased serum LDH levels occur in different tumour entities and indicate a high turnover of tumour cells as well as necrosis in fast-growing tumours. Increased LDH values are associated with high tumour burden and seem to be particularly elevated in liver metastases.

Another prognostic factor for stage VI metastatic melanoma is serum S100B. In immunohistochemistry, routine staining with S100 polyclonal antibody can detect macrophages, monocytes, interdigitating reticulum cells, Langerhans cells and cells from the neural crest including glia, Schwann cells and melanocytes. Serum S100B has been shown to be elevated at stage I/II in 0–12.0%, at stage III in 8.7–31% and at stage IV in 48–100% (Carlson et al. 2005). Weide et al. assessed the use of biomarker in melanoma patients with distant metastases. Serum markers LDH and S100B were found to be independent prognostic factors in melanoma patients with distant metastases, and both factors were associated with similar hazard ratios (Weide et al. 2012).

A retrospective study in 44 patients with stage IV melanoma who were treated with vemurafenib evaluated the potential of the tumour marker S100B as response and progression markers during vemurafenib treatment (Abusaif et al. 2013). Computed tomography scans and measurement of LDH and S100B levels were performed every 6–8 weeks. The correlation between response or progression and LDH and S100B levels was analysed. A good correlation between S100B and LDH decline and a RECIST-confirmed response was observed, especially when S100B and/or LDH were elevated at baseline. However, the correlation in case of tumour progression and S100B/LDH levels was low. Therefore, monitoring the course of the disease with tumour markers is thus not an alternative to monitoring with imaging examinations.

5 Summary and Perspectives

Vemurafenib is a very active drug in unresectable metastatic melanoma. 85% of patients develop tumour regressions up to objective responses in about 50% of patients. The median progression-free survival time is 7 months, up to this duration half of the patients have developed resistance to vemurafenib. A small percentage of patients of 10–15% are now for 18–36 months on treatment, and may develop late or no resistance. A major clinical challenge in vemurafenib treatment is the

development of acquired vemurafenib resistance, and the subsequent often rapid tumour progression. Several mechanisms of resistance to vemurafenib have been reported. The remarkable advances in the direct oncogene therapy in melanoma and the understanding of the mechanisms of vemurafenib resistance has led to the development of novel agents, particularly the combination of BRAF and MEK inhibitors showed superior results. Other kinase inhibitors probably of the PI3K-AKT signalling pathway will likewise be tested in combination with vemurafenib. Furthermore, these concepts of molecular-targeted therapies will be combined with the new immunotherapies in melanoma, and it remains an open question, whether simultaneous or sequential schedules will be used in future.

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Trametinib (GSK1120212)

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Abstract

The mitogen-activated protein kinase (MEK MAPK/ERK kinase) signaling pathways play a critical role in the regulation of diverse cellular activities, including survival, differentiation, proliferation, motility, and angiogenesis. Therefore, MEK inhibition was recognized as a promising target for antineoplastic therapy. Trametinib (GSK1120212), an oral MEK inhibitor which is selective for MEK1 and MEK2, has been approved by the FDA for the treatment of metastatic melanoma in a combination with a BRAF inhibitor. In this overview, preclinical and clinical data for trametinib are presented including mechanisms based on in vitro studies as well as findings from different clinical studies. The future clinical trial in different solid tumor entities will define the therapeutic role of this targeted therapy approach, possibly as a combination with other targeted therapies such as BRAF inhibitors.

Keywords

MAPK/ERK Kinase (MEK) · Trametinib · Small molecules · Kinase inhibition

1 Structure and Mechanism of Action

The mitogen-activated protein kinase (MAPK) signaling pathways involve a family of protein kinases that play critical roles in the regulation of diverse cellular activities, including cell proliferation, survival, differentiation, motility, and angiogenesis (Thompson and Lyons 2005). The MAPK pathways transduce signals from various extracellular stimuli (growth factors, hormones, mitogens, cytokines, and environmental stress), leading to distinct intracellular responses (Chang and Karin 2001) as shown in Fig. 1. Mitogen-activated protein kinase or MAP2K or MAPKK are commonly known as MEK proteins. These MEK enzymes selectively phosphorylate serine/threonine and tyrosine residues within the activation loop of their specific MAP kinase substrates.

Trametinib (GSK1120212, JTP-74057) is a second-generation small molecule inhibitor of MEK kinase. It functions as allosteric, ATP noncompetitive inhibitor with nanomolar activity against both MEK 1 and MEK 2 kinases with a half-maximal inhibitory concentration of 0.7–14.9 nmol/L for MEK1/MEK2 (Gilmartin et al. 2011; Yamaguchi et al. 2011). Inhibitors of MEK1/2 had been previously investigated as targeted therapies for tumors dependent on activating mutations in the MAPK pathway, but prior to trametinib the success of MEK inhibitors with respect to clinical activity was limited due to the dependence of nonmalignant cells on the MAPK pathway which precluded adequate dosing of the inhibitor (Lorusso et al. 2005; Yeh et al. 2007). When compared to other published MEK inhibitors, trametinib has a different pharmacokinetic profile, with a prolonged half-life, and small peak-to-trough ratios, which made it possible to

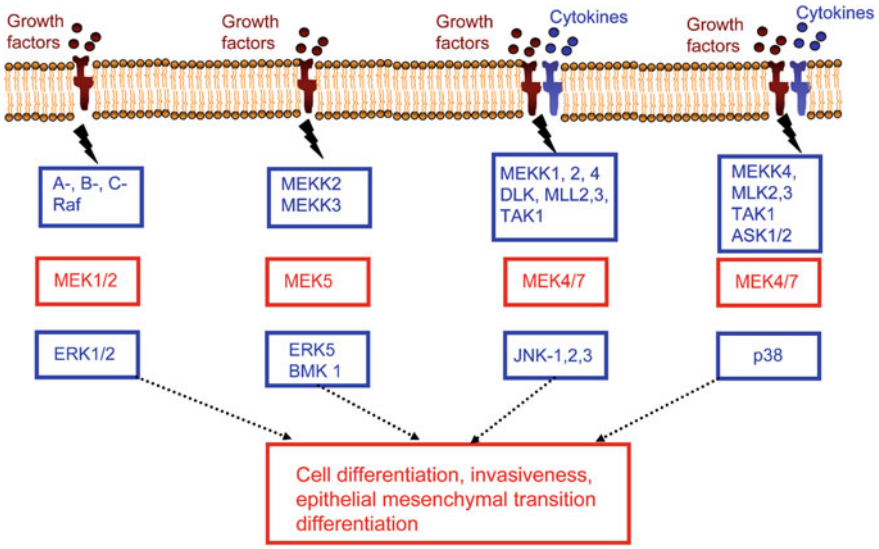

Fig. 1 Displayed are the known MEK enzymes and their four signaling pathways. Brown color: growth factor receptors, blue color: cytokine receptors

overcome the narrow therapeutic index associated with MEK inhibition. Specificity of trametinib for MEK1/2 was confirmed against a panel of more than 180 kinases including B-Raf, C-Raf, and MEK5 the closest kinase homolog (Yamaguchi et al. 2011), adjacent to the active site and defined on one side by the activation loop. The inhibitory effect of trametinib on cell growth was shown to be through inhibition of p-ERK 1/2. Therefore, most significant inhibition was achieved in tumor cell lines with mutant B-RAF or Ras (Yamaguchi et al. 2011). In vitro studies have demonstrated that trametinib decreases cell proliferation, causes G1 cell cycle arrest, and induces apoptosis. The structure and chemical characteristics of trametinib are shown in Fig. 2.

2 Preclinical Data

In the initial studies on trametinib, the proliferation across 94 cancer cell lines was evaluated systematically in vitro (Gilmartin et al. 2011; Yamaguchi et al. 2011). Among the different cell lines evaluated in the study, those with either $BRAF^{V600E}$ mutation or activating mutations in KRAS or NRAS were the most sensitive. In all cancer cell lines evaluated in this study, trametinib inhibited the MEK1/2-dependent activating dual phosphorylation of ERK1/2 on both T202 and Y204 (Gilmartin et al. 2011). The observation that trametinib did not directly inhibit either C-Raf or B-Raf activity in enzymatic assays, led to the conclusion that

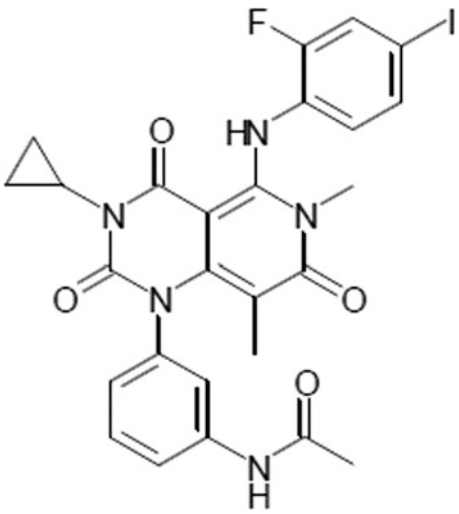

Fig. 2 The structure and chemical characteristics of trametinib. Molecular mass: 615.39 g/mol. Molecular formula: C26H23FIN5O4, chemical name: *N*-(3-(3-Cyclopropyl-5-[(2-fluoro-4-iodophenyl)amino]-6,8-dimethyl-2,4,7-trioxo-3,4,6,7-tetrahydropyrido[4,3-d] pyrimidin-1(2*H*)-yl)phenyl)acetamide

the drug must bind to MEK such that it specifically blocks the accessibility of S217 to Raf kinases. Tumor cell proliferation assay curves for 94 cancer cell lines, evaluating sensitivity to trametinib demonstrated that among cell lines with activating KRAS mutation, 16 of 25 (64%) had a partial response to the drug and 19 of 25 (76%) cell lines had cytotoxic or cytostatic responses (Gilmartin et al. 2011). These findings were then further studied in vivo in nude mice in which the tumor cells were implanted and grown as tumor xenografts (Gilmartin et al. 2011). The treatment with trametinib was initiated when the tumors had reached a volume of 150 mm^3 to mimic the clinical situation. Trametinib or vehicle was administered by oral gavage and the maximum tolerated dose was defined as the highest dose that produced less than 20% mortality and less than 20% weight loss. The activity of trametinib against the tumor was defined according to clinical criteria including growth inhibition, partial remission (PR), or complete remission (CR). The preclinical studies showed efficient inhibition of p-ERK 1/2, which correlated with potent cell growth inhibition in tumor lines with mutant B-Raf or Ras (Gilmartin et al. 2011; Yamaguchi et al. 2011). In xenograft models of HT-29 and COLO205 colorectal tumor cell lines, trametinib demonstrated robust anticancer activity when administered daily for 14 days (Gilmartin et al. 2011; Yamaguchi et al. 2011). Pharmacokinetic profiling in mice indicated a mean effective half-life ($t_{1/2}$) of 33 h, with a low peak:trough ratio of around 1.6–2.8 after single or repeat dosing of trametinib (Gilmartin et al. 2011). This is compatible with other studies showing that pharmacological MEK inhibition completely abrogated tumor growth in BRAF-mutant xenografts, whereas RAS-mutant tumors were only partially

inhibited (Solit et al. 2006). These preclinical findings provided robust evidence that MEK inhibition has in vivo antitumor activity and led to clinical trials.

3 Clinical Data

The rationale for clinical studies was built on the strong in vitro and mouse model data and on the finding in humans that mutated oncogenic forms of RAS are found in approximately 15% of all cancers (Davies et al. 2002) with a variable prevalence of RAS mutations depending on the tumor types. KRAS mutations which predispose for sensitivity to trametinib responsiveness are frequently found in colorectal, lung, pancreatic, and cervical cancer (Schubbert et al. 2007) and activating BRAF mutations have been reported in approximately 60% of cutaneous melanoma, approximately 50% of papillary thyroid, 5–20% of colorectal, approximately 30% of ovarian and approximately 26% of germ cell tumors (Wellbrock et al. 2004; Honecker et al. 2009). Based on these findings, an early phase I dose-escalation trial of trametinib was performed, which enrolled 206 patients with different advanced solid tumors. This clinical trial determined that dose-limiting toxic effects of trametinib were rash, diarrhea, and central serous retinopathy (Infante et al. 2012). While these dose-limiting toxic effects grade 3–4 were infrequent (<8%), common treatment-related adverse events were dermatitis acneiform (80%) and diarrhea (42%). The authors described the effective half-life of trametinib with about 4 days. Based on the results of this study, the recommended dose for the following phase 2 study was 2 mg per day. While the overall objective response rate in the different solid tumor types was 10%, B-Raf mutant melanoma had a higher response rate of 33% (Infante et al. 2012). These encouraging results led to several phase II/III clinical trials of trametinib alone or in combination with other agents. In the first published phase III trial of trametinib (METRIC trial), 322 patients with advanced melanoma previously treated with interferon or chemotherapy with a proven V600E or V600K B-Raf mutation were randomly assigned in a 2:1 ratio to receive oral trametinib or intravenous chemotherapy consisting of either dacarbazine or paclitaxel, every 3 weeks (Flaherty et al. 2012a, b). The median progression-free survival (PFS) of patients who received trametinib was significantly longer than that of patients who received chemotherapy (4.8 vs. 1.5 months, respectively) and at 6 months, the rate of overall survival (OS) was 81% in the trametinib group versus 67% in the chemotherapy group. These findings indicated that trametinib, as compared to chemotherapy, improved rates of PFS and OS among patients who had metastatic melanoma. The response rate was higher in trametinib-treated patients when compared with other MEK inhibitors such as selumetinib which had an response rate of 10% in BRAF-mutant melanoma (Kirkwood et al. 2012), and PD0325901 was poorly tolerated (Haura et al. 2010; LoRusso et al. 2010). Since in vitro studies and analyses of predose and postprogression tumor biopsies in clinical trials have shown both MEK-dependent and MEK-independent resistance following exposure to a BRAF inhibitor (Montagut et al. 2008; Johannessen et al.

2010; Nazarian et al. 2010; Villanueva et al. 2010; Fedorenko et al. 2011) a combination of trametinib with a BRAF inhibitor was a logical next step. In a more recent study, dabrafenib and trametinib were combined and compared with trametinib monotherapy. The rate of pyrexia was increased with combination therapy, whereas the rate of proliferative skin lesions was nonsignificantly reduced. Progression-free survival was significantly improved in the combination therapy compared to monotherapy (Flaherty et al. 2012a, b). Based on the data from the clinical trials, the FDA has approved Mekinist (trametinib) as a single-agent oral treatment for unresectable or metastatic melanoma in adult patients with BRAF V600E or V600K mutations. This result was confirmed by a later study in which significant clinical activity was observed in BRAF-inhibitor-naive melanoma patients while almost no clinical activity was observed as sequential therapy in patients previously treated with a BRAF inhibitor (Kim et al. 2013).

Because BRAF inhibition can lead to paradoxical ERK activation which favors secondary malignancies (Yaktapour et al. 2014) and because the combination of BRAF and MEK inhibition can delay the development of resistance, an open-label, randomized phase III trial compared the outcome of patients that received either a combination of dabrafenib and trametinib or vemurafenib orally as first-line therapy. The study included 704 patients with metastatic melanoma and the primary end point was overall survival (Robert et al. 2015). The overall survival rate at 1 year was 72% in the combination-therapy group and 65% in the vemurafenib group (Robert et al. 2015). Median progression-free survival was 11.4 months in the combination-therapy group and 7.3 months in the vemurafenib group. Consistent with the inhibition of paradoxical ERK activation, cutaneous squamous-cell carcinoma and keratoacanthoma occurred in 1% of patients in the combination-therapy group and 18% of those in the vemurafenib group (Robert et al. 2015). This trial led to the approval of the dabrafenib and trametinib combination for metastatic melanoma. A more recent study analyzed the efficacy of adjuvant dabrafenib plus trametinib in patients with resected, stage III melanoma with BRAF V600 mutations (Long et al. 2017). The authors found that adjuvant use of combination therapy with dabrafenib plus trametinib led to a lower risk of recurrence in patients with stage III melanoma with BRAF V600E or V600K mutations than the adjuvant use of placebo (Long et al. 2017). The 3-year overall survival rate was 86% in the combination-therapy group and 77% in the placebo group. Toxicity included mainly pyrexia (any grade, 63%; grade 3 or 4, 5%), fatigue (any grade, 47%; grade 3 or 4, 4%), and nausea (any grade, 40%; grade 3 or 4, <1%) (Long et al. 2017).

4 Toxicity

In individuals with advanced solid tumors a dose-escalation study with trametinib was performed to define the maximum tolerated dose (Infante et al. 2012). The most common treatment-related adverse events were rash or dermatitis acneiform (80%)

and diarrhea (42%), most of which were grade 1 and 2. Dose-limiting toxicities included rash, central retinopathy, and diarrhea (Infante et al. 2012). Based on this study, the dose of 2 mg/day was chosen for further studies. In patients treated with trametinib for malignant melanoma most common adverse events observed were rash, diarrhea, peripheral edema, fatigue, and dermatitis acneiform (Flaherty et al. 2012a, b). Among the patients with rash, less than 8% had grade 3 or 4 rash. A decreased ejection fraction or ventricular dysfunction was observed in 14 patients (7%) in the trametinib group, of these patients 11 had a decreased ejection fraction and 3 had left ventricular dysfunction (Flaherty et al. 2012a, b). In the light of the reported toxicity data, the dose of 2 mg trametinib once a day was shown to be tolerable and the side effects were manageable. Administration of trametinib in combination with standard gemcitabine dosing (1000 mg/m^2 IV Days 1, 8, and 15 every 28 days) was shown to be feasible (Infante et al. 2013). Though most toxicities were manageable, the addition of trametinib was shown to lead to slightly higher gemcitabine-associated myelosuppression.

5 Drug Interactions

The low C_{max} in plasma with the 2 mg a day dose suggests that trametinib is low risk for drug interactions (unpublished data, mentioned in (Infante et al. 2012)). In vitro and in vivo data suggest that GSK1120212 is unlikely to affect the PK of other drugs.

6 Biomarkers

Biomarkers are essential to identify patients with a better chance to respond to targeted therapies. In order to find predictive biomarkers for the sensitivity to trametinib, a recent study had profiled 218 solid tumor cell lines and 81 hematologic malignancy cell lines (Jing et al. 2012). The authors found that *RAF* and *RAS* mutations were a strong predictor of sensitivity to MEK inhibition by trametinib in solid tumor cells. By using transcriptomics analysis in *KRAS* mutant cell lines, the authors identified cell lines with an gene signature indicative of epithelial-to-mesenchymal transition (EMT) to be less sensitive to trametinib (Jing et al. 2012). Also the gene *DUSP6* was identified to predict for trametinib sensitivity while a lack of expression was associated with resistance to the drug irrelevant of the *RAF/RAS* mutation status. When colon cancer cells had both, *RAF/RAS* mutations and *PIK3CA*/*PTEN* mutations this was predictive for a cytostatic response instead of a cytotoxic response (Jing et al. 2012). The evaluation of trametinib sensitivity within hematological malignancies demonstrated that acute myeloid leukemia and chronic myeloid leukemia cell lines were more sensitive than other entities (Jing et al. 2012). Overall, the different studies for trametinib

sensitivity identified multiple biomarkers including mutant *RAF, RAS, PIK3CA/PTEN* and *DUSP6* in solid tumors and thereby will help in the future to identify patients who could benefit from trametinib treatment.

7 Summary and Perspectives

The MEK inhibitor trametinib in combination with the BRAF inhibitor dabrafenib has shown clinical efficacy for the treatment of metastatic melanoma and as adjuvant therapy in high-risk melanoma. Since targeted therapies attempt to inactivate a mutated oncogenic pathway, critical to survival of cancer cells while sparing normal cells, which do not carry the mutation and are not similarly addicted to the pathway informative biomarkers for trametinib have been identified including *RAF, RAS, PIK3CA/PTEN* and *DUSP6*. Trametinib overcomes paradoxical MEK activation seen in different solid tumors such as melanoma that become resistant to BRAF inhibition and thereby contributes to the solution of a major clinical problem. Current clinical trials study combinations of BRAF and MEK inhibitors with immune checkpoint inhibitors. Preliminary clinical evidence has shown a high toxicity of such combination which led to sequential rather than parallel application of the drugs. Overall the combined blockade of pathways driving tumor progression is an important substitute to our armamentarium against cancer.

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Everolimus

Jens Hasskarl

Contents

Abstract

Everolimus (RAD001) is an oral protein kinase inhibitor of the mTOR (mammalian target of rapamycin) serine/threonine kinase signal transduction pathway. The mTOR pathway regulates cell growth, proliferation and survival, and is frequently deregulated in cancer.

The EMA has approved Everolimus as Afinitor®

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- for the treatment of hormone receptor-positive, HER2/neu-negative advanced breast cancer, in combination with exemestane, in postmenopausal women without symptomatic visceral disease after recurrence or progression following a nonsteroidal aromatase inhibitor,
- for the treatment of unresectable or metastatic, well- or moderately differentiated neuroendocrine tumors of pancreatic origin in adults with progressive disease, and
- for the treatment of unresectable or metastatic, well-differentiated (Grade 1 or Grade 2) nonfunctional neuroendocrine tumors of gastrointestinal or lung origin in adults with progressive disease, and
- for the treatment of patients with advanced renal cell carcinoma, whose disease has progressed on or after treatment with VEGF-targeted therapy

And as Votubia®

- for the treatment of adult patients with renal angiomyolipoma associated with tuberous sclerosis complex (TSC), who are at risk of complications (based on factors such as tumor size or presence of aneurysm, or presence of multiple or bilateral tumors) but who do not require immediate surgery, and
- for the treatment of patients with subependymal giant cell astrocytoma (SEGA) associated with TSC who require therapeutic intervention but are not amenable to surgery, and
- as an add-on treatment in patients from 2 years of age with seizures related to TSC that have not responded to other treatments (https://www.novartis.com/news/media-releases/novartis-drug-votubiar-receives-eu-approval-treat-refractory-partial-onset).

The FDA has approved Everolimus as Afinitor®

- for the treatment of postmenopausal women with advanced hormone receptor-positive, HER2-negative breast cancer in combination with exemestane, after the failure of treatment with letrozole or anastrozole,
- for the treatment of adult patients with progressive neuroendocrine tumors of pancreatic origin (PNET) with unresectable, locally advanced or metastatic disease,
- for the treatment of adult patients with advanced RCC after failure of treatment with sunitinib or sorafenib,
- for the treatment of adult patients with renal angiomyolipoma and tuberous sclerosis complex (TSC), not requiring immediate surgery.
- for the treatment of adult and pediatric patients, 3 years of age or older, with SEGA associated with TSC who require therapeutic intervention but are not candidates for curative surgical resection.

Everolimus shows promising clinical activity in additional indications. Multiple Phase II and Phase III trials of everolimus alone or in combination and

will help to further elucidate the role of mTOR in oncology. For a review on everolimus as immunosuppressant, please consult other sources.

Keywords
RAD001 · mTOR · Everolimus · TSC · Cancer · NET

1 Introduction

Everolimus is an analog of the naturally occurring macrolide rapamycin. Rapamycin (sirolimus) was isolated from a Streptomyces species from the soil of the Easter Island (Rapa Nui) (Sehgal 1995). Rapamycin is a macrolide with antifungal and immunosuppressive properties (Eng et al. 1991). The identification of the mTOR (mammalian target of rapamycin) signaling pathway spurred the development of rapamycin analogs (so-called rapalogs) in the following years. Several rapalogs are under clinical use and further investigation to harness their immunosuppressive and antiproliferative potential are ongoing. These are sirolimus (rapamycin) (Sehgal 1995), temsirolimus (CCI-779) (Geoerger et al. 2001), everolimus (RAD001) (Schuler et al. 1997), and deforolimus (AP23573) (Mita et al. 2008).

Rapalogs bind to the FK506-binding protein-12 (FKBP12). This complex inhibits the mammalian target of rapamycin (mTOR), a protein kinase that regulates cell growth, proliferation, and survival (Fig. 1). mTOR can form two functionally distinct complexes that differ in their sensitivity to rapamycin (Jacinto et al. 2004). mTOR complex 1 (mTORC1) regulates translation and cell growth via phosphorylation of S6 kinase (S6 K) and eukaryotic initiation factor eIF4E binding protein (4E-BP), and is very sensitive to inhibition by rapamycin. The second mTOR complex (mTORC2) is resistant to rapamycin and is involved in (re)organization of the actin cytoskeleton. mTORCs integrate signals from multiple upstream pathways and relay the information through the regulation of multiple downstream pathways (Laplante and Sabatini 2012; Houghton 2010; O'Reilly and McSheehy 2010). In essence, the mTOR pathway is activated via the phosphatidylinositol 3-kinase (PI3 K) pathway and the tuberous sclerosis complex (TSC1/2) (Mak and Yeung 2004; Manning and Cantley 2003; Levine et al. 2006). Mutations in these components or in the tumor suppressor protein PTEN, a negative regulator of PI3 K, may result in their deregulation. Various preclinical models have confirmed the role of this pathway in tumor development (Manning and Cantley 2003; Podsypanina et al. 2001; Chan 2004).

There is evidence that the mTOR pathway holds several feedback loops and that it is interconnected with various other signaling pathways. Inhibition of mTORC1 by everolimus releases the inhibitory action of S6 K on IRS1, allowing further activation of PI3 K and compensatory activation of AKT and its downstream targets (Majumder et al. 2004). Inhibition of mTORC1 by everolimus also results in a feedback activation of the mitogen-activated protein kinase (MAPK) pathway (Carracedo et al. 2008). mTORC1 is mainly regulated by TSC1 and TSC2. Loss of function mutations of the TSC1 or TSC2 genes leads to uncontrolled signaling of mTORC1 and formation of harmatomas throughout the entire body.

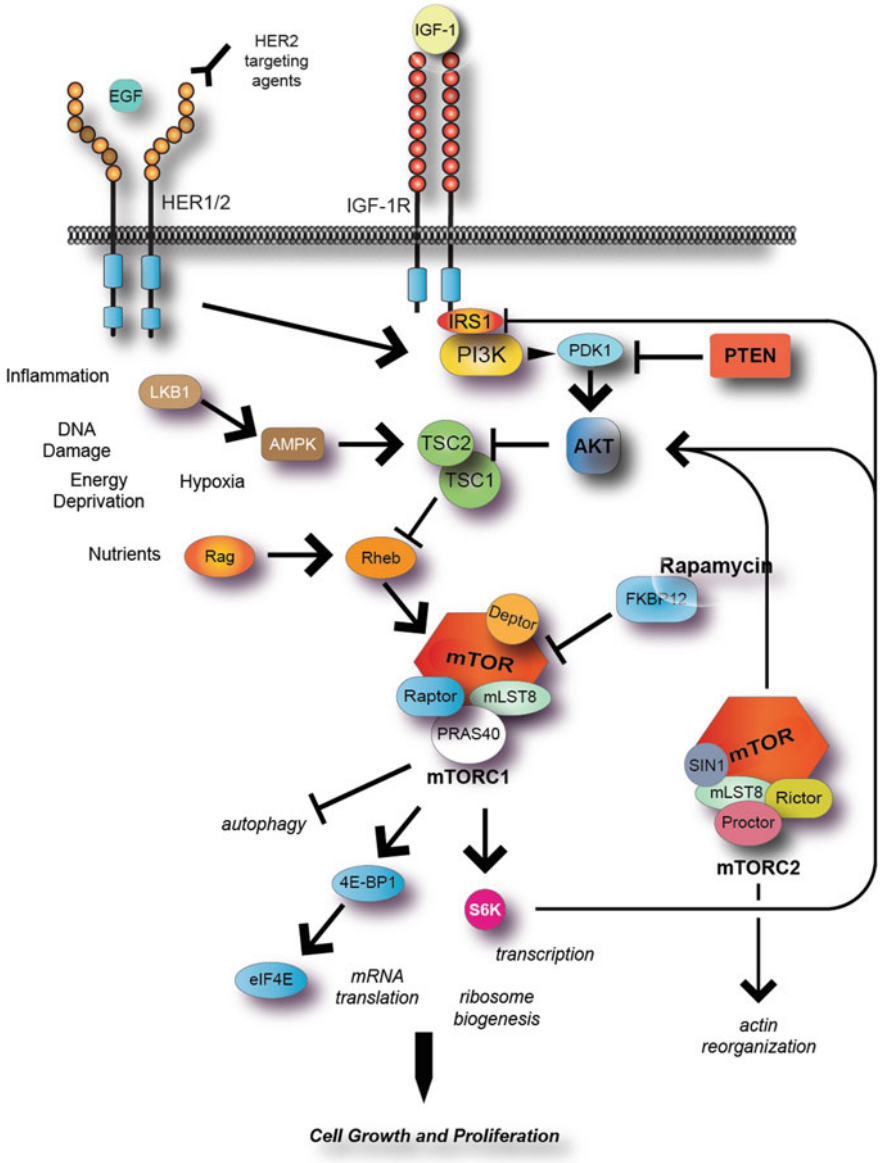


Fig. 1 The mTOR pathway (modified from Laplante and Sabatini 2012; Houghton 2010; O'Reilly and McSheehy 2010; Levine et al. 2006). Deptor: DEP domain-containing mTOR-interacting protein; EGF: epidermal growth factor; eIF4E: Eukaryotic translation initiation factor 4E; 4E-BP: eukaryotic initiation factor 4E (eIF-4E) binding protein; FKBP: FK506 binding protein; HER: human epidermal growth factor receptor; IGF(R): insulin-like growth factor (receptor); IRS1: insulin receptor substrate 1; LKB1: liver kinase B1; AMPK: adenine monophosphate-activated protein kinase, mLST8: mammalian lethal with SEC13 protein 8; mTOR: mammalian target of rapamycin; mTORC: mammalian target of rapamycin complex; PDK1: 3-phosphoinositide-dependent protein kinase-1; PI3 K: Phosphatidylinositide 3-kinase; PRAS: Proline-rich AKT1 substrate 1; Proctor: protein observed with Rictor; PTEN: Phosphatase and tensin homolog; Rag & Rheb: small GTPases; Raptor: regulatory-associated protein of mTOR; Rictor: rapamycin-insensitive companion of mTOR; S6 K: Ribosomal protein S6 kinase; SIN: stress-activated protein kinase interacting protein 1

From an oncologist's perspective, the PI3 K/mTOR pathway is an interesting therapeutic target as it is involved in many cellular processes (Bjornsti and Houghton 2004):

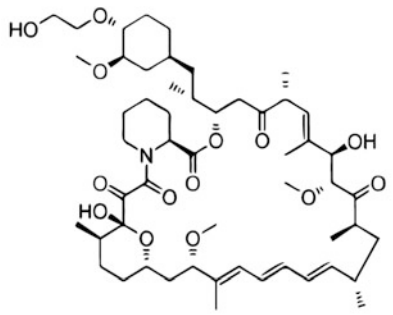
- mTOR functions as a sensor of mitogens, growth factors, and energy and nutrient levels
- mTOR facilitates G1-S cell cycle progression
- The PI3 K/mTOR/PTEN pathway is frequently deregulated in human cancers
- mTOR is involved in the production of pro-angiogenic factors (i.e., VEGF) and inhibition of endothelial cell growth and proliferation,
- mTOR can inactivate eukaryotic initiation factor 4E binding proteins and activate the 40S ribosomal S6 kinases, regulating protein translation, including the HIF-1 proteins.
- Oncogenic transformation may sensitize tumor cells to mTOR inhibition.

2 Structure and Mechanism of Action

Everolimus [RAD001, Afinitor®, (40-O-(2-hydroxyethyl)-rapamycin)] is a derivative of rapamycin (sirolimus) (Fig. 2). It is an orally available selective inhibitor of mTOR. Like Rapamycin it binds FKBP12, and inhibits the mTORC1 complex (Fig. 1), abrogating downstream signaling of this pathway. mTORC1 is a downstream signal transducer of the PI3 K pathway, which is frequently activated in human malignancies. Everolimus, like rapamycin, does not affect the activity of mTORC2 complex. Based on its mechanism of action, everolimus is not expected to induce rapid cell death but rather to slow tumor growth.

3 Preclinical Data

Everolimus and other rapalogs inhibit the proliferation of various human tumor cell lines and human umbilical vein endothelial cells in vitro. The IC_{50} (dose at which growth is inhibited by 50%) ranges from sub-nanomolar to micromolar, depending on the cell type. In vitro everolimus reduces expression of HIF1 and VEGF, suggesting that everolimus may also act as an anti-angiogenic agent. This anti-angiogenic activity of everolimus was confirmed in vivo. Mice with primary and metastatic tumors treated with everolimus showed a significant reduction in blood vessel density, when compared to controls (Lane et al. 2009). The pharmacokinetic profile of everolimus in rats and mice showed sufficient tumor penetration, above what was needed to inhibit the proliferation of endothelial cells and tumor cell lines in vitro, and below concentrations reached in humans (O'Reilly



International non-proprietary name:	Everolimus
Synonyms:	RAD001
Molecular Weight:	958.2 Daltons
Molecular Formula:	$C_{53}H_{83}NO_{14}$

Chemical Name:	(1R,9S,12S,15R,16E,18R,19R,21R,23S,24E,26E,28E,30S,32S,35R)-1,18-dihydroxy-12-(1R)-2-[(1S,3R,4R)-4-(2-hydroxyethoxy)-3-methoxycyclohexyl]-1-methylethyl}-19,30-dimethoxy-15,17,21,23,29,35-hexamethyl-11,36-dioxa-4-aza-tricyclo[30.3.1.04,9] hexatriaconta-16,24,26,28-tetraene-2,3,10,14,20-pentaone
Formulations:	Four strengths of tablets (2.5 mg, 5 mg, 7.5 mg, 10 mg). Store dry at room temperature, away from heat, moisture, and light.

Source:
CSID:24747358,http://www.chemspider.com/Chemical-Structure.24747358.html (accessed 20:52, May 6, 2013)

Fig. 2 Chemical structure of everolimus

et al. 2010). Everolimus administered daily p.o. potently inhibited tumor growth in multiple different mouse and rat xenograft models.

4 Clinical Data

In addition to being a potent immunosuppressive agent, everolimus is currently being investigated as an anticancer agent based on its potential to act directly on the tumor by inhibiting tumor cell growth and proliferation and indirectly by inhibiting angiogenesis (via potent inhibition of tumor cell VEGF production and VEGF-induced proliferation of endothelial cells). At the time of writing 222 active interventional, investigator-initiated, or industry-sponsored Phase II–IV trials were registered at www.cinicaltrials.gov (Table 1). Of those, 110 trials are actively recruiting patients (86 Phase II, 18 Phase III, and 6 Phase IV).

4.1 Pharmacokinetics and Pharmacodynamics

In a dose-escalation study of everolimus in 92 patients with advanced cancer patients everolimus was rapidly absorbed after oral administration, with a median time to peak blood levels (t_{max}) of 1–2 h after administration. Maximum tolerated dose (MTD) was not reached. The blood concentration was dose proportional over the dose range tested while maximum blood concentration C_{max} appeared to plateau at dose levels higher than 20 mg/week (O'Donnell et al. 2008). The terminal

Table 1 Active clinical trials with everolimus

Indication	Phase II	Phase III	Phase IV	Total
Brain tumors	6			6
Breast cancer	23	8	2	33
Gastroesophageal cancer		1		1
GI (CRC, pancreatic cancer, BTC)	2			2
GYN (Ovarian, endometria, cervical cancer)	5			5
Head and neck cancer	3			3
Hepatocellular cancer (HCC)			1	1
Kidney cancer (RCC)	12	3	2	17
Neuroendocrine tumors (NET)	11	3	1	15
NF	1			1
Non-Hodgkin Lymphoma (NHL)	2			2
PKLD		1		1
Prostate cancer	1			1
Sarcoma	2			2
Solid tumors	5			5
Thymoma	1			1
Thyroid cancer	6			6
Tuberous Sclerosis Complex (TSC)	4	2		6
Urothelial cancer	2			2
Total	86	18	6	110

GI: gastrointestinal cancer; HCC: hepatocellular cancer; CRC: colorectal cancer; BTC: biliary tract cancer; GYN: gynecological cancer; NSCLC: non-small-cell lung cancer; PKLD: polycystic kidney/liver disease; CUP: RCC: renal cell carcinoma

half-life was 30 h (range, 26–38 h) similar to that in healthy volunteers. Inter-patient variability was moderate. High-fat meals alter the absorption of everolimus. Everolimus is metabolized and excreted into the feces >80%. Pharmacodynamic modeling based on S6 kinase inhibition in peripheral blood mononuclear cells suggested 5–10 mg daily to be an adequate dose to produce a high degree of sustained target inhibition (O'Donnell et al. 2008).

4.2 Clinical Development of Everolimus

Based on the mode of action, preclinical results and early clinical activity of everolimus across different tumor types, Novartis launched the WIDE (Worldwide Initiative to Develop Everolimus) program to develop everolimus in a broad range of malignancies as well as tuberous sclerosis complex (TSC). Main indications in which everolimus was or is being developed are as follows:

- Breast Cancer (BOLERO: Breast cancer trials of oral everolimus) with BOLERO 1–6
- Gastric Cancer (GRANITE: Gastric antitumor trial with everolimus) with GRANITE 1
- Hepatocellular Cancer (EVOLVE: Everolimus for liver cancer evaluation) with EVOLVE 1
- Lymphoma (PILLAR: Pivotal lymphoma trials of RAD001) with PILLAR 1–2
- Neuroendocrine Tumors (RADIANT: RAD001 in advanced neuroendocrine tumors) with RADIANT 2–4
- Renal Cell Carcinoma (RECORD: Renal cell cancer treatment with oral RAD001 given daily) with RECORD 1–4
- Tuberous Sclerosis Complex (EXIST: Examining everolimus In a Study of TSC) with EXIST 1–3.

In the following, the major indications in which everolimus has been or is being investigated, either as a single agent or in combination with other agents will be discussed.

4.2.1 Clinical Studies in Breast Cancer

Hormone Receptor-Positive, HER2-Negative Breast Cancer

The development of everolimus in breast cancer followed a very strong lead from preclinical results, which translated nicely into early clinical activity. Proliferation of breast cancer cells is driven by the estrogen receptor (ER) and the human epidermal growth factor receptor (HER) family. The PI3 K/AKT/mTOR pathway modulates these signals and can support resistance to endocrine therapy. mTORC1 activates S6 K, which then can phosphorylate and activate the estrogen receptor. Combination of everolimus with aromatase inhibitors inhibited proliferation and induced apoptosis in MCF7 cells (Boulay et al. 2005).

A Phase I trial of everolimus in combination with letrozole reported promising clinical responses, with a manageable safety profile of the combination (Awada et al. 2008). Based on these results, a neoadjuvant, randomized Phase II trial (NCT00107016) was launched. 270 postmenopausal women were randomized to receive either 4 months of letrozole (2.5 mg/d) plus everolimus (10 mg/d) or letrozole plus placebo. Response rate and biomarker inhibition were higher in the everolimus arm (Baselga et al. 2009).

The BOLERO-2 trial was the logical continuation of these trials of everolimus in combination with hormonal therapy. This randomized Phase III trial compared the efficacy of exemestane (25 mg/d) in combination with everolimus (10 mg/d) versus exemestane in combination with placebo. 724 patients with HR-positive, advanced progressive or recurrent breast cancer who were refractory to letrozole or anastrozole were randomized 2:1 to everolimus or placebo. The primary endpoint was progression-free survival. Both arms were well balanced. At time of a preplanned interim analysis after 359 PFS events had been reported, median PFS was 6.9 months with exemestane plus placebo versus 2.8 months with exemestane plus

placebo (HR 0.43; 95% CI 0.35–0.54; $p < 0.001$) based on local assessment, and 10.6 months versus 4.1 months according to central assessment (HR 0.36; 95% CI 0.27–0.47; $p < 0.001$) (Baselga et al. 2012). This led to the approval of everolimus in combination with exemestane for treatment of postmenopausal women with advanced hormone receptor-positive, HER2-negative breast cancer with recurrence or progression after treatment with letrozole or anastrozole in July 2012 by the FDA and the EMA.

Multiple other trials of everolimus in various combinations are currently active, e.g., BOLERO-4 (Open-label, Phase II, Study of Everolimus Plus Letrozole in Postmenopausal Women With ER + Metastatic Breast Cancer), BOLERO-6 (A Phase II Study of Everolimus in Combination With Exemestane Versus Everolimus Alone Versus Capecitabine in Advanced Breast Cancer), and VICTORIA (Study to Compare Vinorelbine In Combination With the mTOR Inhibitor Everolimus versus Vinorelbine monotherapy for Second-line Treatment in Advanced Breast Cancer).

HER2-Positive Breast Cancer

Preclinical studies suggested that PI3 K inhibitors could overcome PTEN loss-induced resistance to trastuzumab in HER2-positive breast cancer cells in vitro and in vivo (Lu et al. 2007; Nagata et al. 2004). Clinical evidence of activity of everolimus in combination with a trastuzumab-containing regimen came from two Phase I/II studies.

Study NCT00426556 was a single-arm, open-label dose-escalation trial designed to evaluate the feasibility, dose, and schedule for combining everolimus with weekly paclitaxel and trastuzumab (Andre et al. 2010). A total of 33 patients with HER2-positive advanced breast cancer previously treated with trastuzumab were treated with everolimus 5 mg/d, 10 mg/d, or 30 mg/week in combination with paclitaxel (80 mg/m^2 days 1, 8, and 15 every 4 week) and trastuzumab (2 mg/kg/week). Neutropenia (Grade 3–4) was the most common toxicity observed ($n = 17$ patients). On the basis of observed dose-limiting toxicities and overall safety considerations, everolimus 10 mg/d was chosen for further development. Among patients with measurable disease ($n = 27$) ORR was 44%. Median PFS was promising (34 week; 95% CI 29.1–40.7 week). The second Phase I/II study (NCT00426530) investigated trastuzumab and vinorelbine plus everolimus. 50 patients with HER2-positive metastatic breast cancer pretreated with trastuzumab were enrolled in this Bayesian dose-escalation study to receive everolimus 5 mg/d, 20 mg/week, or 30 mg/week plus vinorelbine (25 mg/m^2 on day 1 and 8 every 3 week) and trastuzumab (2 mg/kg/week). Again, neutropenia (grade 3/4) was the most frequently observed toxicity (DLT), and everolimus 5 mg/d was selected for further development. Disease control was achieved in 83% of patients; the median duration of response was 32.7 week for CR/PR and 38.6 week for SD (Jerusalem et al. 2011). Based on these results, 2 Phase III trials, BOLERO-1&3 were launched.

A Phase II study investigated the activity of everolimus in combination with paclitaxel and trastuzumab in 55 patients with HER2-positive advanced breast cancer resistant to trastuzumab and pretreated with a taxane (Hurvitz et al. 2013).

Disease control rate was 36.4% with a median PFS of 5.5 months (95% confidence interval [CI]: 4.99–7.69 months). Median OS was 18.1 months (95% CI: 12.85–24.11 months). Most frequent grade 3/4 adverse events (AEs) reported were neutropenia and stomatitis. These findings suggested the clinical activity of the combination of everolimus plus trastuzumab and paclitaxel, which was tested in another Phase III trial.

The BOLERO-1 randomized, double-blind Phase III trial (NCT00876395) compared the efficacy of placebo or everolimus in combination with trastuzumab and paclitaxel, as first-line therapy advanced HER2-positive breast cancer in 719 patients with HER2-positive advanced breast cancer. Patients had to have good performance status (ECOG 0-1) and had not to have received previous trastuzumab or chemotherapy for advanced breast cancer within 12 months of randomization. Randomization (2:1) to receive either everolimus or placebo in combination with weekly trastuzumab and paclitaxel was stratified by prior exposure to trastuzumab and presence of visceral metastases. At a median follow-up of 41.3 months median PFS was 14.95 months (95% CI 14.55–17.91) with everolimus versus 14.49 months (12.29–17.08) with placebo (HR 0.89, 95% CI 0.73–1.08; $p = 0.1166$). In the HR-negative subpopulation ($n = 311$), median PFS with everolimus was 20·27 months (95% CI 14.95–24.08) versus 13.08 months (10.05–16.56) with placebo (HR 0.66, 95% CI 0.48–0.91; $p = 0.0049$); however, the protocol-specified significance threshold ($p = 0{\cdot}0044$) was not crossed.

Most frequent AEs with everolimus were stomatitis (67% vs. 32% in the placebo group), diarrhea (57% vs. 47%), and alopecia (47% vs. 53%). The most frequently reported grade 3 or 4 AEs were neutropenia (25% vs. 35), stomatitis (13% vs. 1%), anemia (10% vs. 3%) and diarrhea (9% vs. 4%). There were 17 AE-related deaths in the everolimus group (4%) and none in the placebo group.

The BOLERO-3 Phase III trial (NCT01007942) compared the combination of trastuzumab and vinorelbine with everolimus versus trastuzumab and vinorelbine with placebo in patients with HER2-positive advanced breast cancer previously treated with a taxane and who were resistant to trastuzumab (Andre et al. 2014). A total of 569 patients were randomized to receive everolimus ($n = 284$) or placebo ($n = 285$). Arms were well balanced. Study treatment was continued until tumor progression or intolerable toxicity. At a median follow-up of 20.2 months the median PFS of 7.0 months (95% CI 6.74–8.18) in the everolimus arm was statistically superior to 5.78 months (5.49–6.90) in the placebo arm (HR 0.78 [95% CI 0.65–0.95]; $p = 0.0067$). The most common grade 3–4 AEs were neutropenia (73% in the everolimus arm vs. 62% in the placebo arm), leucopenia (38% vs. 29%), anemia (19% vs. 6%), febrile neutropenia (16% vs. 4%), stomatitis (13% vs. 1%), and fatigue (12% vs. 4%). Serious adverse events (SAEs) were reported in 42 and 20% of patients. The authors concluded that the clinical benefit should be considered in the context of the adverse event profile in this population (Andre et al. 2014).

In the light of available HER2-targeting treatment options like pertuzumab (Swain et al. 2013; Baselga et al. 2012) and Trastuzumab emtansine (Verma et al. 2012), the clinical value of everolimus in HER2-positive breast cancer seems limited.

Triple Negative Breast Cancer

Data on everolimus in triple negative breast cancer might be of interest but await confirmation in larger patient cohorts (Singh et al. 2014).

4.2.2 Clinical Studies in Gastric Cancer

Based on results from few smaller Phase II trials, which had shown limited activity of everolimus (Doi et al. 2010; Taguchi et al. 2011; Yoon et al. 2012), GRANITE-1 (NCT00879333) was designed.

The GRANITE-1 Phase III trial compared the efficacy of everolimus versus placebo (EVOLVE-1) in adult patients with progressive, histo- or cytologically confirmed gastric adenocarcinoma after one or two previous systemic chemotherapies (Ohtsu et al. 2013). In this trial, 656 patients were randomized 2:1 to receive everolimus plus best supportive care (BSC) or placebo plus BSC. Baseline characteristics were well balanced. At a median follow-up of 14.3 months, the trial failed its primary endpoint with a median OS of 5.4 months with everolimus plus BSC (95% CI, 4.8–6.0 months) versus 4.3 months with placebo plus BSC (95% CI, 3.8–5.5 months; HR for OS, 0.90; 95% CI, 0.75–1.08). Estimated median PFS was 1.7 months with everolimus (95% CI, 1.5–1.9 months) and 1.4 months with placebo (95% CI, 1.4–1.5 months). The most common grade 3/4 AEs with everolimus were anemia, decreased appetite, and fatigue.

4.2.3 Clinical Studies in Liver Cancer (HCC)

Preclinical evidence for a possible role of mTOR in HCC came from xenograft models, in which everolimus suppressed xenograft growth, provided the rationale for investigation of everolimus in HCC (Huynh et al. 2009; Villanueva et al. 2008). One Phase I/II trial in 28 patients with HCC determined 10 mg/d as recommended dose for Phase II. Although possible clinical activity was noted, the trial did not reach its Phase II stage (Zhu et al. 2011).

The EVOLVE-1 Phase III study compared the efficacy of everolimus versus placebo in patients with advanced HCC whose disease progressed during or after sorafenib or who were sorafenib intolerant (Zhu et al. 2014). In this trial, 546 patients were randomized (2:1) to receive everolimus 7.5 mg/d or placebo. At a median follow-up of 24.6 months the primary endpoint, prolongation of OS, was not reached: Median OS was 7.6 months (95% CI, 6.7–8.7) and 7.3 months (95% CI, 6.3–8.7), respectively. Likewise, secondary endpoints failed to show statistically significant differences between the arms, irrespective of prognostic factors or subgroups. Grade 3/4 AEs and serious AEs were more frequently reported in the everolimus arm. Most frequent Grade 3/4 AEs were asthenia (7.8% vs. 5.5%), anemia (7.8% vs. 3.3%), decreased appetite (6.1% vs. 0.5%), HBV (6.1% vs. 4.4%), ascites (5.6% vs. 8.7%), and thrombocytopenia (5.6% vs. 0.5%).

4.2.4 Clinical Studies in Lymphoma

Preclinical results showed increased sensitivity of everolimus-treated diffuse large B-cell lymphoma (DLBCL) cells to rituximab in vitro (Wanner et al. 2006), and an increased cytotoxic effect when combined with other agents in mantle cell

lymphoma (MCL) (Haritunians et al. 2007; Nishioka et al. 2008), and in other models (Crazzolara et al. 2009; Saunders et al. 2011; Xu et al. 2013).

Everolimus showed promising clinical activity as single agent in heavily pretreated Hodgkin lymphoma (HL). Of 19 patients treated with everolimus (10 mg/d), 8 patients achieved a PR and 1 patient achieved a CR. Median time to progression was 7.2 months (Johnston et al. 2010).

Study NCT00516412 evaluated the activity of everolimus in MCL (Renner et al. 2012). In 35 evaluable patients (median age 69) ORR was 20% (95% CI 8–37), median PFS was 5.5 months (95% CI 2.8–8.2). Another Phase II trial investigated everolimus in 77 patients with relapsed/refractory aggressive NHL (47 DLBCL, 19 MCL, 8 FL, 3 other). Median age was 70 years, median number of prior therapies 3 (range 1–15). ORR was 30% (95% CI 20–41%). ORR for patients with DLBCL was 30%, 32% for MCL, and 38% for FL. Median time to progression was 3.4 months (95% CI 2.1–4.2), median progression-free survival was 3.0 months (95% CI 2.1–3.9) and median overall survival was 8.1 months (95% CI 5.3–12.5) (Witzig et al. 2011). Combination of everolimus with rituximab in 26 patients with relapsed DLBCL led to a response rate of 38% (90% CI 21–56). The median duration of response was 8.1 months (Barnes et al. 2013).

The PILLAR-1 trial (NCT00702052) was an open-label, single-arm, Phase II study evaluating everolimus (10 mg/d) in patients with bortezomib-refractory MCL. The primary endpoint was ORR, secondary endpoints included PFS, OS, and duration of response (Wang et al. 2014). In this trial, in 58 patients with heavily pretreated MCL everolimus only showed very modest activity with an ORR of 8.6% (90% CI 3.5–17.3), thus failing the primary endpoint.

PILLAR-2 (NCT00790036) was a randomized, placebo-controlled Phase III trial evaluating everolimus as maintenance therapy in patients with poor risk DLBCL who achieved CR after rituximab-containing first-line therapy (Witzig et al. 2016). The primary endpoint was disease-free survival (DFS). Secondary endpoints were OS, lymphoma-specific survival, and safety. A total of 742 patients with histologically confirmed stage III/IV poor risk (IPI $\geq$ 3) DLBLC with PET/CT-confirmed CR to first-line rituximab-containing chemotherapy were randomized 1:1 to receive everolimus or placebo for 1 year or until disease relapse, unacceptable toxicity, or death. At a median follow-up of 50.4 months everolimus failed to improve DFS compared to placebo (Log-rank $p = 0.276$). The 2-years DFS rates were 78% versus 77%. Common grade 3/4 AEs included neutropenia, stomatitis, lymphopenia, and anemia. Subgroup analyses may imply activity of everolimus in selected patient subgroups and warrant further investigation.

4.2.5 Clinical Studies in Neuroendocrine Tumors (NET)

Phase II Studies in NET

Two initial Phase II studies were conducted in NET. The first trial conducted by J. Yao at the MD Anderson Cancer Center evaluated treatment with everolimus 5 or 10 mg/d plus depot octreotide 30 mg (LAR) every 28 days in patients with metastatic or unresectable, well-differentiated, neuroendocrine tumors (Yao et al.

2008). The overall median PFS of patients treated with octreotide LAR and everolimus was 60 week (95% CI 54–66 week). Stratified by tumor group, median PFS of patients with carcinoid and islet cell tumors was 63 week (95% CI 55–71 week) and 50 week (95% CI 31–70 week), respectively (HR 1.2; 95% CI 0.7–2.2).

An additional open-label, nonrandomized Phase II study in 160 patients with pancreatic NET (PNET) stratified by ongoing octreotide therapy at study entry (Yao et al. 2010). Patients who were not being treated with octreotide at study entry were assigned to Stratum 1 (n = 115, everolimus 10 mg/day), and patients treated with octreotide LAR for at least 3 consecutive months at study entry were assigned to Stratum 2 (n = 45, everolimus 10 mg/day and octreotide LAR every 28 days). Median PFS was 9.7 months (95% CI 8.3–13.3 months) in stratum 1, and 16.7 months (95% CI 11.1 months-NA) in stratum 2. Median OS in Stratum 1 was 24.9 months (95% CI 20.2–27.1 months). Median OS had not been reached for stratum 2 at the time of data cutoff.

Phase III Studies in NET

Three Phase III clinical trials have investigated the efficacy and safety of everolimus in NETs, the RADIANT 2, 3, and 4 trials.

RADIANT-2 was a prospective, randomized, double-blind, multicenter, placebo-controlled Phase III study to evaluate the safety and efficacy of everolimus 10 mg/day plus octreotide LAR or matching placebo plus octreotide LAR in patients with advanced carcinoid tumor (Pavel et al. 2011, 2017). Patients enrolled had to have a progressive, advanced, well-differentiated carcinoid tumors and had to have symptoms related to carcinoid syndrome at enrollment or prior to enrollment ("functional NET"). 492 patients with advanced functional NET were enrolled in this study worldwide, 216 were randomized to treatment with octreotide + everolimus and 213 to treatment with octreotide plus placebo. The primary endpoint was again PFS. This trial was complicated by several factors: Imbalances at baseline and opposing/conflicting results in local and central response assessment interpretations. Results as per the amended primary endpoint (PFS assessed by an independent adjudication radiology committee (IAC)) showed a 5.1-month prolongation in median PFS from 11.3 months for octreotide plus placebo to 16.4 months for octreotide plus everolimus (HR 0.77). Although co-administration of everolimus with octreotide increased the exposure to octreotide, the risk for progression was consistently reduced when everolimus exposure was increased, regardless of octreotide exposure (Pavel et al. 2017). Nevertheless, statistical significance was not reached, as the prespecified statistical boundary was missed. No statistically significant difference was evident in terms of overall survival, although numerically more deaths were reported from the everolimus treatment group (HR 1.22; 95% CI: 0.91, 1.62; p = 0.908). In the final analysis, median OS was not significantly different with 29.2 months (95% CI: 23.8–35.9) for the everolimus arm and 35.2 months (95% CI: 30.0–44.7) for the placebo arm (HR, 1.17; 95% CI, 0.92–1.49) (Pavel et al. 2017).

RADIANT-3 was an international, multicenter, double-blind Phase III study to compare the efficacy of everolimus against placebo in patients with advanced

progressive pancreatic NET (PNET) (Yao et al. 2011). A total of 410 patients from 18 countries were randomly assigned to receive everolimus (207 patients) or placebo (203 patients) until disease progression or intolerable toxicity. Patients assigned to placebo were allowed to cross over to everolimus upon progression. The median PFS (the primary endpoint) by local investigator was 11.0 months (95% CI 8.4–13.9) in the everolimus group, as compared with 4.6 months (95% CI 3.1–5.4) in the placebo group (HR 0.35; 95% CI 0.27–0.45; $p < 0.001$). Median overall survival was not reached, and no significant difference between the groups was observed (HR 1.05; 95% CI 0.71–1.55; $p = 0.59$). At time of final analysis, median OS was 44.0 months (95% CI, 35.6–51.8 months) for patients assigned to everolimus and 37.7 months (95% CI, 29.1–45.8 months) for those assigned to placebo (HR 0.94; 95% CI, 0.73–1.20; $p = 0.30$) (Yao et al. 2016).

Based on this trial, everolimus was approved in 2011 by the FDA for the treatment of progressive neuroendocrine tumors of pancreatic origin (PNET) in patients with unresectable, locally advanced or metastatic disease and by the EMA for the treatment of unresectable or metastatic, well- or moderately differentiated neuroendocrine tumors (NET) of pancreatic origin in adults with progressive disease.

The RADIANT-4 trial (NCT01524783) was a randomized, double-blind, placebo-controlled, Phase III trial in 302 adult patients with advanced nonfunctioning NET of gastrointestinal or lung origin to compare the efficacy of everolimus + BSC versus placebo + BSC (Yao et al. 2016). As this trial excluded patients with functional NET, somatostatin analogs were not allowed as concomitant medication. Median PFS was 11.0 months (95% CI 9.2–13.3) in the everolimus arm versus 3.9 months (95% CI 3.6–7.4) in the placebo arm. Everolimus reduced the risk of progression or death (HR 0·48 [95% CI 0·35–0·67], $p < 0·00001$). Although not statistically significant, the results of the first preplanned interim overall survival analysis indicated that everolimus could be associated with a reduction in the risk of death (HR 0.64 [95% CI 0.40–1.05], one-sided $p = 0·037$). Grade 3 or 4 AEs included stomatitis (9% vs. 0%), diarrhea (7% vs. 2%), infections (7% vs. 0%), anemia (4% vs. 1%), fatigue (3% vs. 1%), and hyperglycemia (3% vs. 0%).

4.2.6 Clinical Studies in Kidney Cancer (RCC)

Based on the strong preclinical rationale and early clinical results, several Phase II and III trials in RCC were launched.

RECORD-1 (NCT00410124) was a randomized, double-blind, placebo-controlled Phase III trial of everolimus in patients with metastatic RCC after progression on VEGF-targeted therapy. 416 patients were randomized 2:1 to receive everolimus (10 mg/d) ($n = 272$) or placebo ($n = 138$). The primary endpoint was PFS, assessed by central review. Results at the second prespecified interim analysis suggested a significant difference in efficacy between arms and the trial was stopped early after 191 PFS events had been observed. Median PFS was 4.0 months (95% CI 3.7–5.5) versus 1.9 months (95% CI 1.8–1.9) (Motzer et al. 2008). Final results confirmed the early results with a median PFS of 4.9 months

(95% CI 4.0–5.5) with everolimus versus 1.9 months (95% CI 1.8–1.9) with placebo (HR 0.33; 95% CI 0.25–0.43; $p < 0.001$). OS was similar in both arms (Median OS 14.8 vs. 14.4 months; HR 0.87; 95% CI 0.65–1.15; $p = 0.162$) but was likely confounded by a high percentage (80%) crossover to everolimus (Motzer et al. 2010). Based on RECORD-1, the FDA and EMA approved everolimus for the treatment of patient with advanced RCC after failure of sunitinib or sorafenib.

The first data on combination of everolimus and bevacizumab in RCC came from trial NCT00323739 (Hainsworth et al. 2010). 80 patients with advanced RCC (50 treatment naïve, 30 previously treated) received bevacizumab (10 mg/kg on days 1 and 15) and everolimus (10 mg/d). Median PFS in treatment naïve and previously treated patients were 9.1 and 7.1 months. Based on promising preliminary data from this trial, two larger randomized studies investigating the combination of everolimus and bevacizumab were launched.

RECORD-2 (NCT00719264) was a randomized, open-label, multicenter Phase II study comparing the efficacy and safety of everolimus in combination with bevacizumab (EB) versus interferon-α in combination with bevacizumab (IB) as first-line treatment for patients with metastatic RCC (Ravaud et al. 2015). Patients were stratified according to their MSKCC risk status (favorable vs. intermediate vs. poor). Primary endpoint was PFS; secondary endpoints included OS, ORR, and duration of response, safety, and QoL. A total of 365 patients were randomized to receive EB ($n = 182$) and IB ($n = 183$) arms. The median PFS was 9.3 and 10.0 months in the EB and B arms, respectively ($P = 0.485$). Median OS was 27.1 months (95% CI 19.9–35.3) in the EB arm, and 27.1 months (95% CI 20.4–30.8) in the IB arm (HR 1.01; 95% CI 0.75–1.34; $p = 0.96$). Both arms showed similar PFS, response rates, and time to definitive deterioration of QoL, and expected safety profiles.

The CALGB-90802 study (NCT01198158), a large randomized Phase III trial, is comparing everolimus plus bevacizumab versus everolimus plus placebo after failure of ≥ 1 prior VEGFR TKI with an expected read out in July 2018.

RECORD-3 (NCT00903175) was a randomized Phase II trial comparing sequential first-line everolimus and secod-line sunitinib versus first-line sunitinib and second-line everolimus in patients with metastatic RCC (Knox et al. 2017). Primary objective was to show PFS non-inferiority of first-line everolimus compared with first-line sunitinib. Secondary objectives included the comparison of combined PFS for the two sequences of treatment, ORR, and OS. 471 treatment-naïve patients with metastatic RCC were enrolled. The trial failed to show non-inferiority (Motzer et al., ASCO 2013 abstract#4504). Median PFS in first line with everolimus was 7.85 months compared to 10.71 months with sunitinib (HR = 1.43; 95% CI 1.15–1.77). ORR clearly favored sunitinib (26.6%; 95% CI 21.1–32.8) over everolimus (8%; 95% CI 4.9–12.2). Median combined PFS was 21.7 months (95% CI: 15.1–26.7) with everolimus-sunitinib and 22.2 months (95% CI: 16.0–29.8) with sunitinib-everolimus [HR 1.2; 95% CI 0.9–1.6]. Median OS was 22.4 months (95% CI: 18.6–33.3) and 29.5 months (95% CI: 22.8–33.1), respectively (HR 1.1; 95% CI: 0.9–1.4). The rates of grade 3 and 4 adverse events

suspected to be related to second-line therapy were 47% with everolimus and 57% with sunitinib (Knox et al. 2017).

The RECORD-4 (NCT01491672) trial assessed efficacy (PFS) of everolimus in second-line treatment of advanced RCC patients with (1) prior cytokines, (2) prior sunitinib, or (3) prior anti-VEGF therapy other than sunitinib (Motzer et al. 2016). A total of 134 patients were enrolled (58 with prior sunitinib, 62 with other prior anti-VEGF therapy, 23 with prior sorafenib, 16 with prior bevacizumab, 13 with prior pazopanib, 5 with prior tivozanib, and 3 with prior axitinib; 14 with prior cytokines). Overall median PFS was 7.8 months (95% CI: 5.7–11.0); in the cohorts, it was 5.7 months (95% CI 3.7–11.3) with previous sunitinib, 7.8 months (95% CI 5.7–11.0) with other previous anti-VEGF therapy, and 12.9 months [95% CI 2.6-not estimable (NE)] with previous cytokines. At final OS analysis, total median OS was 23.8 months (95% CI 17.0-NE) and, in the cohorts, it was 23.8 months (95% CI 13.7-NE) with previous sunitinib, 17.2 months (95% CI 11.9-NE) with other previous anti-VEGF therapy, and NE (95% CI 15.9-NE) with previous cytokine-based therapy. Overall, 56% of patients experienced grade 3 or 4 AEs. The most common grade 3 or 4 AEs were anemia (13%), stomatitis (5%), hyperglycemia (5%), and hypertriglyceridemia (5%).

4.2.7 Clinical Studies in TSC

Tuberous sclerosis complex (TSC) is an autosomal-dominant genetic disorder that results from mutations in the TSC1 or TSC2 genes (Franz 2011). TSC is characterized by the development of benign tumors (harmatomas) throughout the body. Manifestations of TSC vary from individual to individual, ranging from mild symptoms to physical and intellectual disabilities (Orlova and Crino 2010). Approximately one-third of cases are inherited, whereas two-thirds are de novo mutations. TSC1 mutations appear to be more common in familial (inherited) cases of TSC, while mutations in the TSC2 gene occur more frequently in sporadic cases. Inactivating mutations in TSC1 and TSC2 release their inhibitory effect on mTORC1 and subsequent hyperproliferation. Accordingly, mTOR inhibitors were very attractive molecules to find novel treatment options for TSC. Meikle and colleagues demonstrated very good activity of rapalogs in a mouse model for TSC1 (Meikle et al. 2008), where median survival was prolonged from 33 to >100 days. Rapamycin also improved cognitive defects in a TSC2-deficient mouse model (Ehninger et al. 2008). Building on this strong preclinical rationale, an investigator-initiated Phase I/II trial (NCT00411619) in children and adults with TSC suffering from subependymal giant cell astrocytomas (SEGA) was conducted. A total of 28 patients were enrolled to receive everolimus 3 mg/d. There was a clinically meaningful reduction in the volume of the primary SEGA ($p < 0.001$) for baseline versus 6 months (Krueger et al. 2010). Based on these results a full clinical development program (EXIST) was launched.

EXIST-1 was a randomized, double-blind Phase III trial to assess the efficacy and safety of everolimus in patients with SEGA associated with TSC. 117 patients were randomized 2:1 to 4.5 mg/m^2/d (titrated to achieve blood through concentrations of 5–15 ng/ml) everolimus ($n = 78$) or placebo ($n = 39$). 27 (35%) patients

in the everolimus arm had $a \geq 50\%$ reduction in SEGA volume versus none in the placebo group ($p < 0.0001$) (Franz 2013).

EXIST-2 (NCT00790400) was a randomized Phase III trial in adult patients with angiomyolipoma associated with TSC. 118 patients were randomized 2:1 to receive everolimus 10 mg/d ($n = 79$) or matching placebo ($n = 39$). The primary endpoint was the proportion of patients with confirmed $\geq 50\%$ reduction in total volume of target angiomyolipomas relative to baseline. The angiomyolipoma response rate was 42% (95% CI 31–53) for everolimus versus 0% (95% CI 0–9) in the placebo group (Bissler et al. 2013) and was sustained over a long time (Franz et al. 2016).

Based on EXIST-1&2, everolimus was approved for treatment of adults with renal angiomyolipoma and TSC, not requiring immediate surgery, and pediatric and adult patients with TSC who have subependymal giant cell astrocytoma (SEGA) that requires therapeutic intervention but cannot be curatively resected.

There is accumulating evidence that mTOR activation might be involved not only in TSC development but also drive seizures in TSC patients (Wong 2012).

EXIST-3 was a three-arm, randomized, double-blind, placebo-controlled study to assess the efficacy and safety of everolimus as adjunctive therapy in TSC patients with refractory partial-onset seizures. Two trough exposure concentrations of everolimus, 3–7 ng/mL (low exposure) and 9–15 ng/mL (high exposure) were compared with placebo (French et al. 2016). The primary endpoint was changed from baseline in the frequency of seizures during the maintenance period, defined as response rate (the proportion of patients achieving $\geq 50\%$ reduction in seizure frequency) and median percentage reduction in seizure frequency, in all randomized patients. A total of 366 patients were randomized 1:1:1 to receive either everolimus titrated to 3–7 ng/ml ($n = 117$) or to 9–15 ng/ml ($n = 130$), or matching placebo ($n = 119$).

The response rate was 15.1% with placebo (95% CI: 9.2–22.8) compared with 28.2% for low-exposure everolimus (95% CI: 20.3–37.3; $p = 0.0077$) and 40.0% for high-exposure everolimus (95% CI: 31.5–49.0; $p < 0.0001$). The median percentage reduction in seizure frequency was 14.9% (95% CI: 0.1–21.7) with placebo versus 29.3% with low-exposure everolimus (95% CI: 18.8–41.9; $p = 0.0028$) and 39.6% with high-exposure everolimus (95% CI: 35.0–48.7; $p < 0.0001$). Grade 3 or 4 adverse events occurred in 13 (11%) patients in the placebo group, 21 (18%) in the low-exposure group, and 31 (24%) in the high-exposure group. The authors concluded that adjunctive everolimus treatment can significantly reduce seizure frequency with a tolerable safety profile patients with tuberous sclerosis complex and treatment-resistant seizures.

5 Toxicity

Everolimus has been investigated in clinical studies and in post-marketing experience. In cancer patients, the main adverse events reported with everolimus were: stomatitis, non-infectious pneumonitis, infections, and renal failure. In addition,

laboratory abnormalities, mainly hyperglycemia, hyperlipidemia, anemia, neutropenia, and thrombocytopenia were reported. For a recent and complete list of adverse drug reactions please refer to your local drug label or package insert.

6 Drug Interactions

Everolimus is mainly metabolized by CYP3A4 in the liver and to some extent in the intestinal wall. Everolimus is also a substrate of P-glycoprotein (PGP). Therefore, absorption and subsequent elimination of systematically absorbed everolimus may be influenced by medications that interact with CYP3A4 and/or PGP. In vitro studies showed that everolimus is a competitive inhibitor of CYP3A4 and of CYP2D6 substrates, potentially increasing the concentrations of medicinal products eliminated by these enzymes. Strong inhibitors of CYP3A4 (azoles, antifungals, cyclosporine, erythromycin) have been shown to reduce the clearance of everolimus therapy thereby increasing everolimus blood levels. Similarly, Rifampin, a strong inducer of CYP3A4, increases the clearance of everolimus thereby reducing everolimus blood levels. Caution should be exercised when co-administering everolimus with CYP3A4 inhibitors or inducers.

7 Biomarkers

To date, no valid predictive or prognostic biomarker for everolimus across all indications tested has been identified. S6K1 activity in peripheral blood mononuclear cells seems one of the most reliable biomarkers for target inhibition by everolimus (O'Reilly and McSheehy 2010). In RCC, higher expression of phospho-4EBP1 and p70 seems to correlate with better response to everolimus (Nakai et al. 2017).

In a retrospective exploratory analysis from BOLERO-2 302 archival tumor tissue samples (209 from the everolimus arm and 93 from the placebo arm) were analyzed using Next-Generation Sequencing technology (Hortobagyi et al. 2016). The genes most frequently altered were PIK3CA (47.6%), CCND1 (31.3%), TP53 (23.3%), and FGFR1 (18.1%). No predictive marker for response to treatment with everolimus could be identified, as treatment effect was similar in all molecular subgroups analyzed.

8 Summary and Perspectives

Everolimus is an inhibitor of the mTOR pathway, specifically mTORC1. Based on its ubiquitous expression and central role multiple cellular signaling pathways, mTOR is an interesting target for cancer therapy. So far, clinical investigations based on sound preclinical rationale have led to the approval of everolimus for the treatment of advanced hormone receptor-positive, HER2-negative breast cancer, progressive neuroendocrine tumors advanced renal cell carcinoma (RCC), renal angiomyolipoma and tuberous sclerosis complex (TSC), TSC-associated subependymal giant cell astrocytoma (SEGA), and as adjunctive treatment for TSC-associated refractory seizures.

Several registration trials have failed, as the data at the time of the decision to move into a Phase III trial were not too convincing. Nevertheless, compared to other development programs in the industry, the story if everolimus is still a success story. As everolimus reaches the end of its development life cycle on few trial are expected to launch in the future.

I am personally intrigued and grateful to Dr. David Neal Franz, who convinced Novartis to follow the science and develop everolimus in patients with TSC.

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Vismodegib

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Contents

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Abstract

Vismodegib (GDC-0449, Erivedge®) is a small molecule antagonist of the hedgehog (Hh) pathway that binds to smoothened (SMO) and leads to inhibition of an aberrant activation of the Hh pathway. Dysregulated Hh signaling results in uncontrolled proliferation in basal cell carcinoma (BCC) and has also been found present in medulloblastoma, and many other cancers such as those of gastrointestinal tract, brain, lung, breast, and prostate. In January 2012, vismodegib became the first agent to target the Hh pathway to receive approval by the United States Food and Drug Administration (FDA) and in July 2013 approval by the European Medicines Agency (EMA) followed for the treatment of adult patients with symptomatic metastatic BCC, or locally advanced BCC inappropriate for surgery or radiotherapy. The role of vismodegib in other malignancies than BCC has still to be investigated.

Keywords

Vismodegib · Basal cell carcinoma · Medulloblastoma · Hedgehog pathway Smoothened

1 Introduction

The hedgehog (Hh) pathway is a signaling pathway involved in numerous developmental processes, including determination of cell fate, patterning, proliferation, survival, and differentiation (Varjosalo and Taipale 2008). While this pathway is inactive in most adult tissues, aberrant activation of it has been documented in a variety of malignancies (Atwood et al. 2012; McMillan and Matsui 2012). In cancers such as basal cell carcinoma (BCC), ligand-independent mechanisms lead to constitutive Hh pathway activation through mutations in components of the pathway, including patched-1 (PTCH1) or smoothened (SMO) (Rubin et al. 2005; Epstein 2008; Goppner and Leverkus 2011; Ruch and Kim 2013). Moreover, numerous other solid and hematologic tumors have been shown to harbor ligand-dependent activation of the Hh pathway by autocrine or paracrine mechanisms (Atwood et al. 2012; McMillan and Matsui 2012). Therefore, this pathway has been an attractive target for drug development and cancer therapy. While the best-characterized approach is to target the SMO receptor, other rational approaches

for inhibiting the Hh pathway include inhibiting downstream components or directly binding Hh ligands. Vismodegib, a SMO antagonist, showed remarkable activity in phase I and II trials for the treatment of locally advanced and metastatic BCC (Yauch et al. 2008; Robarge et al. 2009; Von Hoff et al. 2009; LoRusso et al. 2011a, b; Sekulic et al. 2012; Tang et al. 2012; Fecher 2013; Sandhiya et al. 2013). In January 2012, vismodegib became the first agent targeting the Hh pathway to receive approval by the United States Food and Drug Administration (FDA) and in July 2013 approval by the European Medicines Agency (EMA) followed for the treatment of adult patients with symptomatic metastatic BCC, or locally advanced BCC inappropriate for surgery or radiotherapy. Despite promising preclinical data with Hh pathway inhibitors in other malignancies, the clinical benefit has been disappointing, not investigated or results are not yet available until now (LoRusso et al. 2011a, b; Atwood et al. 2012; McMillan and Matsui 2012; Belani et al. 2016; Rimkus et al. 2016). Only in a subgroup of medulloblastoma pediatric and adult patients (sonic hedgehog (SHH)-subtype medulloblastoma) antitumor activity could be achieved in the recurrent or refractory therapeutic situation in phase I and II studies (Robinson et al. 2015; Gajjar et al. 2013).

2 Structure and Mechanism of Action

2.1 Structure

Structural modifications of benzimidazole led to the discovery of a functionalized 2-pyridyl amide moiety, which could inhibit the Hh pathway (Robarge et al. 2009; Wong et al. 2009). Further, optimization of pharmacokinetic and pharmacodynamic properties of this molecule finally culminated in the development of vismodegib. The chemical formula for vismodegib is $C_{19}H_{14}Cl_2N_2O_3S$ (Fig. 1). Its chemical name is 2-chloro-*N*-[4-chloro-3-(pyridin-2-yl)phenyl]-4-(methylsulfonyl)benzamide. It is a crystalline-free base with a pKa of 3.8 and a molecular weight of 421.3 g/mol. The solubility as a free base is far greater at an acidic pH (Robarge et al. 2009; Wong et al. 2009).

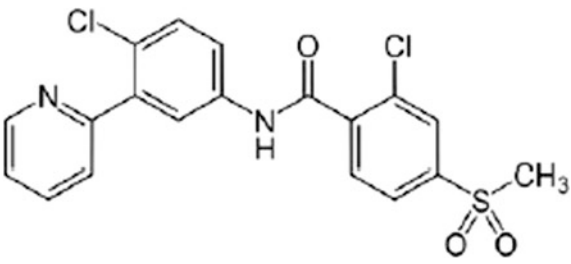

Fig. 1 Structural formula of vismodegib (GDC-0449)

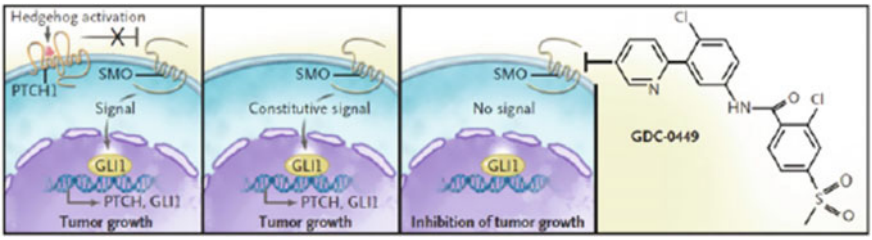

Fig. 2 Hedgehog signal transduction pathway (*left*), loss-of-PTCH1 mutations (*center*), and inhibition of smoothened homolog (SMO) signaling by GDC-0449 (*right*). Hedgehog binding to PTCH1 (*left*) relieves inhibition of SMO activation by PTCH1. In the absence of PTCH1, because of loss-of-PTCH1 mutations, SMO signaling occurs constitutively (*center*). GDC-0449 inhibits SMO signaling through direct interaction with SMO (*right*) (Von Hoff et al. 2009) (reprint with permission of Massachusetts Medical Society)

2.2 Mechanism of Action

Vismodegib acts by targeting the hedgehog (Hh) pathway, which is activated in most BCCs (Rubin et al. 2005; Epstein 2008; McMillan and Matsui 2012; Ruch and Kim 2013). The Hh signaling pathway is an important cascade for cellular growth and differentiation during the embryonic development (Varjosalo and Taipale 2008). The pathway was first identified in the fruit fly, Drosophila, and the name Hh was given to the pathway because of the spiky, hedgehog-like appearance of those fruit fly embryos which had mutated Hh gene (Varjosalo and Taipale 2008). Apart from the Hh ligand, the two receptor proteins involved in the cascade are patched-1 (PTCH1) and smoothened (SMO). PTCH1 is an inhibitory protein and forms an inactive complex with SMO, in the absence of Hh. When Hh binds with PTCH1 and prevents its inhibitory action, SMO becomes free to act (Fig. 2). Activated SMO is then involved in promoting the transcription of different genes, which, during the embryonic development, are responsible for cellular growth and differentiation and, in adults, are involved in tissue repair and stem cell maintenance (Rubin et al. 2005; Epstein 2008; Varjosalo and Taipale 2008; Atwood et al. 2012; McMillan and Matsui 2012; Ruch and Kim 2013).

A dysregulated Hh signaling pathway has not just been attributed to BCC, but also to medulloblastoma, and many other cancers such as those of gastrointestinal tract, brain, lung, breast, and prostate (Varjosalo and Taipale 2008; Atwood et al. 2012; McMillan and Matsui 2012). Aberrant activation of the Hh pathway has been found to cause cellular proliferation and stimulate cancer stem cells. In the stratified epithelium, this disturbs the equilibrium between cellular proliferation and cell cycle arrest, causing epidermal hyperplasia along with uncontrolled proliferation of basal cells leading to BCC (Rubin et al. 2005; Epstein 2008; Ruch and Kim 2013). Inactivation of PTCH1 or oncogenic activation of SMO is a common feature in most of the BCC. So increasing the inhibitory action of the PTCH1 or suppressing the activation of SMO can be targeted for the treatment of BCC and other tumors with hyperactivated Hh pathway. Vismodegib blocks Hh signaling by selectively

inhibiting SMO and thus prevents the consequent induction of target genes and proliferation factors, leading to suppression of BCC growth (Epstein 2008; Goppner and Leverkus 2011; Fecher 2013).

3 Preclinical Data

Preclinical studies demonstrated the antitumor activity of vismodegib in mouse models of medulloblastoma and hepatocellular carcinoma and in xenograft models of colorectal and pancreatic cancer (Yauch et al. 2008; Philips et al. 2011; Atwood et al. 2012; Ferruzzi et al. 2012; McMillan and Matsui 2012).

4 Clinical Data

4.1 Vismodegib in Basal Cell Carcinoma (BCC)

Basal cell carcinoma (BCC) of the skin is the most common cancer worldwide, and its prevalence is increasing, accounting for 80% of non-melanoma skin cancers (Rubin et al. 2005; Epstein 2008; Goppner and Leverkus 2011). Basal cell carcinoma has many clinical subtypes. In the majority of cases, BCC can be treated with surgery, cryotherapy, and laser ablation. Radiotherapy, photodynamic therapy, and topical treatment with imiquimod or 5-fluorouracil are non-surgical therapeutic options in locally circumscribed BCC (Rubin et al. 2005). But BCC can also progress to an advanced state in which surgery or radiation therapy is not considered to be helpful (locally advanced basal cell carcinoma, laBCC) (Rubin et al. 2005; Epstein 2008; Goppner and Leverkus 2011). Such lesions arise either from earlier lesions that have not been treated or from a recurrence of aggressive subtypes of BCC. Metastatic basal cell carcinoma (mBCC) is extremely rare, and the metastasis rate is believed to be less than 0.1% (Rubin et al. 2005; Epstein 2008; Goppner and Leverkus 2011; Lo et al. 1991).

4.1.1 Clinical Trials

An open-label multicenter phase I trial was conducted in 68 patients, which included 33 patients of locally advanced (laBCC) and metastatic BCC (mBCC), to evaluate the drug's safety and tolerability (Von Hoff et al. 2009; LoRusso et al. 2011a, b). In the early stage of the trial, there were only three patients with advanced BCC (LoRusso et al. 2011a, b), but the evidence of clinical benefit in two of these, encouraged the investigators to extend the cohort, increasing the final number of BCC patients to 33 (Von Hoff et al. 2009). Patients who had metastatic disease (n = 18) showed an overall response rate of 50% and those with locally advanced disease (n = 15) showed 60% response rate. Safety and pharmacokinetic studies were performed for three different daily doses of 150 mg (n = 17), 270 mg

(n = 15), and 540 mg (n = 1). The 150 mg daily dose was recommended for the phase II trials, as the higher doses did not produce higher plasma concentration of the drug and the safety profile was found to be acceptable, with no dose-limiting toxic effect (Von Hoff et al. 2009; LoRusso et al. 2011a, b).

A multicenter open-label phase II (Erivance BCC) study was conducted in patients with metastatic BCC (mBCC n = 33) and those with inoperable locally advanced BCC (laBCC n = 63) (Sekulic et al. 2012). A once-daily oral dose of 150 mg vismodegib was given to the patients. The objective response rate, as evaluated by independent reviewers, was 30% in patients with mBCC and 43% in patients with laBCC. Complete response (defined as the absence of residual basal cell carcinoma on assessment of a biopsy specimen) was seen in 13 patients (21%) with laBCC (Sekulic et al. 2012). In 2015 a 12-month update of efficacy and safety of the Erivance BCC study was published (Sekulic et al. 2015). In this analysis the patients had a minimal follow-up time of 21 months (including the primary analysis and an additional 12-month follow up). Objective response rates increased to 33.3% (all partial response, median duration of response 7.6 months) in patients with mBCC, and to 47.6% (22.2% complete response and 25.4% partial response) in the laBCC group and median duration of response increased from 7.6 to 9.5 months in the latter group. A final update of the Erivance BCC study is now available (Sekulic et al. 2017) only reporting investigator-assessed efficacy which already was higher than the independent reviewers evaluation in the previous publications mentioned above (Sekulic et al. 2012, 2015). The objective response rate was 48.5% (90% CI 30.8–66.2) for mBCC and 60.3% (90% CI 47.2–71.7) für laBCC, comparable with the investigator-assessed results of primary analysis (Sekulic et al. 2012).

Another phase II multicentric, randomized, double-blind, placebo-controlled trial (n = 41; vismodegib n = 26; placebo n = 15) tested the efficacy of vismodegib in patients with basal cell nevus syndrome (BCNS, syn: Gorlin syndrome) (Tang et al. 2012). BCNS is an autosomal dominant disorder, and the molecular basis is a mutation in the PTCH1-gene, which results clinically in numerous BCC along with other facial and skeletal abnormalities (Rubin et al. 2005; Epstein 2008; Goppner and Leverkus 2011; Tang et al. 2012). In this trial, the incidence of new BCC after three months of treatment (primary endpoint) in the vismodegib-treated cohort was significantly lower as compared to placebo (2 vs. 29 cases per group per year). The reduction in the size of already existing BCC (secondary endpoint) was also significantly greater with vismodegib than with the placebo (−65% vs. −11%). None of the patients on vismodegib showed progression of existing BCC, and in some patients, all basal cell carcinomas showed complete clinical regression. Biopsies taken from the clinically regressed tumors could not detect any residual BCC in 83% of the samples (Tang et al. 2012). Recently final results of this trial were published (Tang et al. 2016) after the 41 enrolled patients were monitored for a median of 36 months. Patients treated with vismodegib had a mean reduced rate of new surgically eligible BCC compared to patients in the placebo group (2 vs. 34 BCC per patient per year). In 11 patients initially assigned to placebo who crossed over to vismodegib after unblinding (open label phase of the study) developed fewer new surgically eligible BCC as compared to the placebo treatment

(0.4 vs. 30 BCC per patient per year). Vismodegib also reduced the size of existing BCC over an 18 months period (−56% for vismodegib from baseline vs. +13% for placebo). Most patients (74%) had to interrupt vismodegib treatment due to adverse events.

The high percentage of drug interruptions due to side effects despite the need of BCC patients requiring long-term treatment led to the initiation of the phase II MIKIE study (two intermittent vismodegib dosing regimens in patients with multiple basal cell carcinomas) (Dréno et al. 2017). In this randomised, regimen-controlled, double blind trial 229 adult patients with multiple BCC (at least 6 BCC, including BCNS patients) were enrolled. Group A (n = 116) was treated with 150 mg vismodegib per day for 24 weeks, then three rounds of 8 weeks placebo followed by 12 weeks vismodegib. Group B (n = 113) received vismodegib 150 mg daily for 24 weeks, then three rounds of 8 weeks placebo followed by 8 weeks vismodegib. The overall treatment phase was 72 weeks in both groups and per protocol planed total drug exposure was similar (48 weeks of vismodegib and 24 weeks of placebo). The mean number of BCC lesions was reduced from baseline by 62.7% (95% CI 53.0–72.3) in group A and 54.0% (95% CI 43.6–64.4) in group B.

Another nonrandomised phase II trial evaluated the activity of vismodegib in operable BCC by measuring the rate and durability of complete histological clearance (CHC) in 3 different dosing cohorts (cohort 1, n = 24: 24 weeks of vismodegib followed by Mohs surgery (MS); cohort 2, n = 25: 12 weeks of vismodegib and 24 weeks of observation thereafter MS; cohort 3, n = 25: two 8 weeks treatment periods separated by 4 weeks drug holiday followed by MS) (Sofen et al. 2015). In this study predefined primary efficacy end points of a >50% CHC (cohort 1 and 3) respectively >30% CHC (cohort 2) were not met in either cohort (cohort 1: 42%, cohort 2: 26%, cohort 3: 44%).

Meanwhile more data on clinical activity are profided by an expanded access study (n = 119) and the STEVIE (The SafeTy Events in VIsmodEgib) study (n = 1215) (Chang et al. 2014; Basset-Séguin et al. 2017). These studies provide insight in the efficacy in a patient population representative of clinical practice. Objective responses occurred in 46.4–68.5% of laBCC patients and in 30.8–36.9% of mBCC patients respectively (investigator-assessed according to RECIST v1.1) (Chang et al. 2014; Basset-Séguin et al. 2017).

4.2 Vismodegib in Colorectal Cancer

Based on the observed evidence for Hh activation in human colorectal cancer (CRC) tissues (Carpenter and Lo 2012; Hong et al. 2013), preclinical Hh ligand-dependent CRC models, D5123, and 1040830 were used to test the in vivo activity of vismodegib (Wong et al. 2011). In these models, oral treatment with vismodegib at a dose of 92 mg/kg twice daily caused tumor regression (Wong et al. 2011). In these mouse xenograft models, pathway modulation was linked to efficacy of vismodegib. To test whether these preclinical findings may be translatable

into the human situation, the efficacy and toxicity of vismodegib were studied in a randomized phase II trial including patients with CRC. Vismodegib was combined with FOLFOX or FOLFIRI and bevacizumab in patients with previously untreated metastatic (m)CRC (Berlin et al. 2013). In this trial, a total of 199 patients with mCRC were treated on protocol (124 FOLFOX, 75 FOLFIRI). Although Hh activity had been found in CRC, the overall response rates for placebo-treated and vismodegib-treated patients were comparable with 51% (90% CI 43–60) and 46% (90% CI 37–55), respectively. Also, the level of Hh expression in the CRC tissue did not correlate with a clinical benefit by vismodegib treatment. Overall no vismodegib-associated benefit was observed in combination with either FOLFOX or FOLFIRI. Based on the data from this clinical trial, a combination of vismodegib with FOLFOX-/FOLFIRI-based chemotherapy regimens is currently not justified in mCRC.

4.3 Vismodegib in Ovarian Cancer

In ovarian cancer (OC), aberrant activation of Hh signaling was observed to be correlated with unfavorable prognosis and the Hh pathway marker Gli1 was suggested to function as a negative prognostic marker in advanced serous OC (Ciucci et al. 2013). Consistent with the expression data, and in vitro data showing that Hh signaling pathway regulates the growth of OC spheroid forming cells (Ray et al. 2011), pharmacological inhibition of Hh signaling was shown to reduce serous OC growth in a primary xenograft model (McCann et al. 2011). Based on these preclinical data, a phase II, randomized, double-blind, placebo-controlled trial on vismodegib was performed to determine the efficacy in patients with OC in second or third complete remission as a maintenance therapy (Kaye et al. 2012). In this clinical trial, patients with recurrent epithelial OC were treated with either vismodegib (150 mg daily) or placebo according to their randomization after completing chemotherapy (Kaye et al. 2012). The treatment was discontinued when radiographic progression or toxicity occurred (Kaye et al. 2012). One hundred and four patients were treated with vismodegib or placebo (both arms: $n = 52$), and the median PFS was 7.5 and 5.8 months, respectively. The most frequent AEs in the vismodegib arm were dysgeusia, ageusia, muscle spasms, and alopecia (Kaye et al. 2012). Grade 3/4 AEs occurred in 12 patients (23.1%) with vismodegib and six (11.5%) with placebo (Kaye et al. 2012). The unexpected low advantage of the vismodegib-treated group with respect to PFS could have been due to the low Hh expression, which was found only in 13.5% of archival OC tissues.

4.4 Vismodegib in Small Lung Cell Cancer

The presence of Hh activity in SCLC (Watkins et al. 2003) fueled hope that a targeted disruption of this pathway could overcome therapy resistance. Using a SCLC mouse model not only an active Hh signaling pathway was described but

also its pharmacological blockade inhibited the growth of mouse and human SCLC (Park et al. 2011). Moreover it was shown that Hh pathway inhibition radiosensitizes non-small cell lung cancers (Zeng et al. 2013) and Hh pathway inhibition may delay or prevent the recurrence of residual disease after chemotherapy (Park et al. 2011). A phase I trial of vismodegib in patients with refractory, locally advanced solid tumors reported three patients with SCLC treated with vismodegib (LoRusso et al. 2011a, b). On the basis of this encouraging preclinical and early phase clinical data a trial of the ECOG-ACRIN cancer research group (E1508) was initiated. Unfortunately the trial did not show a significant improvement of PFS and OS with the addition of vismodegib (n = 52) or cixutumumab (n = 52) to chemotherapy with cisplatin and etoposide (n = 48) in extensive stage SCLC (Belani et al. 2016).

4.5 Vismodegib in Hematologic Diseases

Inhibition of the Hh pathway was considered most promising in chronic myeloid leukemia (CML) because is was shown to be required for maintenance of myeloid cancer stem cells (Dierks et al. 2008; Zhao et al. 2009). Treatment of the BCR-ABL-positive cell line OM9; 22 cells with vismodegib caused cell growth inhibition and induced apoptosis (Okabe et al. 2012). Besides these results with a cell line, vismodegib also inhibited the colony growth of Philadelphia-chromosome (Ph)-positive primary CML patient samples (Okabe et al. 2012). A combination of vismodegib and the established drug dasatinib resulted in a synergism with an increase in the cytotoxic effects of dasatinib in the presence of feeder cells in vitro (Okabe et al. 2012). A synergism was also described for the combination of ponatinib with vismodegib for therapy-resistant BCR-ABL1-positive leukemia (Katagiri et al. 2013). Also, B cell malignancies were shown to be responsive to Hh inhibition (Dierks et al. 2007), in particular when combined with Bcl2 inhibition (Kunkalla et al. 2013). However, there is currently no clinical trial published on the efficacy of vismodegib in leukemias or lymphomas.

4.6 Vismodegib in Medulloblastoma

The ontogenesis of medulloblastoma (MB) is regulated by Sonic Hh signaling, and Gli3 as a marker of Hh pathway activity is found in MB tissues (Miyahara et al. 2013). In an early phase I trial of vismodegib in patients with different types of solid tumors one patient with MB treated with vismodegib was reported (LoRusso et al. 2011a, b). Another phase I trial in pediatric patients with recurrent or refractory MB reported vismodegib to be well tolerated and the recommended phase II study dose was 150 or 300 mg of vismodegib, depending on the patients BSA (Gajjar et al. 2013). In this study antitumor activity could be observed in 1 of 3 patients with SHH-subtype disease. In 2015 efficacy of vismodegib in a phase II study in pediatric (n = 12) and adult (n = 31) patients with recurrent MB was reported (Robinson et al. 2015). Three adult patients and one pediatric patient exhibited protocol-defined

responses, all of these patients with SHH-subgroup MB, no clinical efficacy was observed in Non-SHH MB patients. Clinical data is still very limited for MB patients, but for the subgroup of SHH-MB patients with recurrent or refractory MB vismodegib should be considered as a therapeutic option (Lou et al. 2016).

5 Toxicity

Safety data are available from numerous studies (Table 1). Nearly all patients treated with vismodegib report at least one treatment related adverse event (AE). The most frequent AEs are muscle spasms/cramps, alopecia, dysgeusia, weight loss, fatigue, nausea, loss of appetite, and diarrhea (Table 1). Most AEs are mild to moderate and occur early in the course of treatment (Lacouture et al. 2016). Longer duration of treatment does not seem to increase frequency and severity of AEs (Basset-Séguin et al. 2017; Sekulic et al. 2017). Nevertheless the long-term nature of these AEs may result in impaired quality of life, treatment interruption and in some cases in discontinuation. In the early phase I study one patient displayed QTc interval prolongation, but further studies in healthy volunteers could not find an apparent relationship between the administration of vismodegib and QT prolongation (Von Hoff et al. 2009; LoRusso et al. 2011a, b; Graham et al. 2013). Discontinuation rate due to all AEs vary across studies: in the adjuvant BCC trial (Sofen et al. 2015) with a short exposure to vismodegib the discontinuation rate was only 5% compared to 31% in the STEVIE study (Basset-Séguin et al. 2017) with a median treatment duration of 8.6 months. The majority of the most common treatment related AEs resolved within 12 months after discontinuation. Because the

Table 1 Summary of safety data from selected vismodegib studies

	Erivance BCC			Chang et al. (2014) $n = 119$	Basset-Séguin et al. (2017) $n = 1215$	Sofen et al. (2015) $n = 74$
	Sekulic et al. (2012) $n = 104$	Sekulic et al. (2015) $n = 104$	Sekulic et al. (2017) $n = 104$			
Any AE (%)	100	100	100	98	98	99
Muscle spasms/cramps	68	71	71	71	66	76
Alopecia	64	65	66	58	62	58
Dysgeusia	51	54	56	71	55	50
Weight loss	46	50	52	16	41	–
Fatigue	36	40	43	19	17	20
Nausea	29	33	33	19	18	18
Loss of appetite	23	27	28	–	25	11
Diarrhea	22	26	27	25	16	8

frequent occurrence of AEs patient education is mandatory and existing management guidelines of vismodegib related AEs should be followed by the physicians (Lacouture et al. 2016). If AEs result in dose interruptions despite of sufficient AE management the clinical activity of vismodegib seems not to be effected as shown by the results of the intermittent vismodegib dosing regimens evaluated in the MIKIE study (Dréno et al. 2017).

Besides the above-mentioned side effects and toxicities vismodegib, based on its mechanism of action is known to be teratogenic, embryotoxic, and fetotoxic (Varjosalo and Taipale 2008; Atwood et al. 2012; McMillan and Matsui 2012). In rats at maternal exposures lower than human exposures at the recommended dose of 150 mg/day, malformations included craniofacial anomalies, an open perineum, and absent or fused digits; moreover, fetal retardations and variations were also noted (Genentech Inc. 2017). Both male and female patients must be advised of this risk. In addition, before initiating treatment with vismodegib, physicians must verify a female patient's pregnancy status and must advise female patients of the need for contraception. Male patients must be informed of the potential risk of exposing their partners to vismodegib through semen (Genentech Inc. 2017).

6 Drug Interactions

The elimination of vismodegib involves several pathways. Vismodegib is predominantly excreted as an unchanged drug, but several minor metabolites are produced by cytochrome P450 isoenzymes (Genentech Inc. 2017). In parallel with safety/efficacy assessments conducted during clinical development, the drug–drug interaction (DDI) potential as well as the assessment of absorption, distribution, metabolism, and excretion has extensively been evaluated to anticipate/avoid unwanted side effects or reductions in efficacy due to concomitant drug administration (Wong et al. 2009; LoRusso et al. 2011a, b; Sharma et al. 2013). In standard in vitro assays, metabolism-based DDIs were assessed, and vismodegib had moderate potential for inhibition of cytochrome P450 (CYP) 2C8 and CYP2C9 and to a lesser extent of CYP2B6, CYP2C19, and CYP2D6. Vismodegib does not induce CYP1A2, CYP2B6, or CYP3A4/5 in cultured human hepatocytes, nor is it a strong binder of human pregnane X receptor (PXR) (Wong et al. 2009; LoRusso et al. 2011a, b; Sharma et al. 2013). Oxidative metabolites of vismodegib were primarily formed by CYP3A4/5 and CYP2C9 in vitro (Wong et al. 2009), but in vivo coadministration of CYP3A4/5 inducers and inhibitors did not alter steady-state plasma concentration (Genentech Inc. 2017; LoRusso et al. 2011a, b). In vitro studies identified vismodegib as an inhibitor/substrate (Zhang et al. 2009) of the efflux transporter *P*-glycoprotein, while others could not reproduce this observation (Wong et al. 2009). The clinical relevance of the effect on the *P*-glycoprotein therefore remains unclear, but the prescribing information indicates a potential interaction by coadministration of vismodegib and drugs inhibiting *P*-glycoprotein

(e.g., clarithromycin, erythromycin, azithromycin) leading to an increased incidence of adverse events (Genentech Inc. 2017).

7 Summary and Perspectives

The association between PTCH1 mutations in Gorlin syndrome and aberrant pathway activity in BCC and the development of a small molecule that specifically inhibits this aberrant signaling is an exceptional example of successful translational research. Targeting the Hh pathway is a promising strategy in cancer therapy, and the efficacy of vismodegib in BCC patients has led to its approval by the FDA and the EMA for adult patients with symptomatic metastatic BCC, or locally advanced BCC inappropriate for surgery or radiotherapy. A concern in patients being treated with vismodegib is the side effect profile which, even though these side effects are mostly low grade, seems to make a long-term treatment difficult to tolerate for some patients. Side effect management has therefore been of major interest and side effect management guidelines have been published recently and should be followed by physicians to reduce impairment of quality of life and improve adherence to therapy. The role of vismodegib in a neoadjuvant treatment regimen might reduce surgical defect area and thus could impact morbidity, scarring and function of vital structures. To date there is only limited data available from good quality studies for the neoadjuvant use of vismodegib in BCC and long term outcomes are not yet available. In many other cancers, involvement of the Hh pathway has been postulated. Apart from the reported clinical efficacy in a small subgroup of recurrent MB patients (SHH-MB) negative clinical results in other tumor entities raise the question of the clinical significance in these tumor entities. Despite this disappointing results multiple clinical trials are ongoing at the moment addressing these questions; therefore, vismodegib and also other Hh inhibitors will still be of future interest in the treatment of cancer.

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Larotrectinib (LOXO-101)

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Contents

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Abstract

One of the most challenging issues in oncology research and treatment is identifying oncogenic drivers within an individual patient's tumor which can be directly targeted by a clinically available therapeutic drug. In this context, gene fusions as one important example of genetic aberrations leading to carcinogenesis follow the widely accepted concept that cell growth and proliferation are driven by the accomplished fusion (usually involving former proto-oncogenes) and may therefore be successfully inhibited by substances directed against the fusion. This concept has already been established with oncogenic gene fusions like BCR-ABL in chronic myelogenous leukemia (CML) or anaplastic lymphoma kinase (ALK) in lung cancer, including special tyrosine kinase inhibitors (TKIs) which are able to block the activation of the depending downstream proliferation pathways and, consequently, tumor growth. During the last decade, the NTRK1, 2, and 3 genes, encoding the TRKA, B, and C proteins, have attracted increasing attention as another significant and targetable gene fusion in a variety of cancers. Several TRK inhibitors have been developed, and one of them, Larotrectinib (formerly known as LOXO-101), represents an orally available, selective inhibitor of the TRK receptor family that has already shown substantial clinical benefit in both pediatric and adult patients harboring an NTRK gene fusion over the last few years.

Keywords

NTRK genes · TRK receptor family · Larotrectinib · LOXO-101

1 Introduction

The NTRK1, NTRK2, and NTRK3 genes encode the TRKA, TRKB, and TRKC proteins, all of which belong to the tropomyosin receptor kinase (TRK) family (Khotskaya et al. 2017).

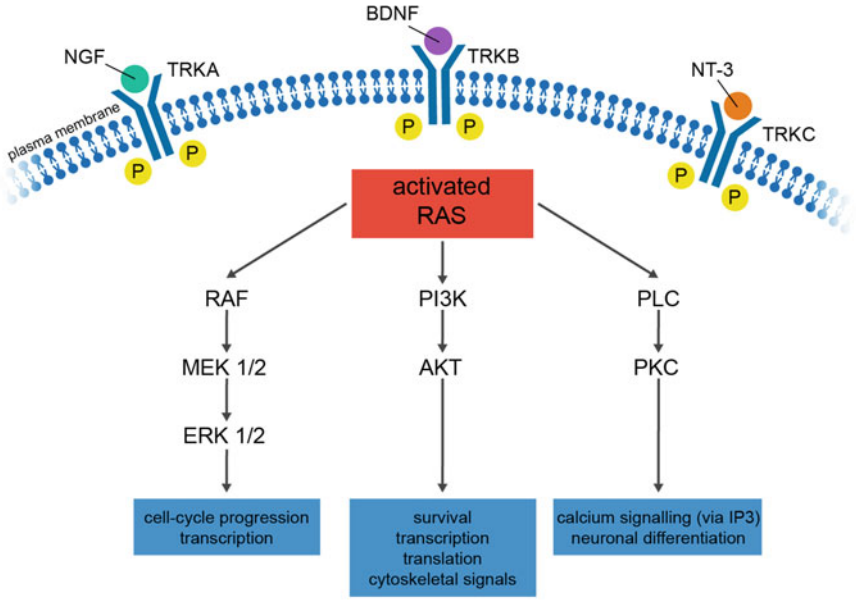
TRKA, B, and C are three different transmembrane proteins (Kaplan et al. 1991). TRK proteins are formed by an extracellular domain for ligand binding, a single transmembrane segment, and an intracellular tyrosine kinase domain (Khotskaya et al. 2017). The receptors are activated by the binding of ligands (nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophin-3 (NT-3)), inducing receptor dimerization, phosphorylation, and, consequently, activation of downstream signaling pathways (Klein et al. 1991). These include such significant pathways as RAS-RAF-MAPK-ERK, PI3K-AKT-mTOR, and PLC-γ/PKC.

The RAS-RAF-MAPK-ERK pathway promotes cell-cycle progression and proliferation, PI3K-AKT-mTOR is involved in protein synthesis and survival, and PLC-γ/PKC in differentiation. The RAS-RAF-MAPK pathway is probably the most

important one of these three (Kaplan et al. 1991; Klein et al. 1991; Roccato et al. 2002).

For an illustration of these processes, interactions, and the depending downstream pathways, please refer to Fig. 1.

TRK signaling is physiologically involved in and critical for neuronal development and maturation of the central and peripheral nervous system (Ardini et al. 2014; Chao 2003; Doebele et al. 2015), and it is also involved in perception of pain, thermoregulation, mood regulation, memory processes, and proprioception (Snider 1994). The TRK receptors are expressed in cells of the immune system, lung, and



TRK downstream signalling pathways: The neurotrophins NGF, BDNF and NT-3 bind to their receptors TRKA/B/C, resulting in conformational changes, like receptor dimerization, phosphorylation and activation of signalling pathways, thereby leading to cell proliferation, survival and other downstream effects.
Abbreviations: NGF, nerve growth factor; BDNF, brain-derived neurotrophic factor; NT-3, neurotrophin-3; TRKA/B/C, tropomyosin-receptor kinase A/B/C; RAS, rat sarcoma; RAF, rat fibrosarcoma; MEK, mitogen-activated kinase; ERK, extracellular-regulated kinase; PI3K, phosphoinositide-3-kinase; AKT, activated kinase; PLC, phospholipase C; PKC, protein kinase C; IP3, inositol-trisphosphate (second messenger)

Partly adopted and rearranged from **Downward 2013, Khotskaya et al. 2017** and **Ricciuti et al. 2017**

Fig. 1 TRK downstream signaling pathways

bone, additionally to their expression in nerve cells, under physiological circumstances (Coppola et al. 2004).

The TRK protein had originally been identified from a colorectal cancer sample over 30 years ago (Martin-Zanca et al. 1986), but for a long time thereafter, no subsequent report of what the underlying oncogene might imply in colorectal cancer has been made. Only in the last 15 years, the importance of aberrant TRK signaling as an oncogenic driver has become the focus of attention (Roccato et al. 2002; Tognon et al. 2002).

Aberrant TRK signaling has been implicated in cancer development in a lot of different types of cancer, although the detection of altered TRK signaling is a relatively rare event in most cancers.

The detected aberrations resulting in altered TRK signaling include gene fusions, single-nucleotide alterations, in-frame deletions, and splice variants, with gene fusions being the most frequent and most important sort of aberration (Khotskaya et al. 2017; Miranda et al. 2014; Tacconelli et al. 2004; Vaishnavi et al. 2015).

The typical oncogenic fusion is that the 3′ region of a former proto-oncogene is juxtaposed to 5′ sequences of an unrelated gene via intra- or interchromosomal rearrangement (Vaishnavi et al. 2015). Consequently, the newly formed oncogene disposes of a constitutive activation of the kinase domain, resulting in constitutive activation of downstream pathways of cell growth and proliferation as well (Rubin and Segal 2003).

The concept of oncogenic gene fusion is not a novel one. In 1982, the first oncogenic gene fusion was discovered: The ABL proto-oncogene is translocated to the BCR gene which results in a BCR-ABL fusion gene that encodes a constitutively activated kinase and represents the pathomechanism of the development of chronic myelogenous leukemia (CML). Only recently, the anaplastic lymphoma kinase (ALK) gene and ROS-1 proto-oncogene were detected as further candidates for potential oncogenic gene fusion, resulting in special forms of lung cancer carcinogenesis (Soda et al. 2007).

Oncogenic NTRK fusions can be found in about 20 different types of tumors, including adenocarcinoma of the lung, colorectal cancer, papillary thyroid cancer, brain cancers (glioma, pilocytic astrocytoma, and glioblastoma), spitzoid neoplasms, intrahepatic cholangiocarcinoma, special forms of sarcoma, head and neck squamous cell carcinoma, acute myeloid leukemia, gastrointestinal stromal tumor (GIST), and others (Frattini et al. 2013; Hyman et al. 2017; Khotskaya et al. 2017; Knezevich et al. 1998; Ross et al. 2014; Vaishnavi et al. 2015).

Intriguingly, NTRK fusions appear with a rather high frequency in some special and rare cancer types, such as congenital/infantile fibrosarcoma, secretory breast cancer, or mammary analog secretory carcinoma (MASC), which represents a cancer of the salivary gland (Hyman et al. 2017). In these tumors, the detection rate of an NTRK gene fusion is 90–100%, whereas common cancer types display a rather low NTRK fusion frequency (lung cancer about 3%, colorectal cancer <2%) (Farago et al. 2015; Ricciuti et al. 2017; Vaishnavi et al. 2013). While NTRK rearrangements have been found in maximally 12% of samples of invasive breast cancer, in one study, they have been detected in as much as 40% of breast cancer

brain metastases, which might help enlightening the role of NTRK in bringing metastases to the CNS (Bollig-Fischer et al. 2015; Gao et al. 2013).

NTRK gene fusion variants include the LMNA-NTRK1 gene fusion (found in spitzoid tumors, colorectal cancer, and soft tissue carcinoma), the TPM3-NTRK1 gene fusion (found in colorectal cancer and papillary thyroid carcinoma), the PAN3-NTRK2 gene fusion (found in head and neck squamous cell carcinoma), the ETV6-NTRK3 gene fusion (found in congenital fibrosarcoma, secretory breast carcinoma, acute myeloid leukemia, and mammary analog secretory carcinoma (MASC)), and others. Whereas NTRK1 and NTRK2 have several fusion partners which cannot all be mentioned here (entire list available in Khotskaya et al. 2017), for NTRK3, only the ETV6 fusion partner has been described so far.

2 Structure and Mechanism of Action

As mentioned above, oncogenic gene fusions are a recurrent event in carcinogenesis, and by inhibiting the activated tyrosine kinase domain by a tyrosine kinase inhibitor (TKI), they represent attractive targets in oncology. Several TKIs (Bosutinib, Dasatinib, Imatinib, Nilotinib, and Ponatinib) have already demonstrated efficacy in the treatment of CML, Crizotinib in the treatment of lung tumors harboring ROS-1 or ALK gene fusions, and Ceritinib and Alectinib in tumors bearing ALK gene fusions (Califano et al. 2015).

Similarly to ALK and ROS-1 rearrangements, NTRK fusions are characterized by a high variability in the 5′ gene fusion partner, which makes the resulting chimeric protein sensitive to TRK-directed TKIs (Ricciuti et al. 2017; Stransky et al. 2014).

Larotrectinib (LOXO-101) is a highly selective pan-TRK inhibitor that shows no significant activity outside of the TRK family (Vaishnavi et al. 2015). It is characterized by the molecular formula of C21H22F2N6O2 and is a 3-urea-substituted pyrazolo[1,5a]pyrimidine (Ricciuti et al 2017; Vaishnavi et al. 2013). The molecular weight is 428,444 g/mol. Larotrectinib is orally bioavailable in 25 and 100 mg capsules. Pharmacokinetics detected good systemic exposure after oral dosing (Burris et al. 2015a, b), and they also showed that maximum plasma concentrations were reached 30–60 min after dosing (Hong et al. 2015).

Larotrectinib preferentially blocks the ATP-binding site of TRKA, TRKB, and TRKC, thereby inhibiting the TRK catalytic activity, the autophosphorylation, and the activation of downstream pathways (Doebele et al. 2015; Vaishnavi et al. 2013). It works with a 2–20 nmol/l cellular potency against all TRK kinases and is characterized by IC50 values in the low nanomolar range (Burris et al. 2015a; Hong et al. 2015; Khotskaya et al. 2017; Nagasubramanian et al. 2016).

3 Preclinical Data

Doebele et al. (2015) performed proliferation assays on three cell line models harboring NTRK gene fusions (a lung carcinoma cell line, the KM 12 cell line derived from colorectal cancer, and a cell line from an AML patient). They observed a dose-dependent inhibition of cell proliferation by Larotrectinib in all three cell lines. The IC50 was less than 100 nmol/l for the lung cancer cell line and less than 10 nmol/l for the colorectal cancer cell line. Vaishnavi and colleagues (2013) had formerly shown that Larotrectinib brings no effect to cell lines lacking an NTRK gene fusion.

In preclinical in vivo xenograft mouse models harboring NTRK fusions, Larotrectinib also demonstrated potent tumor growth inhibition: Doebele and colleagues injected the KM 12 cell line into athymic nude mice and then treated them with Larotrectinib orally. They found dose-dependent tumor growth inhibition (Doebele et al. 2015; Hong et al. 2015).

Taken together, there is evidence that Larotrectinib can inhibit proliferation in vitro and in vivo, but is only effective in the presence of an NTRK gene fusion.

4 Clinical Data

As NTRK gene fusions are relatively rare events in carcinogenesis, and as Larotrectinib represents a substance that has only been investigated for a few years, most clinical data are derived from the pooled interim analysis of three clinical trials that were presented at the ASCO annual meeting in Chicago in June 2017.

Here, Hyman et al. (2017) reported that 55 patients (29 male and 26 female), 12 of them under the age of 15, had been enrolled in the Larotrectinib TRK fusion development program. This involves three still ongoing trials: an adult phase I trial (8 adult patients enrolled), a pediatric phase I/II trial, called "SCOUT" (12 patients under the age of 21 years enrolled), and an adult/adolescent phase II basket trial, called "NAVIGATE" (35 patients, at least 12 years old, enrolled). Basket trials recruit patient with specific genomic or molecular aberrations (here: NTRK gene fusion) independently of the underlying histology (Okimoto and Bivona 2016).

The aim of the trials is to test the safety, side effects, and dosing of Larotrectinib (administered as a single agent 100 mg twice daily) in combination with response to the treatment. All participants are characterized by advanced solid tumors (17 different types of cancer, including infantile fibrosarcoma, thyroid cancer, sarcoma, and salivary gland tumors) which are fusion-positive.

Of all the enrolled patients with confirmatory response data available (others: confirmatory scans pending), the objective response rate was 76%, with 12% complete responses and 64% partial responses. 12% experienced stable disease, only 12% progressive disease (Hyman et al. 2017; Laetsch et al. 2017). The efficacy of the treatment was regardless of the patients' age (responses seen in pediatric and adult patients), of the tumor type, and of the gene fusion partner (responses seen in 12 different partners, including ETV6, TPM3, and LMNA).

75% of all patients still remain on treatment (by the time of June 2017) or underwent surgery with curative intent (for example, one patient with fibrosarcoma on the leg could, after treatment with four cycles of Larotrectinib, undergo surgical resection with no functional deficit post-surgery and showed pathologic complete response). Responses to the therapy have generally been long-lasting, with the median duration of response and the median progression-free survival not reached yet (time of June 2017).

A case report was published about a 14-year-old female patient from Bangladesh, diagnosed with secretory breast carcinoma (Shukla et al. 2017). As an ETV6-NTRK3 oncogenic gene fusion represents the pathognomonic alteration in this cancer type (Tognon et al. 2002), this was molecularly tested and confirmed. Having exhausted all available treatment options in Bangladesh, a therapy with Larotrectinib was initiated as part of the Larotrectinib TRK fusion development program, resulting in a significant and rapid tumor reduction with near-complete resolution after 2 months of therapy.

Another case report is about the successful use of Larotrectinib in a pediatric patient with infantile fibrosarcoma (IFS) (Nagasubramanian et al. 2016). IFS represents a very rare pediatric cancer, usually presenting in the first 2 years of life, which is characterized by a translocation t(12; 15) (p13; q25), fusing the ETV6 gene with the NTRK3 gene. This female patient had been born with a mass at the right side of her neck which was later diagnosed as IFS, treated with surgery and chemotherapy, but repeatedly experienced recurrence of the disease. She was then treated with Larotrectinib as part of the Larotrectinib TRK fusion development program and showed a partial response to the treatment, which is clearly superior to the previous treatment.

The third case report, published by Doebele et al. (2015), is about a 41-year-old woman diagnosed with soft tissue sarcoma metastasized to the lungs, harboring an LMNA-NTRK1 gene fusion in her tumor. She was treated with Larotrectinib as part of the program and experienced massive tumor regression, accompanied by notable reduction of dyspnea.

Larotrectinib is able to penetrate the blood–brain barrier, thereby possibly achieving tumor regression in patients with CNS tumors as well, as described in the fourth case report (Hong et al. 2016), where a patient with non-small cell lung cancer, characterized by a TPR-NTRK1 rearrangement, and metastases to the brain achieved a radiographic response in his brain lesions.

Taken together, these data show that Larotrectinib can be effective in a variety of cancers.

5 Toxicity

As reported before in the section “Clinical Data”, most information about the toxicity of Larotrectinib has been gained as part of the Larotrectinib TRK fusion development program, and has been presented at the ASCO annual meeting in Chicago, 2017 (Hyman et al. 2017).

These treatment-emergent adverse events (AEs) were reported: Fatigue in 38% of the participants (mostly grade 1 and 2), dizziness in 27% (mostly grade 1), nausea in 26%, and vomiting in 24%. Anemia was seen in 26%; remarkably, 9% of the participants experienced grade 3 anemia, which, according to the common terminology criteria for adverse events, denotes a hemoglobin level of less than 8 g/dl, requiring a blood transfusion. An increase in liver enzymes was seen in 23% (AST) and 20% (ALT). Other adverse events were constipation (22%), cough (21%), diarrhea (20%), and dyspnea (18%).

When only the treatment-related adverse events were regarded, percentages were remarkably lower (fatigue 18%, dizziness 20%, nausea 18%, vomiting 13%, anemia 10%, increased AST/ALT 18%/17%, constipation 12%, cough 2%, and diarrhea 6%).

Only 13% of the participants required a dose reduction; no patient discontinued therapy due to adverse events.

Previously (Burris et al. 2015b), AEs had been described as fatigue (47%), dizziness (27%), anemia (33%), constipation (20%), dry mouth (20%), diarrhea (13%), nausea and vomiting (both 13%), and syncope (13%). Notably, one patient experienced delirium. Severe adverse events (SAEs) had not been reported.

As the TRK receptors play an important role in pain mediation in the CNS, a potential side effect of TRK inhibitors may be a decrease in pain, which might be particularly beneficial in tumor patients who often suffer from pain (Vaishnavi et al. 2015).

Interestingly, the adverse events mentioned above partly involve the CNS, corresponding to the fact that TRK signaling is physiologically involved in neuronal development and is activated by neurotrophins.

6 Drug Interactions

In all clinical trials, Larotrectinib was used as monotherapy against tumor proliferation. Drug interactions with concomitant chemotherapy or other targeted drugs were therefore not described.

Larotrectinib is “not a significant inhibitor or inducer of cytochrome P450 3A4 isoenzyme” (statement of Loxo Oncology that distributes Larotrectinib) which is why there may be a reduced risk of drug interactions.

To our knowledge, no drug interactions of critical relevance have been described until now, but as NTRK gene fusions are rare events in carcinogenesis, few patients have undergone treatment with Larotrectinib so far.

7 Biomarkers

To our knowledge, no significant special biomarker has been detected so far which could be helpful to decide in advance if Larotrectinib achieves response. Actually, the best predictive biomarker probably is the detection of an NTRK gene fusion

itself—if present, this might refer to a successful treatment with Larotrectinib (Ricciuti et al. 2017).

In the data on the three clinical trials, presented at the ASCO annual meeting 2017, 76% of the enrolled patients, all of which harbored an NTRK gene fusion, responded to the therapy, which means that the detection of this gene fusion could predict response in a very high percentage.

8 Summary and Perspectives

In a restricted amount of trials (due to the small number of patients harboring an NTRK gene fusion in their tumors), Larotrectinib has demonstrated encouraging clinical effectiveness in inhibiting tumor growth and progression in both pediatric and adult patients with a very high response rate of over 75%.

As far as can be assumed by the available data, Larotrectinib is also well tolerated by patients, and the toxicity profile, in general, seems manageable, with most adverse events being reported as grade 1 or 2, and no SAEs being reported so far.

Obviously, Larotrectinib can only unfold its effect on tumors which are NTRK gene fusion-positive. Therefore, it is pivotal that tumor samples are tested for this gene fusion, which, at present, is not routinely accomplished and should be expanded in oncological diagnostics.

Both the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have granted orphan drug designation for Larotrectinib for the treatment of patients with soft tissue sarcoma harboring NTRK fusions. Additionally, the FDA granted breakthrough therapy designation to Larotrectinib in 2016 "for the treatment of unresectable or metastatic solid tumors with NTRK fusion proteins in adult and pediatric patients who require systemic therapy and who have either progressed following prior treatment or who have no acceptable alternative treatments," meaning an indication solely based on the molecular basis of the tumor. Loxo Oncology, which distributes Larotrectinib, plans to submit an application to the FDA by the beginning of 2018 for the approval of Larotrectinib.

As it can be expected that, during the next years, more and more patients will be treated with Larotrectinib, more information about safety and side effects will be obtained simultaneously. Only recently, another important issue has become apparent under ongoing treatment with Larotrectinib: Hyman et al. (2017) observed that some tumors were able to acquire mechanism of resistance against Larotrectinib, which in four out of six patients were caused by the TRKA G595R resistance mutation. Two of them could successfully be treated with the TRK tyrosine kinase inhibitor LOXO-195 which was able to overcome the resistance (Drilon et al. 2017).

In conclusion, observations on the fascinating impact of Larotrectinib on inhibiting tumor growth in NTRK gene fusion-positive tumors have only recently been initiated, and undoubtedly, during the next years, we will hear and learn a lot more about applications and implications of this promising anticancer therapy.

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Palbociclib—The First of a New Class of Cell Cycle Inhibitors

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Abstract

During the last decades, much has been learned about with cyclin-dependent kinases (CDK) playing a pivotal role in the cell cycle regulation. CDK4/6 is the key regulator of the G1-S transition. Palbociclib (PD 0332991, Ibrance®) is the first oral CDK4/6 inhibitor showing a substantially improved median progression-free survival (PFS) in advanced estrogen receptor (ER) positive and human epidermal growth factor receptor 2 (HER2) negative breast cancer. This PFS prolongation was seen both with letrozole as first-line therapy (24.8 vs. 14.5 months [PALOMA 2]) and with fulvestrant in endocrine pretreated patients (9.2 vs. 3.8 months [PALOMA-3]). The main toxicity is neutropenia due to cell cycle arrest which can be easily managed with dose interruption or dose reduction leading to a favorable safety profile with delayed deterioration of global quality of life (QoL). Palbociclib is approved by the Federal Drug Administration (FDA) and the European Medicines Agency (EMA) for ER-positive/HER2-negative advanced breast cancer. Despite the well-understood mode of action of palbociclib, predictive biomarkers are not yet defined. In conclusion, inhibition of CDK4/6 using palbociclib in combination with endocrine therapy is an efficient and well-tolerated treatment option in ER-positive/HER2-negative advanced breast cancer. Ongoing clinical trials are investigating the role of palbociclib in early breast cancer as well as in other types of cancer.

Keywords

Palbocilib · Breast cancer · Metastatic · Advanced · Endocrine

1 Introduction

Endocrine therapy is a mainstay in the treatment of endocrine receptor (ER) positive patients with breast cancer. This is especially true in patients with advanced metastatic disease. The therapeutic goal is control of the disease and preservation of quality of life (QoL). It is well accepted that metastatic breast cancer is incurable but treatable (Cardoso et al. 2017). In this setting, different endocrine agents like tamoxifen, aromatase inhibitors (AI), and fulvestrant are often used in patients with hormone receptor-positive breast cancer. However, endocrine resistance occurs in

the majority of the patients either primarily or secondarily. Besides estrogen receptor 1 (ESR1) mutations, interactions with growth factor receptors are important mediators of endocrine resistance. In addition to cell signaling pathway activation like phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) or RAF/mitogen-activated protein kinase kinase (MEK)/extracellular signal-regulated kinase (ERK), cell cycle checkpoint alterations play a crucial role in endocrine-resistant breast cancer (Murphy and Dickler 2016). Cell cycle alterations gained considerable interest in recent years. Cyclin-dependent kinases (CDK) have a crucial role in orchestrating the tightly regulated cell cycle. Early CDK inhibitors like flavopiridol showed broad activity upon several CDKs but had considerable toxicities and limited efficacy (Ingham and Schwartz 2017). Recently, more specific CDK inhibitors targeting especially CDK4/6 were developed. Palbociclib (PD 0332991, Ibrance®) is the first compound of this class. It is approved for advanced estrogen receptor (ER) positive and human epidermal growth factor 2 (HER2) breast cancer in combination with endocrine therapies like aromatase inhibitors or fulvestrant.

2 Structure and Mechanism of Action

Palbociclib (Fig. 1) is a potent and highly specific inhibitor of CDK4 (inhibitory concentration 50% [IC50], 0.011 μmol/L) and CDK6 (IC50, 0.016 μmol/L) without activity against a panel of 36 additional protein kinases (Table 1) (Fry et al. 2004).

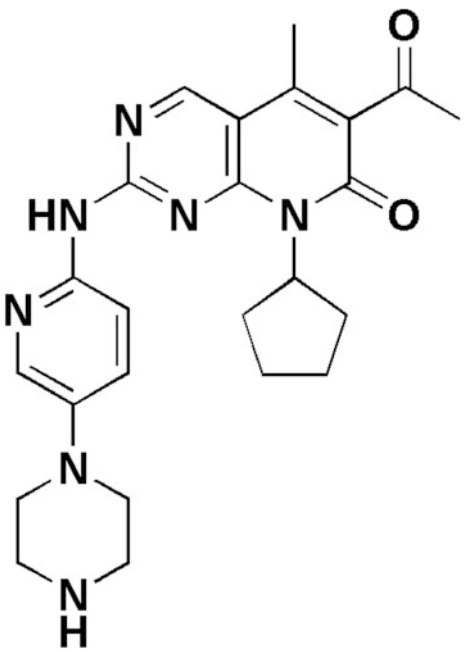

Fig. 1 Molecular structure of palbociclib (PD-0332991) (Fry et al. 2004)

Table 1 Inhibitory activity of palbociclib against a panel of protein kinases (Fry et al. 2004)

Protein kinase	IC50 (μmol/L)
Cdk4/cyclin D1	0.011
Cdk4/cyclin D3	0.009
Cdk6/cyclin D2	0.015
Cdk2/cyclin E2	>10
Cdk2/cyclin A	>10
Cdk1/cyclin B	>10
Cdk5/p25	>10
Epidermal growth factor receptor	>10
Fibroblast growth factor receptor	>10
Platelet-derived growth factor receptor	>10
Insulin receptor	>10
Lymphocyte kinase	>10
Vascular endothelial growth factor receptor	>10
AMP-activated protein kinase	>10
Checkpoint kinase-1	>10
Casein kinase-1	>10
Casein kinase-2	>10
c-Src kinase	>10
C-terminal Src kinase	>12
Dual-specificity tyrosine phosphorylation-regulated kinase 1A	2.0
Glycogen synthase kinase-3β	>10
c-Jun NH2-terminal kinase	>10
Mitogen-activated protein kinase 2/Erk2	>10
Mitogen-activated protein kinase–activated protein kinase 1a	8.0
Mitogen-activated protein kinase–activated protein kinase 2	>10
Mitogen-activated protein kinase kinase	>10
Mitogen and stress-activated protein kinase 1	>10
p70 Ribosomal protein S6 kinase	>10
3-Phosphoinositide-dependent protein kinase 1	>10
Phosphorylase kinase	>10
Cyclic AMP-dependent protein kinase	>10
Protein kinase B	>10
Protein kinase C	>10
p38-Regulated/activated kinase	>10
Rho-dependent protein kinase	>10
Stress-activated protein kinase 2a	>10
Stress-activated protein kinase 3	>10
Stress-activated protein kinase 4	>10
Serum- and glucocorticoid-induced kinase	>10

CDK cyclin-dependent kinase; *IC50* inhibitory concentration 50%

CDK4/6 plays a pivotal role in the regulation of the cell cycle. Disrupted cell cycle regulation is one of the hallmarks of cancer (Hanahan and Weinberg 2011). Notably, tumor cells are evading growth suppressors and circumvent programs negatively regulating cell proliferation. This negative regulation is largely maintained by tumor suppressor genes like retinoblastoma (RB). RB is of critical importance for the transmission of growth-inhibitory signals originating outside the cell. Hence, RB is a critical gatekeeper of cell cycle progression whose absence permits persistent cell proliferation.

Cells must progress through the four phases of the cell cycle to divide and replicate: G1, S phase (DNA synthesis), G2, and M phase (mitosis). The key players for regulation of the cell cycle are the cyclin-dependent kinases, a group of serine/threonine kinases that form active heterodimeric complexes following binding to cyclins, thereby facilitating the transition of the cell cycle (Malumbres and Barbacid 2001). CDK4/6 is the key regulator of the G1-S transition. In complex with cyclin D, CDK4/6 phosphorylates retinoblastoma protein (Rb) and drives cell cycle progression, a process inhibited by p16 (Fig. 2). In its active hypo-phosphorylated state, Rb inhibits the transcription factor E2F which is of pivotal importance for the progression from G1 to S phase (reviewed by Shapiro 2006; Schmidt 2016; Ingham and Schwartz 2017). In response to mitogenic signals, cells synthesize cyclin D which forms a complex with CDK4/6 leading to phosphorylation of Rb. When Rb is phosphorylated, the inhibition of the transcription factor E2F is lost. Thereby, cell cycle progression occurs. Obviously, cell cycle progression can be successfully blocked with CDK4/6 inhibitors. However, cell

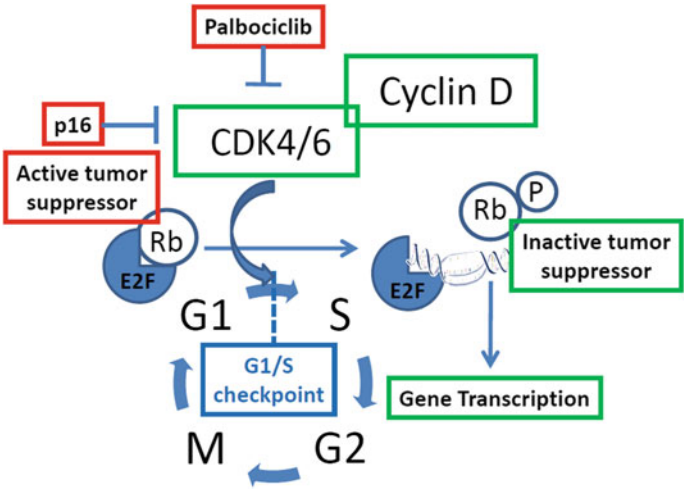

Fig. 2 Role of CDK4/6 in cell cycle regulation (reprinted with permission Schmidt 2016, S. Karger AG). Cyclin-dependent kinase (CDK); E2 promoter binding factor (E2F); gap1 phase (G1); gap2 phase (G2); mitotic phase (M); phosphorylation (P); protein 16/cyclin-dependent kinase inhibitor 2A/multiple tumor suppressor 1 (p16); retinoblastoma protein (Rb); and synthetic phase (S)

cycle regulation has a lot of redundancy built in. This means that cells can proliferate even in the absence of CDK4/6 using CDK2 instead. Transcription of cyclin E serves as a positive feedback loop, because cyclin E forms a complex with CDK2. Either way, it leads to phosphorylation of Rb, thus disrupting the binding of Rb to E2F. This again leads to E2F activation and the transcription of genes necessary for S phase entry and cell cycle progression. However, there are also negative regulators of cell cycle progression. For instance, p16 plays a major role in the induction of cellular senescence in breast cells (Bazarov et al. 2012). p16 is of critical importance, because it inhibits CDK4/6 and leads to release and degradation of cyclin D as well as to a redistribution of p21 and p27 to the cyclin E—CDK2 complex which contributes to G1 arrest. In addition, antiproliferative signals induce p21 and p27 that also inhibit the cyclin E—CDK2 complex leading to G1 arrest. However, this tightly regulated pathway is frequently disrupted in breast cancer through p16 loss, CDK4/6 amplification, cyclin D overexpression, or Rb loss leading to inactivation of the G1 to S phase checkpoint (Shapiro 2006; Ingham and Schwartz 2017).

3 Preclinical Data

This well-known frequent activation of the pathway described above in cancer led to efforts to block it using CDK inhibitors (Baughn et al. 2006). The first-generation CDK inhibitors like flavopiridol were nonselective CDK inhibitors with significant side effects. However, in recent years, several selective CDK4/6 inhibitors were developed and entered clinical studies. The most advanced drug in this class is palbociclib, formerly known as PD 0332991 (Ibrance®, Pfizer, Inc.).

Palbociclib is a potent oral inhibitor of CDK4 and CDK6 that prevents downstream phosphorylation of Rb thereby leading to G1 arrest. Fry and co-workers showed that oral administration of palbociclib to mice produced marked tumor regression in a broad spectrum of human tumor xenografts in vivo (Fry et al. 2004). Additionally, they showed that palbociclib led to elimination of phospho-Rb and the proliferative marker Ki-67 in tumor tissue as well as downregulation of genes under the transcriptional control of E2F. Given the high efficacy and specificity of palbociclib, this drug was scheduled early for clinical trials to answer the question whether selective inhibitors of CDK4/6 can provide a therapeutic benefit in cancer patients.

In further preclinical studies, it was shown that palbociclib achieved specific inhibition of CDK4/6 and induced G1 arrest in primary bone marrow myeloma cells ex vivo and prevented tumor growth in disseminated human myeloma xenografts (Baughn et al. 2006). Furthermore, the authors showed that the efficacy of palbociclib was markedly increased when used in combination with a second agent.

Building on these early results showing the efficacy of palbociclib in myeloma cells, Menu et al. (2008) reported that the combination of palbociclib with bortezomib, a proteasome inhibitor widely used in myeloma treatment, increased tumor

suppression and survival in the immunocompetent 5T33MM myeloma model. Indeed, induction of G1 arrest by palbociclib sensitized 5T33MM tumor cells to killing by bortezomib. The authors speculated that this combination therapy should prove useful in myeloma patients.

The cell cycle regulation by CDK6 but not CDK4 plays a key role in several acute leukemias. In MLL-rearranged acute myeloid leukemia, CDK6 depletion induced myeloid differentiation of the AML cells (Placke et al. 2014). Palbociclib showed efficacy in MLL-rearranged AML cell lines enhancing differentiation. A Phase Ib/II trial is currently testing palbociclib in this indication. Furthermore, palbociclib with different combination partners is tested in relapsed/refractory AML. Internal tandem duplications of the FLT3 receptor are frequently found in AML cells (approx. 30%). Uras et al. (2016) demonstrated the induction of apoptosis of FLT3-ITD cells and a synergistic cytotoxicity of FLT3 inhibitors in combination with Palbociclib. The combination seems to be promising in FLT3-ITD AML.

Focusing on gynecological cancer, Konecny and co-workers investigated the role of CDK4/6 inhibition in human ovarian cancer using a panel of 40 established human ovarian cancer cell lines (Konecny et al. 2011). As expected, CDK4/6 inhibition induced G0/G1 cell cycle arrest and blocked Rb phosphorylation. Rb-proficient cell lines with low p16 expression were most responsive to CDK4/6 inhibition. Furthermore, expression of p16 and Rb was studied in a large clinical cohort of ovarian cancer patients. Indeed, this biomarker profile was associated with poor progression-free survival in ovarian cancer patients. The authors concluded that Rb and p16 expression might be useful biomarkers in selecting patients most likely to benefit from CDK4/6 inhibition in ovarian cancer. Furthermore, in four endometrial cancer cell lines expressing Rb protein, palbociclib showed G0/G1 cell cycle arrest in two cell lines (Tanaka et al. 2017). The authors came to the conclusion that palbociclib had therapeutic potential against endometrial cancer cell lines expressing Rb protein. Another preclinical study utilized a tamoxifen-inducible phosphatase and tensin homolog (PTEN) knockout mouse model to assess the antitumor effects of cyclin D1 knockout and CDK4/6 inhibition by palbociclib on endometrial tumors (Dosil et al. 2017). This study convincingly showed that palbociclib reduced tumor cell proliferation, triggered shrinkage of endometrial tumors, and significantly increased the survival of PTEN-deficient mice. Uterine leiomyosarcomas (uLMS) are a rare disease with a poor prognosis. Comprehensive genomic profiling of 279 advanced uLMS revealed potentially actionable targets in approximately 57% (Elvin et al. 2017). Interestingly, CDKN2A mutations inactivating p16INK4a were identified in 11% of uLMS. Elvin and co-workers described a single case of an advanced uLMS harboring a CDKN2A mutation experiencing clinical benefit from treatment with palbociclib. The authors furthermore showed that 19% of uLMS had mutations affecting the cyclin-dependent kinase pathway.

Obviously, alterations of CDK4/6 are important in a wide variety of cancer entities. Finn et al. (2009) described in their seminal preclinical work the in vitro sensitivity to palbociclib across a panel of molecularly characterized human breast

cancer cell lines. They used 47 breast cancer cell lines representing known molecular subtypes (i.e., luminal, HER2, basal) and determined the inhibitory concentration 50% (IC50) for each cell line. These results were then compared with gene expression data of these cell lines to characterize genes associated with in vitro sensitivity to palbociclib. In general, ER-positive luminal cell lines showed the highest sensitivity. This was also true when HER2 was amplified. In contrast, ER-negative basal cell lines displayed the lowest sensitivity. Using gene expression analysis (Agilent Human 1A V1), Finn and co-workers identified 450 differentially expressed genes between sensitive and nonsensitive cell lines. 253 genes were upregulated in sensitive cell lines, and 197 genes were upregulated in resistant lines. Sensitivity correlated positively with increased Rb and cyclin D1 as well as decreased p16. This pattern of expression is in accordance with the mechanism of CDK4/6 inhibition as outlined above. Expectedly, cell cycle analyses revealed a G0/1 arrest in sensitive cell lines and western blot analyses demonstrated that phosphorylation of Rb was blocked in sensitive cell lines. In addition, they could demonstrate that the combination of palbociclib with tamoxifen in ER-positive cell lines and with trastuzumab in HER2 amplified cell lines, respectively, showed a strong synergism in vitro. Moreover, treatment of a cell line with acquired resistance to tamoxifen with palbociclib reversed this resistance either when used as monotherapy or when these two agents were used in combination. The authors concluded their comprehensive preclinical investigations with the notion of a strong rationale for clinical development of palbociclib focusing on ER-positive luminal as well as HER2 amplified breast cancer. Furthermore, they proposed a combination of palbociclib with antiestrogen or anti-HER2 therapy, respectively (Finn et al. 2009). Conversely, in another preclinical study palbociclib decreased the efficacy of DNA-damaging cytotoxic drugs like carboplatin in breast cancer xenografts in mice (Roberts et al. 2012). In line with these findings, well-known toxicities of carboplatin like thrombocytopenia were ameliorated when mice were also treated with palbociclib. Thus, the authors concluded that CDK4/6 inhibitors should not be combined with DNA-damaging therapies in tumors that require CDK4/6 activity for proliferation.

4 Clinical Data

4.1 Phase I

To proceed with the clinical development of this drug, phase I trials were performed. In the first-in-human phase I study, Schwartz et al. (2011) enrolled 33 patients with Rb-positive advanced solid tumors or non-Hodgkin's lymphoma refractory to standard therapy. Patients received palbociclib once daily for 14 days followed by 7 days off treatment. The maximum tolerated dose (MTD) was 200 mg once daily. The drug was slowly absorbed and eliminated with a mean half-life of 26.7 h resulting in its accumulation following repeated dosing. Extensive

penetration of peripheral tissue was suggested by the large volume of distribution (mean 3241 L). Adverse events were generally mild to moderate. Dose-limiting toxicities (DLT) were mostly myelosuppression. In this phase I trial, early signs of efficacy were noticed with one partial remission and nine patients with stable disease (SD).

A second phase I trial investigated DLT and MTD of palbociclib administered once daily for 21 of 28 days (3/1 schedule) in patients with Rb-positive advanced solid tumors and described pharmacokinetic–pharmacodynamic relationships relative to drug effects (Flaherty et al. 2012). Flaherty et al. enrolled 41 patients, including five with breast cancer, in this open-label phase I trial with standard 3 + 3 design with provision for cohort expansion to six evaluable patients if a DLT was observed in the first cycle of treatment among the initial three patients. Similar to the phase I trial reported by Schwartz and co-workers, palbociclib was slowly absorbed (median T(max), 5.5 h), and slowly eliminated (mean half-life of 25.9 h) with a large volume of distribution (mean, 2793 L). MTD and recommended phase II dose of palbociclib was 125 mg once daily. Neutropenia was the only dose-limiting effect with 12% grade 3 neutropenia after cycle 1. Pharmacodynamics for absolute neutrophil count (ANC) and platelet levels showed a nadir occurring at the end of the dosing period in cycle 1 and cycle 2 for both cell types with a rebound of both ANC and platelet levels during the off-drug period that continued up to day 8 of the following dosing cycle. Other adverse events were mostly mild to moderate and included fatigue (34%), nausea (24%), and diarrhea (15%) with only few grade 3 events. The drug showed signs of clinical efficacy in this heavily pretreated group of patients with SD $\geq$ 4 cycles in 27%. Taken together, this phase I study fulfilled its primary objective of establishing the safety profile of palbociclib and identifying a recommended 3/1 schedule dose for further investigation in phase II studies.

Human papillomavirus (HPV) negative HNSCC frequently shows Rb inactivation with an underexpression of p16INK4a. In a phase I trial, the combination of palbociclib and cetuximab in locally advanced or metastatic head and neck squamous cell carcinoma (HNSCC) appeared to be safe and well tolerated (Michel et al. 2016). The observation of a high disease control rate in 89% of the patients including two partial responses led to a phase II trial, testing the combination versus cetuximab monotherapy in an HPV-negative population with advanced and refractory HNSCC.

4.2 Breast Cancer

Considering the proposed mechanism of action with Rb as a potential biomarker, 37 patients with advanced breast cancer with positive Rb protein were enrolled in a single-arm phase II trial (Demichele et al. 2015). Patients were treated with single-agent palbociclib 125 mg daily in a 3/1 schedule as proposed in a preceding phase I trial (Flaherty et al. 2012). Primary objectives were tumor response and tolerability. Secondary objectives included progression-free survival (PFS) and a

comprehensive biomarker assessment including Rb expression/localization, KI-67, p16 loss, and cyclin D amplification. The majority of the patients (84%) were hormone receptor-positive and HER2-negative, 5% were ER-positive with co-expression of HER2, and 11% were triple-negative. Patients had received a median of two prior cytotoxic therapies. Palbociclib showed clinical activity in this heavily pretreated cohort of breast cancer patients with a clinical benefit rate of 19% overall. Median PFS overall was 3.7 months, but significantly longer for those with hormone receptor positive versus negative disease ($P = 0.03$) and those who had previously progressed through endocrine therapy for advanced disease ($P = 0.02$). The major toxicity was neutropenia (51% grade III/IV) which could be easily managed with dose reduction. The authors concluded that palbociclib demonstrated single-agent activity in this heavily pretreated population of patients with advanced breast cancer.

Building on the encouraging results of palbociclib in estrogen receptor-positive breast cancer a series of studies (PALOMA) was launched (Table 2). An open-label randomized phase II trial enrolled 165 postmenopausal ER-positive and HER2-negative breast cancer patients who had not received any systemic therapy for advanced disease (PALOMA-1) (Finn et al. 2015). Patients were randomized either to letrozole or letrozole plus palbociclib (125 mg, given once daily for 3 weeks followed by 1 week off over 28-day cycles). Patients were enrolled sequentially in two separate cohorts to investigate a predictive influence of cyclin D or p16 alterations. For the whole cohort of patients, median progression-free survival was 10.2 months for the letrozole group and 20.2 months for the palbociclib plus letrozole group (hazard ratio [HR] 0.488, 95% confidence interval [CI] 0.319–0.748; $P = 0.0004$). These results clearly show that inhibition of CDK4/6 is a promising way to improve the efficacy of endocrine treatment in ER-positive breast cancer patients with comparably little side effects.

Recently, more detailed efficacy and safety analyses based on several specific patient and tumor characteristics were presented (Finn et al. 2016a). The substantial improvement in PFS was seen in every subgroup evaluated. Palbociclib plus letrozole improved median PFS regardless of age, tumor type, prior neoadjuvant/adjuvant systemic treatment or site of metastases. For instance, median PFS in patients without prior neoadjuvant/adjuvant systemic treatment was

Table 2 Efficacy of palbociclib in three randomized PALOMA studies

Study	Phase	*n*	Experimental arm	Median PFS (months)	HR (95% CI)
PALOMA-1	II	165	Letrozole ± palbociclib	20.2 versus 10.2	0.488 (0.319–0.748)
PALOMA-2	III	666	Letrozole ± palbociclib	24.8 versus 14.5	0.58 (0.46–0.72)
PALOMA-3	III	521	Fulvestrant (+goserelin if premenopausal) ± palbociclib	9.2 versus 3.8	0.42 (0.32–0.56)

CI confidence interval; *HR* hazard ratio; *PFS* progression-free survival

24.4 months with palbociclib plus letrozole and 8.2 months with letrozole alone (HR 0.341, 95% CI 0.194–0.599; $P = 0.00004$). Median PFS in patients with prior systemic treatment was 16.1 months with palbociclib plus letrozole and 10.9 months with letrozole alone (HR 0.539, 95% CI 0.302–0.962; $P = 0.0169$).

Building on these encouraging results of PALOMA-1, a confirmatory phase III trial (PALOMA-2) was launched. In this double-blind study, 666 postmenopausal women with ER-positive, HER2-negative breast cancer, who had not had prior treatment for advanced disease, were randomly assigned to receive palbociclib plus letrozole or placebo plus letrozole (Finn et al. 2016b). Median PFS was 24.8 months in the palbociclib plus letrozole group, as compared with 14.5 months in the placebo plus letrozole group (HR 0.58, 95% CI 0.46–0.72, $P < 0.001$). All predefined subgroups examined derived a similar benefit from the addition of palbociclib to letrozole. For instance, patients with visceral (HR 0.63, 95% CI 0.47–0.85) or nonvisceral (HR 0.50, 95% CI 0.36–0.70) disease had an improved PFS. Furthermore, patients with (HR 0.53, 95% CI 0.40–0.70) and without (HR 0.63, 95% CI 0.44–0.90) prior hormonal therapy derived benefit from palbociclib. The rate of confirmed objective response was 55.3% versus 44.4%. Again, these results confirmed both high clinical activity and favorable toxicity profile of palbociclib in advanced breast ER+ HER2−breast cancer.

The studies described above enrolled postmenopausal advanced ER-positive/HER2-negative patients treated in a first line setting. In the PALOMA-3 study, patients that had relapsed or progressed during prior endocrine therapy were randomized between fulvestrant ± palbociclib ($n = 521$) (Turner et al. 2015). This study included also premenopausal patients who additionally received ovarian function suppression using goserelin. The median age was 57 years, 59.7% of the patients had visceral disease, 79.3% were postmenopausal, and 78.7% had cancers that were sensitive to prior endocrine therapy. Median progression-free survival was 9.2 months versus 3.8 months. Hazard ratio for disease progression or death was 0.42 (95% CI 0.32–0.56, $P < 0.001$). The rate of clinical benefit was increased with palbociclib (34.0% vs. 19.0%, $P < 0.001$). Subgroup analyses according to stratification factors and demographic or prognostic factors revealed consistent results. Notably, both premenopausal or perimenopausal patients and postmenopausal patients derived similar benefit from the addition of palbociclib (HR 0.44 and 0.41, respectively). There was no interaction between the study-drug assignment and menopausal status ($P = 0.94$). The results of this randomized trial clearly showed that the addition of palbociclib improved PFS also in endocrine pretreated as well as in premenopausal patients.

The final analysis of the PALOMA-3 study revealed a sustained benefit in terms of PFS (HR 0.46, 95% CI 0.36–0.59, $P < 0{\cdot}0001$) (Cristofanilli et al. 2016). Again, the most common grade 3 or 4 adverse events were neutropenia (65% vs. 1%), as well as leucopenia and anemia.

A further analysis of PALOMA-3 comparing patient-reported outcomes (PROs) showed that the greater efficacy and favorable safety profile of palbociclib plus fulvestrant translate to a relatively better QoL compared with placebo plus fulvestrant (Harbeck et al. 2016). The addition of palbociclib to fulvestrant resulted in

a significant delay in deterioration of global QoL ($P < 0.025$) and pain symptoms ($P < 0.001$), a significantly greater improvement from baseline in emotional functioning and pain, and no significant increase in systemic therapy side effects.

Since PALOMA-3 also enrolled premenopausal patients, a recent analysis focused especially on the impact of palbociclib in premenopausal patients (Loibl et al. 2017). The authors assessed whether safety profile and PFS improvement also apply to premenopausal women. Potential drug–drug interactions and ovarian suppression with goserelin were assessed via plasma pharmacokinetics and biochemical analyses, respectively. PFS was improved in premenopausal patients in a range similar to postmenopausal patients (HR 0.50, 95% confidence interval 0.29–0.87). Hormone concentrations were similar between treatment arms and confirmed sustained ovarian suppression. Clinically relevant drug–drug interactions were not observed. In conclusion, both the significant PFS gain and tolerable safety profile strongly support the use of this regimen in premenopausal women with endocrine-resistant disease.

A recently published preplanned subgroup analysis of the PALOMA-3 study included premenopausal and postmenopausal Asians taking palbociclib plus fulvestrant ($n = 71$) or placebo plus fulvestrant ($n = 31$) (Iwata et al. 2017). Efficacy, QoL, and safety were similar in Asians and non-Asians. The authors concluded that palbociclib plus fulvestrant was a reasonable treatment option in Asians with ER-positive/HER2-negative advanced breast cancer patients that have progressed on prior endocrine therapy.

Considering the efficacy of palbociclib in advanced breast cancer, a tempting question is whether palbociclib is a better therapeutic option than chemotherapy in these patients. At present, randomized trials comparing palbociclib with chemotherapy in ER-positive/HER2-negative advanced breast cancer are still pending. Meanwhile, a recent systematic review and network meta-analysis comparing palbociclib with chemotherapy agents consistently showed statistically significant improvements in PFS versus capecitabine and mitoxantrone, and trended toward improvements versus paclitaxel, docetaxel, and other monotherapy or combination chemotherapy agents (Wilson et al. 2017). However, only randomized clinical trials will help to clarify this matter.

4.3 Germ Cell Tumors

A phase II clinical trial using palbociclib in patients with retinoblastoma protein-expressing germ cell tumors which also included female patients reported early results (Vaughn et al. 2015). 30 patients with refractory Rb expressing germ cell tumors were treated with palbociclib. The estimated 24-weeks progression-free survival rate was 28%. Patients with unresectable teratomas and teratomas with malignant transformation had a better progression-free survival than patients with nonteratomatous germ cell tumors. Recently, a retrospective analysis of this phase II trial with long-term follow-up data of the patient cohort with unresectable mature teratoma was reported (Narayan et al. 2016). Four of the 12 patients were female.

The median progression-free survival was 5.3 months. Furthermore, the median event-free survival duration was 16.2 months. The authors postulated that palbociclib might result in a clinically meaningful delay in disease-related major clinical events in this incurable patient population.

4.4 Liposarcoma

Liposarcoma is a common soft tissue sarcoma. A high amplification of CDK4 is seen in more than 90% of the subgroup of well-differentiated liposarcoma (WDLS) or dedifferentiated liposarcoma (DDLS) (Conyers et al. 2011). Systemic standard treatment with cytotoxic agents like doxorubicin or ifosfamide has modest activity. Two single-arm phase 2 trials tested palbociclib in different treatment regimens in patients with advanced/metastatic liposarcoma (WDLS/DDLS) refractory to standard chemotherapy or treatment-naive. Of the 90 patients treated in both trials, one patient had a CR and one patient experienced a PR. Median PFS was 17.9 weeks in both trials (Dickson et al. 2013, 2016).

4.5 Non-Small Cell Lung Cancer (NSCLC)

Deregulation of CDK4/6 activity is frequently seen in NSCLC. A Phase II single-arm trial in patients with p16 IHC-negative or unknown p16 staining tested the activity of palbociclib after failure of previous treatment (Gopalan et al. 2014). No responses were found, a stabilization of disease as best response was found in 50% of the population. Current trials investigate the activity of palbociclib in combination with different MEK inhibitors in KRAS-mut NSCLC, to date no results have been reported but a press release stated that phase 3 trial JUNIPER (Goldman et al. 2016) comparing Abemaciclib with Erlotinib in patients harboring a KRAS-mutation after failure of a platinum-based chemotherapy, failed in showing an OS benefit.

4.6 Multiple Myeloma

The prolongation of early G1 cell cycle arrest can be achieved by palbociclib and sensitizes myeloma cell in vitro to cytotoxic killing induced by bortezomib and dexamethasone. A phase 1/2 trial evaluated tolerability and efficacy of the combination bortezomib/dexamethasone and palbociclib in patients with refractory or relapsed multiple myeloma (Niesvizky et al. 2015). Dose-limiting toxicity was mainly cytopenias; disease control was seen in 64%, partial response in 20% of the patient population. Currently, there is no further development of palbociclib in this indication in clinical trials.

4.7 Active Clinical Trials

Up to December 2017, 147 clinical trials using palbociclib for the treatment of solid tumors and hematological malignancies have been registered (http://www.clinicaltrials.gov). 79 studies are actively recruiting patients. Of these, the vast majority (n = 56) are currently enrolling patients with breast cancer.

5 Toxicity

Targeting kinase activity drug development is often hindered by toxicities. Furthermore, predicting drug behaviors is often influenced by redundant kinase activities, a lack of unique substrates, and cell-specific signaling networks (Chen et al. 2016). According to the mode of action of palbociclib, the most common adverse reactions described in the three randomized studies in breast cancer were hematological toxicities, mostly neutropenia (Table 3). In the randomized

Table 3 Adverse events of palbociclib in two phase 3 studies in advanced breast cancer

Adverse event	PALOMA-2 Palbociclib–letrozole (n = 444)			PALOMA-3 Palbociclib–fulvestrant (n = 345)		
	Any grade	Grade 3	Grade 4	Any grade	Grade 3	Grade 4
	Number of patients (%)					
Neutropenia	353 (79.5)	249 (56.1)	46 (10.4)	272 (78.8)	184 (53.3)	30 (8.7)
Leukopenia	173 (39.0)	107 (24.1)	3 (0.7)	157 (45.5)	85 (24.6)	2 (0.6)
Fatigue	166 (37.4)	8 (1.8)	0	131 (38.0)	7 (2.0)	0
Nausea	156 (35.1)	1 (0.2)	0	100 (29.0)	0	0
Alopecia	146 (32.9)	0	0	51 (14.8)	NA	NA
Diarrhea	116 (26.1)	6 (1.4)	0	66 (19.1)	0	0
Constipation	86 (19.4	2 (0.5)	0	58 (16.8)	0	0
Stomatitis	68 (15.3)	1 (0.2)	0	40 (11.6)	2 (0.6)	0
Dizziness	63 (14.2)	2 (0.5)	0	37 (10.7)	1 (0.3)	0
Decreased appetite	66 (14.9)	3 (0.7)	0	44 (12.8)	3 (0.9)	0
Vomiting	69 (15.5)	2 (0.5)	0	50 (14.5)	1 (0.3)	0
Dyspnea	66 (14.9)	5 (1.1)	0	37 (10.7)	0	1 (0.3)
Upper respiratory infection	59 (13.3)	0	0	67 (19.4)	1 (0.3)	0

open-label phase II study, the incidence of all-causality grade 3–4 AEs as well as of treatment discontinuation with palbociclib plus letrozole was generally similar in all subgroups (Finn et al. 2015, 2016a). Neutropenia was the most common adverse event. In the palbociclib plus letrozole arm, 75.9% of patients had any grade neutropenia, 49.4% had grade 3 neutropenia, and 6.0% had grade 4 neutropenia. Approximately half (51.8%) of the patients who had any grade neutropenia had dose reductions, dose interruptions, or cycle delays in the palbociclib plus letrozole arm but only five patients (6%) were required to permanently discontinue treatment due to grade 3–4 neutropenia. There was a downward trend in grade 3–4 neutropenia over time, suggesting that there was no cumulative toxicity and that early dose modifications were likely effective in reducing the frequency of severe neutropenia. Furthermore, the majority (71.7%) of patients with grade 3–4 neutropenia in the palbociclib plus letrozole arm had no overlapping infections of any grade demonstrating a favorable safety profile of palbociclib.

Similarly to PALOMA-1, the most common grade 3 or 4 adverse events in the pivotal phase III study were neutropenia (66.4% vs. 1.4%), leukopenia (24.8% vs. 0%), anemia (5.4% vs. 1.8%), and fatigue (1.8% vs. 0.5%) (Finn et al. 2016b). Other adverse events of any grade for which the incidence was higher in the palbociclib plus letrozole group were diarrhea (26.1% vs. 19.4%), cough (25.0% vs. 18.9%), and stomatitis (15.3% vs. 5.9%). Febrile neutropenia was reported in 1.8% of patients in the palbociclib plus letrozole group and in none of the patients in the placebo plus letrozole group. However, there was no substantial difference with respect to infections of grade 3 or higher. Permanent discontinuation as a result of adverse events occurred in 9.7% in the palbociclib plus letrozole group compared to 5.9% in the placebo plus letrozole group. Again, these results confirmed a favorable toxicity profile of palbociclib in advanced breast ER+ HER2− breast cancer.

In the PALOMA-3 study, patients that had relapsed or progressed during prior endocrine therapy were randomized between fulvestrant ± palbociclib ($n = 521$) (Turner et al. 2015). This study included also premenopausal patients who additionally received ovarian function suppression using goserelin. Again, the most common grade 3 or 4 adverse events in the palbociclib–fulvestrant group were neutropenia (62.0% vs. 0.6%), leukopenia (25.2% vs. 0.6%), anemia (2.6% vs. 1.7%), thrombocytopenia (2.3% vs. 0%), and fatigue (2.0% vs. 1.2%). Febrile neutropenia was reported in 0.6% of palbociclib-treated patients and 0.6% of placebo-treated patients. The most common nonhematologic adverse events of all grades were fatigue (38.0% vs. 26.7%), nausea (29.0% vs. 26.2%), and headache (21.2% vs. 17.4%). The incidence of infections was increased in the patients receiving palbociclib (34.2% vs. 24.4%). However, the rate of discontinuation due to adverse events was 2.6% with palbociclib and 1.7% with placebo. Global QoL was generally maintained with palbociclib/fulvestrant but deteriorated significantly with placebo/fulvestrant ($P = 0.03$).

The safety of Palbociclib in combination with fulvestrant (+goserelin in premenopausal patients) was compared by Verma and co-workers in detail (Verma et al. 2016). In PALOMA-3 neutropenia was the most common grade 3 (55%) and 4 (10%) adverse event; median times to onset and duration of grade ≥ 3 episodes

were 16 and 7 days, respectively. Multivariate analysis revealed that both Asian ethnicity and a below-median value for absolute neutrophil count conferred a significantly increased risk for developing grade 3–4 neutropenia in the palbociclib arm. For the palbociclib arm, 28% of patients had one dose reduction, and 6% of patients had two dose reductions. Dose modification appeared to be effective at reducing the risk for subsequent grade 3–4 neutropenia. The median duration of dose interruption or dose delay in the palbociclib arm was 6.0 or 2.5 days, respectively. Remarkably, neither dose modifications for grade 3–4 neutropenia (HR 0.87, 95% CI 0.61–1.25) nor dose interruption or cycle delay (HR 0.84, 95% CI 0.61–1.17) had adverse effect on progression-free survival. Although grade 3–4 neutropenia occurred in 221 (65%) of 340 patients in the palbociclib arm, febrile neutropenia was reported in only 3 (0.9%) patients in the palbociclib arm. All-grade infections were more common in patients treated with palbociclib (42% vs. 30%). Multivariate analysis performed to assess the association between grade 3–4 neutropenia and infection showed that infection status was not significantly related to the presence of grade 3–4 neutropenia ($P = 0.17$). The authors concluded that palbociclib-related neutropenia differed in its clinical time course, patterns, and consequences from those seen with chemotherapy. Neutropenia was effectively managed by dose reduction, interruption, or cycle delay and without routine use of granulocyte-colony stimulating factor (G-CSF).

However, a recent mechanistic study of bone marrow suppression associated with palbociclib showed striking differences as compared to the bone marrow suppression with cytotoxic chemotherapies (Hu et al. 2016). Utilizing an in vitro assay with human bone marrow mononuclear cells (hBMNC), the authors elegantly showed that palbociclib-induced bone marrow suppression occurred through cell cycle arrest without DNA damage and apoptotic cell death, which is usually seen with cytotoxic chemotherapies. Furthermore, palbociclib-induced bone marrow suppression was reversible upon palbociclib withdrawal. These results show that palbociclib causes reversible bone marrow suppression, differentiating it from apoptotic cell death caused by cytotoxic chemotherapeutic agents. These in vitro findings were recently confirmed in vivo, using data from 185 advanced cancer patients receiving palbociclib in three clinical trials (Sun et al. 2017). The nadir of the absolute neutrophil count was reached approximately 21 days after palbociclib treatment initiation. Consistent with their mode of action, neutropenia associated with palbociclib (cytostatic) was rapidly reversible and noncumulative as compared to cytotoxic chemotherapies. Very obviously, palbociclib is not a cytotoxic chemotherapy, which has important implications for the management of patients.

6 Drug Interactions

Palbociclib is metabolized primarily by CYP3A and SULT2A1 enzymes and is a time-dependent inhibitor of CYP3A (Dhillon 2015). Administration of palbociclib with a strong CYP3A inhibitor (e.g., itraconazole) should be avoided as well as

Table 4 CYP3A inhibitors and inducers

CYP3 inhibitors	CYP3A inducers
Ketoconazole	Phenytoin
Itraconazole	Rifampin
Fluconazole	St. John's wort
Cimetidine	Carbamazepine
Clarithromycin	Enzalutamide
Erythromycin	Mitotane
Troleandomycin	Dexamethasone
Grapefruit juice	Ritonavir

administration with strong (e.g., phenytoin) or moderate (e.g., modafinil) CYP3A inducers (Table 4) (Dhillon 2015). CYP3A inhibitors may increase and CYP3A inducers might decrease plasma exposure to palbociclib which in turn could lead to an increased toxicity or decreased efficacy, respectively. Yu and co-workers developed a physiologically based pharmacokinetic (PBPK) model of palbociclib (Yu et al. 2017). They verified this model with clinical drug–drug interaction (DDI) results of palbociclib with strong CYP3A inhibitor (itraconazole), inducer (rifampin), and a sensitive CYP3A substrate (midazolam). Furthermore, they predicted the DDI risk of palbociclib with moderate/weak CYP3A inhibitors. Their results have clearly shown that weak CYP3A inhibitors (e.g., fluoxetine) had an insignificant DDI risk with palbociclib, whereas moderate CYP3A inhibitors (e.g., verapamil) increase plasma palbociclib by ~40%. Conversely, a moderate CYP3A inducer (e.g., efavirenz) decrease plasma palbociclib by ~40%. Reassuringly, there was no drug interaction of palbociclib with letrozole or fulvestrant and goserelin when administered concomitantly in breast cancer patients (Dhillon 2015; Ettl and Harbeck 2017; Loibl et al. 2017).

7 Biomarkers

Potential biomarkers for resistance or sensitivity against CDK4/6 inhibitors like palbociclib are an increasingly important topic of research. The regulation of the cell cycle is well understood. Cyclin D1 (CCND1) through CDK4 and CDK6, initiates cell cycle entry by phosphorylating and inactivating the retinoblastoma protein (pRb) releasing E2F transcription factors. This in turn initiates an S phase transcriptional program promoting E-type cyclin and CDK2 expression and cell cycle progression.

Based on this method of action, analysis of alterations in cyclin D1 and p16 are comprehensible as potential biomarkers. Considering the proposed mechanism of action with Rb as a potential biomarker, 37 patients with advanced breast cancer with positive Rb protein were enrolled in a single-arm phase II trial (Demichele et al. 2015). Patients were treated with single-agent palbociclib 125 mg daily in a

3/1 schedule. Secondary objectives included a comprehensive biomarker assessment including Rb expression/localization, KI-67, p16 loss, and cyclin D amplification. Contrary to the assumed mechanism of action as outlined above, neither analysis of p16 nor cyclin D were significantly associated with clinical benefit or PFS. An association of these biomarkers and response to palbociclib was also investigated in the PALOMA-1 study (Finn et al. 2015). Patients were enrolled sequentially in two separate cohorts: in cohort 1, patients were enrolled on the basis of their ER-positive and HER2-negative biomarker status alone, whereas in cohort 2 they were also required to have amplification of the cyclin D1 gene (CCND1) or loss of p16. However, preliminary results suggested that further patient selection based on CCND1 amplification or p16 loss was unlikely to further improve patient outcome over the use of ER and HER2 status alone (HR with CCND1 or p16 copy changes 0.37, 95% CI 0.10–1.40, $P = 0.13$ vs. HR with no CCND1 or p16 copy changes 0.19, 95% CI 0.05–0.67, $P = 0.0045$).

Loss of RB function is an established mechanism of primary resistance to CDK4/6 inhibitors in vitro (Fry et al. 2004; Finn et al. 2009; O'Leary et al. 2016). Accordingly, the retinoblastoma pathway as a key player in cell cycle regulation was investigated in a comprehensive gene expression analysis (Malorni et al. 2016). A gene expression signature of RB loss-of-function (RBsig) was able to identify palbociclib resistant and sensitive breast cancer cells. Signatures of RB loss might be helpful in personalizing treatment of patients with ER-positive/HER2-negative breast cancer. Further validation in patients receiving palbociclib is warranted.

Studies analyzing the expression levels of hormone receptors and response to endocrine therapies in advanced breast cancer suggest that an increased expression is associated with an improved response (Elledge et al. 2000). The PI3K/AKT/mTOR pathway is an intracellular signaling pathway important in regulating the cell cycle. Mutation of phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA) is a common genetic event in breast cancer which is present in more than one-third of luminal breast cancer (Zardavas et al. 2014). Preclinical work shows that therapies that combined targeting of both CDK4/6 and PI3K triggered cancer cell apoptosis in vitro and in patient-derived tumor xenograft (PDX) models, resulting in tumor regression and improved disease control (Herrera-Abreu et al. 2016). Inhibition of the PI3K/AKT/mTOR pathway synergizes with CDK4/6 inhibitors through blockade of early adaptation combined with apoptosis induction. These preclinical findings support the development of triplet combinations of CDK4/6 inhibitors, PI3K inhibitors, and fulvestrant in ER-positive breast cancer.

Building on these preclinical findings, PIK3CA mutations were analyzed in the PALOMA-3 study using circulating tumor DNA (ctDNA) (Cristofanilli et al. 2016). Baseline plasma samples were available for 395 patients (76%). PIK3CA mutation was detected in the plasma DNA of 129 (33%) of 395 patients for whom these data were available. PIK3CA mutations were associated with worse PFS in the whole cohort of patients (5.8 vs. 9.2 months). However, the PIK3CA status was not predictive for palbociclib ($P_{\text{interaction}} = 0.83$). The expression levels of hormone receptors were not predictive either ($P = 0.77$). In conclusion, neither PIK3CA

status nor hormone receptor expression level significantly affected treatment response.

ESR1 mutations are often selected by aromatase inhibitors in advanced breast cancer. Patients with tumors harboring these mutations might respond better to an endocrine therapy as compared with an aromatase inhibitor. Plasma samples of the PALOMA-3 study were used to investigate whether ESR1 mutations affect also the response to palbociclib (Fribbens et al. 2016). ESR1 mutations were found in the plasma of 25.3% of patients with available circulating tumor DNA (ctDNA). However, the benefit from palbociclib was seen regardless of the ESR1 mutation status ($P_{\text{interaction}} = 0.74$).

Taken together, these results underline that, despite a thorough understanding of the mode of action of CDK4/6 inhibitors, a predictive biomarker for palbociclib other than ER remains to be found.

8 Summary and Perspective

Cell cycle regulation plays a pivotal role in cancer. After initial disappointment with the early development of pan-CDK inhibitors, drug development focused on CDK4/6 as a key regulator of the G1-S transition. The highly specific CDK4/6 inhibitor palbociclib is the first drug of this novel class of cell cycle inhibitors. Palbociclib showed in phase III trials a substantial prolongation in addition to letrozole or fulvestrant in advanced ER-positive/HER2-negative breast cancer. It was approved by the Federal Drug Administration (FDA) and the European Medicines Agency (EMA) for this indication. Ongoing clinical studies are also investigating the role of palbociclib in ER-positive early breast cancer. In addition, the impact of palbociclib in several other tumor entities is currently examined.

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Cobimetinib (GDC-0973, XL518)

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Abstract

The mitogen-activated protein kinase cascade (MAPK/ERK pathway) is a signaling pathway activated as a cellular response to various stimuli and for regulating the proliferation and survival of several types of eukaryotic cells, among others a wide variety of tumor cells. Mutations of the proteins involved in this pathway have been discovered in several tumor entities, indicating their inhibition as a potential therapeutic target. BRAF inhibitors have been in the clinical use since 2011. Several MEK inhibitors have been studied for metastatic cancer treatment in the recent past. After trametinib, cobimetinib is another potent, selective oral MEK1/2 inhibitor that was approved by European Medicine Agency (EMA) and Food and Drug Administration (FDA) in 2015 for treatment of malignant melanoma in a combination with the BRAF inhibitor vemurafenib.

Keywords

Cobimetinib · MAPK/ERK pathway · MEK inhibitors · BRAF/MEK inhibition · Combination therapy

1 Structure and Mechanism of Action

Cobimetinib is an orally bioavailable small-molecule inhibitor of mitogen-activated protein kinase kinase 1 (MAP2K1 or MEK1). This kinase is a part of the MAPK/ERK signaling pathway (also known as the Ras–Raf–MEK–ERK pathway) that affects cell cycle, proliferation, differentiation, and secretion as a response to diverse stimuli (e.g., growth factors, cytokines, and proto-oncogene) (Boulton et al. 1990; Cobb et al. 1991; Robbins et al. 1992; Moodie et al. 1993). Since the discovery of BRAF mutation in 66% of melanomas and approximately 15% of other tumors (Davies et al. 2002), which results in a constitutive activation of this pathway, its inhibition on different levels has been studied. The MAPK/ERK pathway is depicted in Fig. 1.

Similar to trametinib, cobimetinib (GDC-0973, XL518) is a carboxamide-based allosteric MEK inhibitor, which binds to and selectively inhibits MEK1 and MEK2. The inhibition results in decreased ERK1/2 phosphorylation. Cobimetinib maintains its inhibitory effect even when MEK is already phosphorylated. The half-maximal inhibitory concentration was established 4.2 nmol/L for MEK1. Cobimetinib is a very selective MEK inhibitor, its sensitivity is more than 100-fold higher for MEK compared to over 100 other serine–threonine and tyrosine kinases. The predisposition to sensitivity to cobimetinib in the in vitro studies was a mutation in RAF or RAS gene. Nevertheless, not all RAF- or RAS-mutated cell lines were sensitive to cobimetinib, in contrast to some wild-type cells. This indicates that the sensitivity to cobimetinib is multifactorial (Hoeflich et al. 2012). The efficacy of MEK inhibitors in BRAF-mutated versus BRAF wild-type and KRAS-mutated tumors depends on the form of interaction with MEK. Some MEK

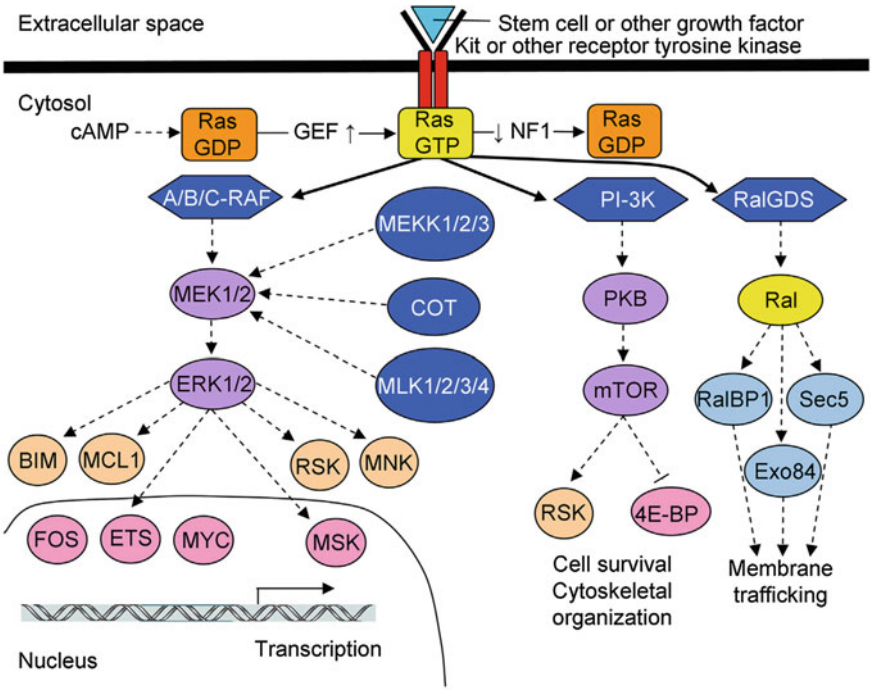

Fig. 1 Signaling pathways downstream RAS activation, including RAS/RAF/MEK/ERK signaling pathway. Modified after Roskoski (2017)

inhibitors form a strong hydrogen-bond interaction with S212 part of the kinase and, therefore, prevent its phosphorylation by wild-type RAF. Cobimetinib, on the other hand, has a stronger binding capacity to phosphorylated MEK and, therefore, shows a higher efficacy in the BRAF-mutated tumors (Hatzivassiliou et al. 2013). The drug elimination of cobimetinib is mostly intestinal (Han et al. 2015; Takahashi et al. 2015), in the liver it is metabolized via CYP3A and UGT2B7 (Musib et al. 2013). An impaired renal function does not have an effect on its elimination (Han et al. 2015).

The structure and chemical characteristics of cobimetinib are shown in Fig. 2.

2 Preclinical Data

In the first preclinical studies, cobimetinib showed a strong inhibition of cellular viability in several tumor cell lines, particularly those harboring a mutation in the RAS or RAF gene. Altogether 80% of the cells lines carrying BRAF mutation (V600E or non-V600E) and 54% carrying NRAS or KRAS mutation were sensitive to cobimetinib. Nevertheless, 35% of wild-type cells responded as well

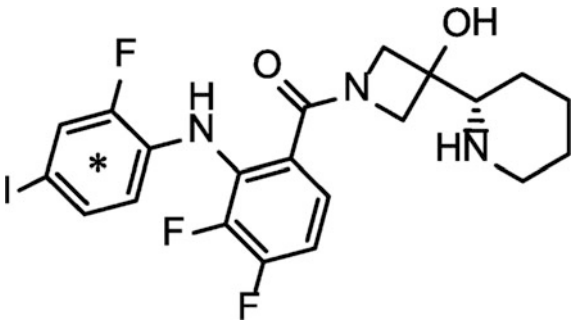

Fig. 2 The structure and chemical characteristics of cobimetinib. Mol. mass: 531.318 g/mol. Molecular formula: $C_{21}H_{21}F_3IN_3O_2$, chemical name: 3,4-difluoro-2-(2-fluoro-4-iodoanilino) phenyl]-[3-hydroxy-3-[(2*S*)-piperidin-2-yl]azetidin-1-yl]methanone

(Hoeflich et al. 2012). The single-agent efficacy and pharmacodynamics of cobimetinib were tested in xenograft models of acute myeloid leukemia, melanoma, non-small cell lung cancer, pancreatic, colorectal, ovarian, and breast cancer. Cobimetinib was administered in three different doses per oral gavage during 21 days after the subcutaneous tumor inoculation. The tumor size was measured on consecutive days. A response was observed under different doses (1–10 mg/kg body weight) in different tumor types. The highest efficacy was observed in the BRAF V600E-mutated melanoma model. Apart from this mutation, no other mutations strictly correlated with the response. The mechanism of action was inhibition of ERK phosphorylation (Hoeflich et al. 2012). In a combination therapy with a PI3K inhibitor GDC-0941, induction of pro-apoptotic proteins Bim and cleaved PARP caused apoptosis in the examined cell lines and a potent inhibition of tumor growth in xenograft models (Hoeflich et al. 2012). Exposure to the combination of these two inhibitors led to increased phosphorylation of proteins involved in DNA damage response (Kirkpatrick et al. 2013). The combination of cobimetinib with GDC-0941 and gemcitabine led to a remarkable tumor growth inhibition compared to gemcitabine alone treatment in a KRAS-driven genetic mouse model for pancreatic cancer (Junttila et al. 2015). In a pharmacokinetics–pharmacodynamics study of cobimetinib, immunodeficient mice were inoculated subcutaneously with a BRAF V600 mutated A375 melanoma cell line or a BRAF V600E mutated, PTEN deficient WM-266-4 melanoma cells (Wong et al. 2012). When the tumors reached the size of 100–120 mm^3 (day 11 or 13), they were randomized into eight groups treated with either vehicle or different doses of cobimetinib. Mice were sacrificed at different timepoints and the tumor tissue was analyzed for ERK inhibition and the inhibitor concentrations in the tumor and plasma. The BRAF V600 mutated melanomas were very sensitive to MEK inhibition. The WM-266-4 xenografts responded only moderately to cobimetinib, yet the slower response allowed for a more sufficient tumor mass for further analysis. The concentrations of the inhibitor were higher in tumor tissue than the plasma and were detectable in tumor for a longer period of time. The in vivo IC50 values were between 0.52 and

3.89 mmol/L and the response rate increased with a higher concentration of cobimetinib in the tumor (Wong et al. 2012).

3 Clinical Data

Mutations in BRAF gene occur in approximately 15% of all tumors (Davies et al. 2002). RAS mutations are variable throughout the tumor spectrum and the type of Ras protein (K-Ras, N-Ras and H-Ras), overall about 30% of all tumors carry a mutation in one of the RAS genes (Forbes et al. 2011). Therefore, the inhibitors of the Ras/Raf/MEK/ERK pathway have been subject of preclinical and clinical studies in the last 15 years. The first FDA and EMA approved BRAF inhibitors were vemurafenib (McArthur et al. 2014) and dabrafenib (Hauschild et al. 2012) for metastatic malignant melanoma. The BRAF inhibitors proved to be very potent, nevertheless, virtually all treated patients developed resistance throughout the course of treatment. A significant part of the resistance mechanisms was MEK-dependent, therefore a need of combination therapy emerged. So far two combination treatments showed superiority over the single-agent treatment with a BRAF inhibitor. One of them combines the BRAF inhibitor dabrafenib with a MEK inhibitor trametinib (Robert et al. 2015; Long et al. 2015). The other combination treatment included cobimetinib with a BRAF inhibitor vemurafenib (Ribas et al. 2014; Larkin et al. 2014; Ascierto et al. 2016). In a phase Ib trial, 129 patients who displayed a tumor progress under vemurafenib (66 patients), or never received any BRAF-targeted treatment (63 patients) were treated with vemurafenib and cobimetinib. The endpoint of the trial was safety and efficacy. The maximum tolerated doses was established to 960 mg vemurafenib twice daily and 60 mg of cobimetinib once a day for 21 days of a 28-day treatment period. The most common adverse events (AE) included diarrhoea (64%), non-acneiform rash (60%), increased liver enzymes (50%), fatigue (48%), nausea (45%), and photosensitivity (40%) with most of them being mild to moderate. Response rates reached 15% in patients with a progressive disease under vemurafenib and 87% in patients never treated with a BRAF inhibitor, with median progression-free survival 13.7 months. A complete response was achieved by 10% of the patients (Ribas et al. 2014). In a multicentric, randomized, double blind phase III trial co-BRIM, 495 previously untreated patients with stage III or IV BRAF-mutant melanoma were randomized to receive either vemurafenib and cobimetinib combination treatment, or vemurafenib with a placebo (Ascierto et al. 2016). The response rate, overall survival and progression-free survival data showed a clear advantage of the combination treatment, with response rate 70% versus 50%, overall survival 22.3 versus 17.4 months (HR 0.70, 95% CI 0.55–0.90; $p = 0.005$) and progression-free survival 11.0 versus 8.8 months (HR 0.58, 95% CI 0.46–0.72, $p < 0.0001$) in favor of the combined vemurafenib and cobimetinib treatment. The combination treatment showed slightly higher levels of toxicity, where serious adverse events occurred in 37% of the patients, compared to 28% of the patients in the vemurafenib arm.

However, the incidence of secondary dermatological malignities typical for vemurafenib treatment was lower in the combination arm. The occurrence of cutaneous squamous cell carcinoma, keratoacanthoma, or Bowen's disease was only 6% in the combination arm compared with 20% in the vemurafenib arm. This can be explained by blocking the paradoxical ERK activation, following BRAF inhibition, by adding a Mek inhibitor (Ascierto et al. 2016).

Currently, combinations of cobimetinib with other targeted therapies are being studied in clinical trials. Cobimetinib with duligotuzumab, an inhibitor of both epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor 3 (HER3) were tested in various KRAS-mutated solid tumors in a phase Ib study (Lieu et al. 2017). However, the trial was associated with limited efficacy and high toxicity accompanying the use of the drug combination. Ongoing or terminated, yet unpublished trials include combinations of cobimetinib with Akt inhibitors, different PI3K inhibitors, bevacizumab, and checkpoint inhibitors, such as atezolizumab, in different types of solid tumors. The vemurafenib/cobimetinib combination is being tested for melanoma brain metastases and in the neoadjuvant setting. In the malignant hematology, the trials testing the combination of cobimetinib with venetoclax and ±atezolizumab in relapsed multiple myeloma and cobimetinib with venetoclax or idasanutlin for elderly patients with acute myeloid leukemia are currently recruiting. For more detailed information, visit https://clinicaltrials.gov.

4 Toxicity

A maximum tolerated dose for cobimetinib 60 mg for 21 days of a 28-day cycle was estimated in a dose escalation study together with vemurafenib (Ribas et al. 2014). The data from the dose escalation study with cobimetinib alone were not published (NCT00467779). The treatment-related adverse events were evaluated from the phase III trial of cobimetinib in combination with vemurafenib versus vemurafenib and placebo (co-BRIM) in a detailed safety analysis report (Dréno et al. 2017). The most frequent AE for both drugs included rash, photosensitivity, diarrhoea, serous retinopathy, increase in blood creatine kinase and alanine aminotransferase, fatigue, nausea and vomiting, alopecia, hyperkeratosis, and a decrease in left-ventricular ejection fraction. More frequent adverse events in the combination arm in comparison to patients treated only with vemurafenib were the increase of creatine kinase (+32.4%) and aspartate aminotransferase (+11.7%), diarrhoea (+27.4%), serous retinopathy (+23.4%), nausea (+16.5%), vomiting (+11.4%), and photosensitivity (+10%), grade 3 and 4 being the increase in liver enzymes and diarrhoea. The most common serious adverse events for both arms were pyrexia and dehydration (both 2% of the total number of patients). In most cases, dose reduction and supportive therapy was a sufficient AE management. The therapy had to be discontinued in less than 20% of patients.

5 Drug Interactions

Cobimetinib is mostly eliminated intestinally (Han et al. 2015; Takahashi et al. 2015). In the liver, the drug was metabolized via CYP3A and UGT2B7 in healthy volunteers (Musib et al. 2013). However, another study showed that CYP3A is responsible for ca. 78% of the total clearance of cobimetinib (Budha et al. 2016) and moderate (erythromycin and diltiazem) and strong (itraconazole) inhibitors of this enzyme lead to three- to seven-fold increase in cobimetinib exposure (area under the plasma-time curve, AUC). Similarly, CYP3A inducers, such as efavirenz and rifampicin lead to decrease in cobimetinib exposure.

6 Biomarkers

The mutated BRAF is a strong predictor of sensitivity to MEK inhibition and only patients with BRAF mutation were included in the clinical trial of vemurafenib and cobimetinib. Biomarker analysis of the phase 1b trial of vemurafenib and cobimetinib could show that the pERK inhibition was reflected by the decrease in the proliferation marker Ki67. S6 inhibition was much more variable across the groups (Yan et al. 2014). In the analysis of the co-BRIM trial, patients receiving vemurafenib with a high Ki67 expression had a shorter overall survival. On the contrary, the response of the patients receiving the combination therapy was not dependent on Ki67 expression. The levels of pERK and S6 did not have any association with the overall survival (Ascierto et al. 2016). In the further analysis, mutations in RAS, PTEN and RTK did not have an effect on the progression-free survival. Interestingly, the loss of PTEN was a negative biomarker in the progression-free survival of the patients receiving only vemurafenib, however, it did not have any effect on the PFS in the combination group (unpublished data, presented at ASCO 2015).

7 Summary and Perspectives

Based on the latest preclinical studies and clinical trials, the use of cobimetinib has proven beneficial in the combination therapy, especially in the combination with the BRAF inhibitor vemurafenib in the treatment of stage III and IV BRAF-mutated malignant melanoma. The adverse effects of this combination were slightly higher than in the monotherapy with vemurafenib, yet manageable with supportive therapy and dose adjustment. Therefore, the targeted therapy in combination is a serious candidate for the first line treatment in metastatic melanoma. The current discussion in the scientific community is about the superiority of this treatment as the first line option for BRAF-mutated melanoma in comparison to the checkpoint inhibition in different subgroups of patients. The clinical trials studying the combination of

BRAF + MEK inhibitor with checkpoint inhibitors are underway, the main concern is the toxicity of such combination. The combination of vemurafenib and cobimetinib is currently tested in the treatment of brain metastases. Based on the preclinical data, cobimetinib may be effective with other drugs, such PI3K inhibitors in various solid tumors.

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Lenvantinib: A Tyrosine Kinase Inhibitor of VEGFR 1-3, FGFR 1-4, PDGFRα, KIT and RET

Stefanie Zschäbitz and Carsten Grüllich

Contents

Abstract

Lenvatinib is an oral receptor tyrosine kinase inhibitor (TKI) with activity against vascular endothelial growth factor (VEGF) receptors 1-3, fibroblast growth factor receptors (FGFR) 1-4, platelet-derived growth factor receptor-alpha (PDGFRα), and RET and KIT proto-oncogenes. Lenvatinib is

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approved for the treatment of radioiodine-refractory differentiated thyroid cancer and in combination with everolimus for the treatment of advanced renal cell carcinoma following anti-VEGF treatment. In hepatocellular carcinoma lenvatinib was non inferior to sorafenib in first line with an improved progression-free survival and approval in this indication is expected. Lenvatinib is currently investigated for further indications as single agent and in combinations. Side effects include typical TKI induced toxicities such as hypertension, diarrhea, hypothyroidism, and fatigue.

Keywords
Lenvantinib · Renal cell carcinoma · Hepatocellular carcinoma · Differntiated thyroid cancer

1 Introduction

Lenvantinib has activity against vascular endothelial growth factor (VEGF) receptors 1-3, fibroblast growth factor receptors (FGFR) 1-4, platelet-derived growth factor receptor-alpha (PDGFRα), and RET and KIT proto-oncogenes (Tohyama et al. 2014; Yamamoto et al. 2014).

Activation of tyrosine kinase receptors initiates intracellular signaling pathways among them RAS and PI3K/AKT leading to cell growth and differentiation. Overexpression, mutation, and/or constitutive activation of tyrosine kinase receptors can lead to pro-cancerous effects.

In renal cell carcinoma (RCC) two major events can be observed in almost all cases. First, the loss of function of von Hippel–Lindau (VHL) gene, a gene that functions as a tumor-suppressor gene, and second, activation of mTOR (mechanistic target of Rapamycin). Reduced activity of the VHL gene results in a continuous hypoxic response also in the absence of hypoxia, leading to an upregulation of genes encoding for growth factors such as VEGF and PDGF (Hickey and Simon 2006). Vice versa, those growth factors activate mTOR and promote in turn HIF (hypoxia inducible factor) expression inducing a positive feedback loop between VHL/HIF and mTOR signaling systems (Guo et al. 2015).

However, combination strategies of the mTOR inhibitors everolimus and temsirolimus together with tyrosine kinase inhibitors (TKIs) and the anti-VEGF directed antibody bevacizumab investigated in the past for the treatment of metastatic RCC (mRCC) have been disappointing (Rini et al. 2014; Flaherty et al. 2015; Ravaud et al. 2015). In contrast, preclinical data on the combination of lenvatinib and everolimus in human RCC xenograft mouse models showed anti-proliferative and anti-angiogenic action.

Almost all papillary thyroid cancers (PTCs) have at least one driver genomic alteration as analyzed in The Cancer Genome Atlas (TCGA; https://cancergenome.nih.gov/). Most mutations involve the MAPK and the PI3K–mTOR pathways. Binding of factors such as RET, PDGFR, cKIT, FGFR, or VEGFR leads to receptor

dimerization and auto-phosphorylation of transmembrane receptor tyrosine kinases. This results in activation of the MAPK and the PI3K–mTOR pathways and finally in proliferative, pro-angiogenic and pro-metastatic signals. Expression of members of the FGFR and VEGF families in thyroid cancer has been associated with a more aggressive subtype, larger tumor size, higher extent of extra-thyroidal extension, and BRAF mutations (Klein et al. 2001).

2 Structure and Mechanism of Action

Lenvatinib, formerly known as E7080, or 4-[3-chloro-4-(cyclopropylcarbamoylamino)phenoxy]-7-methoxyquinoline-6-carboxamide is an oral receptor TKI (Fig. 1).

TKIs directed against VEGFR2 bind to the ATP binding site. TKIs are classified into different types of inhibitors (I, II, III, IV) depending on the conformation of VEGFR2 in complex with the TKI. Each type of inhibitor expresses distinct association and dissociation kinetics. Co-crystal structure analysis of lenvatinib revealed that lenvatinib binds to the ATP binding site and to the neighboring allosteric region adopting a specific conformation (Asp-Phe-Gly, so called "DFG-in" conformation), resulting in high kinase selectivity. It is further characterized by rapid association and relative slow dissociation kinetics. Okamoto et al. (2015) hence suggested to categorize lenvatinib as a new inhibitor type V.

Numerous in vitro and xenograft tumor models have shown antitumor, anti-angiogenic and anti-lymphangiogenic activity of lenvatinib as single agent via FGFR 1-4, VEGFR 1-3, PDGFRα, RET, and c-KIT. Tumor models include different entities, among them non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC), breast cancer, mesothelioma, sarcoma, thyroid cancer and

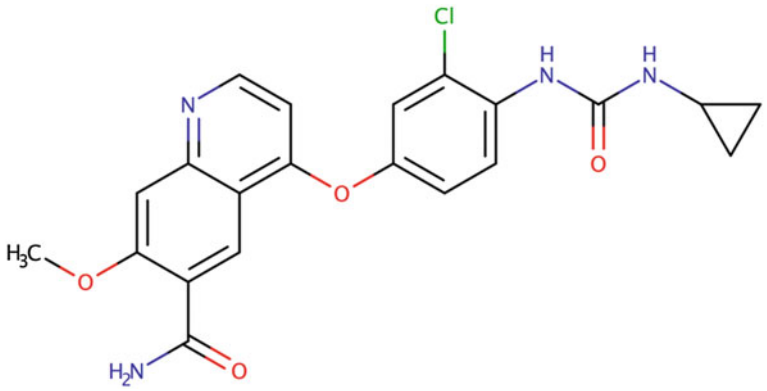

Fig. 1 Structure of lenvatinib (4-[3-chloro-4-(cyclopropylcarbamoylamino)phenoxy]-7-methoxyquinoline-6-carboxamide)

Table 1 Preclinical in vivo models of lenvatinib as a single agent

Tumor type	References
Breast cancer	(Matsui et al. 2008a)
Colorectal cancer	(Wiegering et al. 2014; Yamamoto et al. 2014)
Melanoma	(Yamamoto et al. 2014)
Mesothelioma	(Ikuta et al. 2009)
Non-small cell lung cancer	(Yamamoto et al. 2014)
Ovarian cancer	(Yamamoto et al. 2014)
Pancreatic cancer	(Yamamoto et al. 2014)
Prostate cancer	(Yamamoto et al. 2014)
Sarcoma	(Bruheim et al. 2011)
Small cell lung cancer	(Matsui et al. 2008b; Ogino et al. 2011)
Thyroid cancer	(Tohyama et al. 2014)

Table 2 Targets of TKIs in thyroid cancer

	BRAF	CKIT	CMET	EGFR	FGFR	FLT3	PDGFR	RET	VEGFR
Axitinib							X		X
Cabozantinib		X	X			X		X	X
Lenalidomide									X
Lenvatinib		X			X		X	X	X
Pazopanib		X					X		X
Sorafenib	X	X					X	X	X
Sunitinib		X				X	X	X	X
Vandetanib				X				X	X
Vemurafenib	X								

colorectal carcinoma (CRC) (Table 1). Table 2 lists targets of different TKIs for the example of thyroid cancer.

3 Clinical Data

3.1 Pharmacokinetics, Clinical Safety Profile

Lenvatinib is orally administered. The peak of its concentration (C_{max}) is reached within 3 h following oral administration. Plasma concentrations decline bi-exponentially following C_{max}. Half-life is approximately 28 h. Lenvatinib is highly (>96%) bound to plasma proteins. It is metabolized mainly in the liver through CYP3A4. Pharmacokinetic studies showed that neither food nor drugs elevating the

gastric pH nor CYP3A4 inductors/inhibitors had an effect on plasma peak levels for lenvatinib (Boss et al. 2012; Gupta et al. 2016).

The excretion is primarily via feces (64%) and a minor proportion (25%) with urine (Leonetti et al. 2017).

In healthy individuals a prolongation of the QTc interval was not observed (Shumaker et al. 2014), however it was noted in oncologic patients. Within the SELECT trial prolongation of the QTc interval was 8% for any grade and 1.5% for grade ≥ 3 (Schlumberger et al. 2015).

Pharmacokinetics were studied also in patients with mild or moderate hepatic impairment. Increase of AUC and $t_{1/2}$ to 37 h was noted (Shumaker et al. 2015). In patients with preexisting hepatic impairment dose modification is necessary in severe disease (Child-Pugh class C). Recommended dose is thus 10 mg once daily in patients with RCC and 14 mg once daily in differentiated thyroid cancer (DTC), respectively.

Renal impairment seemed not to have an effect on pharmacokinetics of lenvatinib in healthy individuals and the pooled dataset of 779 cancer patients from 15 clinical trials receiving lenvatinib doses of 3.2–32 mg (Gupta et al. 2016). However, vice versa, events of renal impairment were reported in 14% of patients on lenvatinib versus 2% with placebo (3% vs. 1% grade ≥ 3) in patients with DTC and in 18% of patients on lenvatinib plus everolimus versus 12% with everolimus alone (10% vs. 2% grade ≥ 3) in patients with RCC.

In patients with preexisting creatinine clearance <30 mL/min a dose adjustment to 10 mg once daily in patients with RCC and 14 mg once daily in patients with DTC, respectively, is recommended by the United States prescribing information.

There is no data in patients with end-stage renal disease (ESRD). Due to high protein binding, Lenvatinib is not expected to be dialyzable.

Baseline and periodic urine analyses to screen for proteinuria are recommended during treatment with lenvatinib. Interruption or discontinuation of therapy for patients who develop moderate to severe proteinuria (defined as ≥ 2 g per 24 h for lenvatinib) is recommended.

There is no data on lenvatinib in children, and pregnant or breastfeeding women. As administration of lenvatinib during pregnancy could cause fetal harm, effective contraception in female patients is recommended. Breastfeeding should be discontinued while female patients are treated with lenvatinib.

Lenvatinib has a predictable safety profile similar to other TKIs. Very common side effects include infectious, hematologic (thrombocytopenia), endocrine (hypothyroidism), cardiac (myocardial infarction, heart failure, and prolonged QT time hypertension), gastrointestinal (diarrhea, abdominal pain, nausea, emesis, and stomatitis), dermatologic (palmar-plantar erythrodysesthesia, alopecia) toxicities as well as electrolyte disorders (hypocalcaemia, hypokalemia), and general symptoms (such as decreased appetite, weight loss, insomnia, dizziness, dysgeusia, and dysphonia). For full safety profile, refer to product characteristics.

3.2 Phase I Trials

Antitumor activity of lenvatinib in 27 patients was first shown by a phase I, dose-escalation study (Yamada et al. 2011). Included were various solid tumor entities. Medication was applied starting from a dose of 0.5 mg twice daily in a 2-week-on/1-week-off rhythm. The maximum tolerated dose (MTD) was determined to be 13 mg twice daily. In a patient with CRC a partial response (PR) was achieved. In most patients (n = 21), however, stable disease (SD) was the best response reported. Adverse events (AE) reported in >50% of participants included hematuria (74.1%), fatigue (70.4%), hypertension (66.7%), AST/ALT increased (63.0 and 55.5%), headache (63.0%), proteinuria (63.0%), diarrhea (55.5%), and lactate dehydrogenase (LDH) increased (51.9%).

In a subsequent phase I, dose-escalating study, 82 patients with different solid malignancies (among others CRC, sarcoma, melanoma, RCC, gastric carcinoma, and pancreatic carcinoma) were treated with lenvatinib on a continuous schedule with doses between 0.2 and 32 mg. MTD was determined to be 25 mg once daily. However, dose reductions were necessary in 54% of patients at this dosage. Clinical benefit occurred in 45 patients (55%). In n = 7 patients (9%) with RCC, sarcoma, or malignant melanoma a PR was achieved. In n = 38 patients (46%), stable disease was the best response reported. Prolonged disease stabilization and objective responses were observed in patients with RCC, sarcoma, and malignant melanoma (Boss et al. 2012).

A Japanese multicenter, open-label, phase 1 dose-finding study evaluated the combination of up to six cycles of paclitaxel (200 mg/m^2) and carboplatin (area under the curve (AUC) 6.0 min × mg/ml) intravenously on day 1 every 3 weeks in combination with lenvatinib in patients with metastatic NSCLC. Lenvatinib maintenance therapy was continued when chemotherapy was discontinued for reasons other than progressive disease (PD). 28 patients were included. Of a total of 22 patients at the 4 mg level, n = 14 patients (64%) had a PR and n = 1 patient (5%) had a complete response. Progression-free survival (PFS) was 9 months. The observed AE profile was comparable to that previously described for NSCLC patients treated with carboplatin/paclitaxel (Nishio et al. 2013).

A phase Ib trial tested the combination of lenvatinib plus everolimus in n = 20 patients with mRCC. The MDT was determined to be 24 mg for lenvatinib (plus everolimus 5 mg once daily). Grade 3–4 toxicities were noted in n = 15 patients (75%), the most common being hypertriglyceridemia (15%) and proteinuria (15%). Disease control rate was about 83% across all dose levels with n = 6 patients (33%) achieving a PR. The median PFS was 10.9 months.

Another phase 1b study tested the combination of lenvatinib 24 mg/day with temozolomide 150 mg/m^2/day (days 1–5) for the treatment of advanced malignant melanoma (stage 4 or unresectable stage 3). 32 patients were enrolled. MTD was not reached. Best overall response was a PR in six patients (18.8%), and SD lasting $\geq$ 16 weeks in nine patients (28.1%). In conclusion, clinical activity was shown but proved inferior to those observed with ipilimumab in combination with nivolumab or nivolumab alone (Hong et al. 2015).

3.3 Lenvatinib in the Treatment of Thyroid Cancer: Phase II/III Trial Data

Between October 2008 and February 2010, $n = 58$ patients with advanced radioiodine-refractory DTC were enrolled in a phase II trial investigating efficacy of lenvatinib at a dose of 24 mg daily until disease progression, toxicity, withdrawal, or death. A significant proportion of patients (29%) had received prior anti-VEGFR therapy. Objective response rate (ORR) as primary endpoint was 50% (95% confidence interval (CI): 37–63%) with 29 patients (50%) achieving a PR, 25 patients (43%) SD (≥ 7 weeks), and 16 patients (28%) durable SD (≥ 23 weeks). Median response duration was 12.7 months (95% CI: 8.8 months—not evaluable (NE)). Median PFS was 12.6 months (95% CI: 9.9–16.1 months). Prior anti-VEGFR TKI therapy did not result in significant differences in outcomes. AEs of all grades were noted in all patients; grade ≥ 3 toxicity was observed in $n = 42$ patients (72%). Dose reductions and drug withdrawal were necessary in 74% of patients and 26%, respectively. Among serious AEs (SAEs) that occurred in at least two patients pulmonary embolism, hypertension, and cardiac failure (3% each) were noted. Two fatal SAEs (one arterial hemorrhage and one cardiac arrest) were reported (Cabanillas et al. 2015).

The medullary thyroid cancer (MTC) study population consisted of $n = 59$ patients. ORR was 36% (95% CI: 24–49%), median PFS was 9.0 months (95% CI: 7.0–NE), and median overall survival (OS) was 16.6 months (95% CI: 16.4–NE). Again, prior anti-VEGFR TKI therapy did not result in significant differences in outcomes. Dose reduction and treatment withdrawal due to lenvatinib-associated AEs was necessary in 59% and 24% of patients, respectively. Three deaths were reported during or within 30 days post lenvatinib treatment, one of them (respiratory failure) deemed treatment-related (Schlumberger et al. 2016).

Within the phase III SELECT trial patients with radioiodine-refractory DTC were randomized in a 2:1 strata to either receive lenvatinib 24 mg daily or placebo. The primary endpoint was PFS. Secondary endpoints were ORR and OS. In total, $n = 392$ patients from 21 countries were recruited between August 2011 and October 2012. Median PFS was 18.3 months (95% CI: 15.1–NE) with lenvatinib compared to 3.6 months (95% CI: 2.2–3.7) with placebo (hazard ratio (HR) for progression or death = 0.21; 99% CI: 0.14–0.31; $p < 0.001$). The beneficial results with lenvatinib were independent of histological subtype (papillary, poorly differentiated, follicular, and Hürthle-cell), BRAF or RAS mutation status or prior TKI treatment (Schlumberger et al. 2015).

For OS, no statistical difference between lenvatinib and placebo arms were noted (HR for death = 0.73; 95% CI: 0.50–1.07; $p = 0.10$). To note, crossover was allowed being a potential confounder.

Tumor response to study drug was significant (64.8% vs. 1.5%; odds ratio, 28.87; 95% CI: 12.46–66.86; $p < 0.001$). In $n = 4$ patients (1.5%) who received lenvatinib a complete remission was noted whereas none such event was noted in the placebo arm.

AEs of CTC grade ≥ 3 were noted in 75.9% of patients in the lenvatinib group and 9.9% in the placebo group. They included hypertension (42.9%), proteinuria (10.0%), arterial thromboembolic effects (2.7%), and venous thromboembolic effects (3.8%). Six of 20 deaths (2.3%) in the lenvatinib arm were considered to be related to treatment with lenvatinib (including one case of pulmonary embolism and hemorrhagic stroke each).

Lenvatinib has received market authorization worldwide due to the positive results of the SELECT trial.

3.4 Lenvatinib in the Treatment of Renal Cell Carcinoma: Phase II Trial Data

A randomized, phase II, open-label, multicenter trial was conducted to explore efficacy and safety of lenvatinib alone or in combination with everolimus compared to single-agent everolimus as a second-line treatment. Between March 2012 and June 2013, $n = 153$ patients with mRCC with clear cell histology were recruited. 51 patients received the combination therapy (lenvatinib 18 mg plus everolimus 5 mg daily) in 28-day continuous cycles, $n = 52$ patients were treated with single-agent lenvatinib (24 mg daily) and $n = 50$ patients with single-agent everolimus (10 mg daily). Primary endpoint was PFS. Combination therapy prolonged median PFS significantly compared with everolimus alone but not lenvatinib alone (14.6 months (95% CI: 5.9–20.1) vs. 5.5 months (95% CI: 3.5–7.1, HR = 0.40, 95% CI: 0.24–0.68; $p = 0.0005$) vs. 7.4 months (95% CI: 5.6–10.2, HR = 0.66, 95% CI: 0.30–1.10; $p = 0.12$)). Single-agent lenvatinib also increased median PFS compared to single-agent everolimus (7.4 vs. 5.5 months; HR = 0.61; 95% CI: 0.38–0.98; $p = 0.048$).

The ORR was 43% for patients receiving the combination therapy compared with 27% for lenvatinib alone (rate ratio 1.6; 95% CI: 0.9–2.8; $p = 0.10$) and 6% for everolimus alone (rate ratio 7.2; 95% CI: 2.3–22.5; $p < 0.0001$). Single-agent lenvatinib yielded a significantly higher ORR when compared to single-agent everolimus (rate ratio 4.5; 95% CI: 1.4–14.7; $p = 0.0067$).

Median OS between groups was not significant in the original report. However, updated median OS was reported in the post hoc updated analysis as 25.5 months for lenvatinib plus everolimus, 19.1 months for lenvatinib, and 15.4 months for everolimus and became statistically significant between lenvatinib plus everolimus versus everolimus (HR = 0.51, 95% CI: 0.30–0.88; $p = 0.024$), but not between lenvatinib and everolimus or the combination treatment and single-agent lenvatinib (19.1 vs. 15.4 months; HR = 0.68; 95% CI: 0.41–1.14; $p = 0.12$, and 25.5 vs. 19.1 months; HR = 0.75, 95% CI: 0.43–1.30; $p = 0.32$).

71 and 24% of patients receiving lenvatinib plus everolimus and 62 and 25% of those assigned to single-agent lenvatinib needed a lenvatinib dose reduction or treatment discontinuation due to AEs. Drug toxicities were consistent with the preknown class-specific AEs of the two agents. Grade 3 and 4 AEs occurred in 71% of patients receiving the combination, 79% receiving single-agent lenvatinib and

50% of patients receiving everolimus monotherapy. Two fatal SAEs were deemed related to the study drug (one cerebral hemorrhage in the lenvatinib plus everolimus group and one myocardial infarction with single-agent lenvatinib).

The study design was criticized for everolimus being the comparison arm in the light of more potent TKIs and checkpoint inhibitors being already approved or emerging as effective treatment options for mRCC on the horizon.

Lenvatinib received US FDA and EMA approval in 2016 for the treatment of mRCC after failure of a prior TKI therapy at the dose of 18 mg/daily in combination with everolimus 5 mg/daily.

3.5 Lenvatinib in the Treatment of Hepatocellular Cancer: Phase II/III Trial Data

In the phase II study, evaluating lenvatinib in hepatocellular carcinoma (HCC) $n = 46$ patients were included. Patients were predominantly classified Child A ($n = 45$, 97.8%) and BCLC C ($n = 27$, 58.7%). ORR was 37% ($n = 17$; all patients had PR as best response), and median OS was 18.7 months (95% CI: 12.7–25.1).

Perceived toxicity levels in HCC patients in phase I trials led to the decision to reduce the dosage in the phase II trial to 12 mg for this patient population. However, also with this dose in $n = 34$ patients (74%) treatment-related AEs occurred. Treatment was terminated early in $n = 10$ patients (22%) due to drug-related toxicity. Patients requiring dose reductions or permanent interruptions of therapy had a significantly lower body weight and higher lenvatinib serum levels (Ikeda et al. 2016, 2017). The relationship between lower body weight and higher serum drug levels was hence analyzed in healthy individuals and cancer patients. However, only in HCC patient, this proved so relevant that two different dose levels dependent on patients' body weight were chosen to be used in further trials (Tamai et al. 2017).

The REFLECT trial, a phase III trial, compared lenvatinib (8 mg once daily when body weight <60 kg, 12 mg once daily when ≥ 60 kg) with sorafenib 400 mg twice daily in patients with unresectable HCC, previously not systemically treated and BCLC stage B or C and CHILD A liver function (Finn et al. 2017). Results were presented on ASCO 2017: Primary endpoint (non-inferiority in OS) was met. Patients treated with lenvatinib had a median OS of 13.6 months (95% CI: 12.1–14.9) compared to 12.3 months (95% CI: 10.4–13.9) when treated with sorafenib (HR = 0.92; 95% CI: 0.79–1.06). Also, secondary endpoints (PFS, time to progression, and ORR) yielded statistically significant results favoring lenvatinib.

3.6 Active Clinical Trials

3.6.1 Completed Trials, not Reported to Date

Endometrial cancer, phase II, single-agent lenvatinib, NCT01111461.
Melanoma, phase I/II, lenvatinib ± dacarbazine, NCT01133977.
Ovarian cancer, platinum-sensitive recurrence, phase Ib/II, Carboplatin + Gemcitabine ± lenvatinib, NCT01133756.

3.6.2 Ongoing Trials

Active phase Ib, II, or III trials include patients with breast cancer (NCT03168074), neuroendocrine tumors (NCT02678780), anaplastic thyroid cancer (NCT02726503), 131 I-refractory differentiated thyroid cancer (NCT02966093), pheochromocytoma or paraganglioma (NCT03008369), osteosarcoma (NCT02432274) and non-squamous non-small cell lung cancer (NCT01529112).

Combination therapies currently investigated for different treatment lines in curative and palliative settings include everolimus/lenvatinib in patients with differentiated thyroid cancer (NCT03139747), in patients with renal cell carcinoma (NCT02811861) and in patients with non clear cell renal cell carcinoma (NCT02915783), eribulin/lenvatinib in solid tumors (NCT02640508), paclitaxel/lenvatinib in endometrial or ovarian cancer (NCT02788708), capecitabine/lenvatinib plus external radiation therapy in rectal adenocarcinoma (NCT02935309), letrozole/lenvatinib in breast cancer (NCT02562118), pembrolizumab/lenvatinib in solid tumors (NCT02501096), renal cell carcinoma (NCT02811861) hepatocellular carcinoma (NCT03006926) and gastroesophageal cancer (NCT03321630).

4 Conclusion and Future Perspectives

Positive trials in RCC and differentiated radioiodine-refractory DTC have led to the approval of lenvatinib in combination with everolimus in RCC and as a single agent treatment in DTC, respectively. Approval for treatment of HCC is expected in the near future. Multiple clinical trials testing efficacy of lenvatinib as single agent or in combination in various tumor entities are still underway.

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Afatinib

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Contents

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Abstract

Afatinib (BIBW 2992, US: Gilotrif™, other countries: Giotrif©) is an irreversible blocker of the ErbB family, acting at the tyrosine kinases of these proteins. In 2013, it was approved by the FDA and the EMA for the treatment of adults with advanced, EGFR mutation-positive non-small-cell lung cancer. Further investigations for the treatment of many other tumors with afatinib, e.g., HNSCC and breast cancer, are ongoing.

Keywords

Afatinib · EGFR · Mutation · NSCLC · Solid tumors

1 Introduction

Afatinib targets the EGFR (epidermal growth factor receptor), a protein that belongs to the ErbB protein family. Further proteins belonging to this family are EGFR1/HER1 (ErbB-1), HER2/c-neu (ErbB-2), HER3 (ErbB-3), and HER4 (ErbB-4) (Roskoski 2014). Point mutations or amplifications of the gene locus can be detected in various tumors (Modjtahedi and Dean 1994), leading to enhanced signaling.

The EGF receptor is a transmembrane receptor with intrinsic tyrosine kinase activity. In canonical signaling, the extracellular activation of the receptor is initiated by the binding of EGF (epidermal growth factor), TGF-α (transforming growth factor-α), and others. After ligand binding, further activation is mediated by dimerization of two EGF receptors. The two kinases of the receptors induce an allosteric stimulation of downstream pathways. The involved pathways include phosphatidylinositol-3-kinase/Akt (PKB), Ras/Raf/MEK/ERK1/2, and phospholipase Cγ (PLC γ) (Cohen 1983; Lemmon and Schlessinger 2010). Besides this canonical pathway, other factors, e.g., environmental or cellular stress, activate noncanonical EGFR signaling. (Sigismund et al. 2017). Activated canonical and/or noncanonical signaling of EGFR can be found in various malignancies.

2 Structure and Mechanism of Action

Afatinib is an oral, selective inhibitor of the receptor tyrosine kinases (RTK) of the ErbB family (Fig. 1). The substance irreversibly binds to cysteine 797 of the EGF receptor and the corresponding cysteines 805 and 803 in HER2 and HER4,

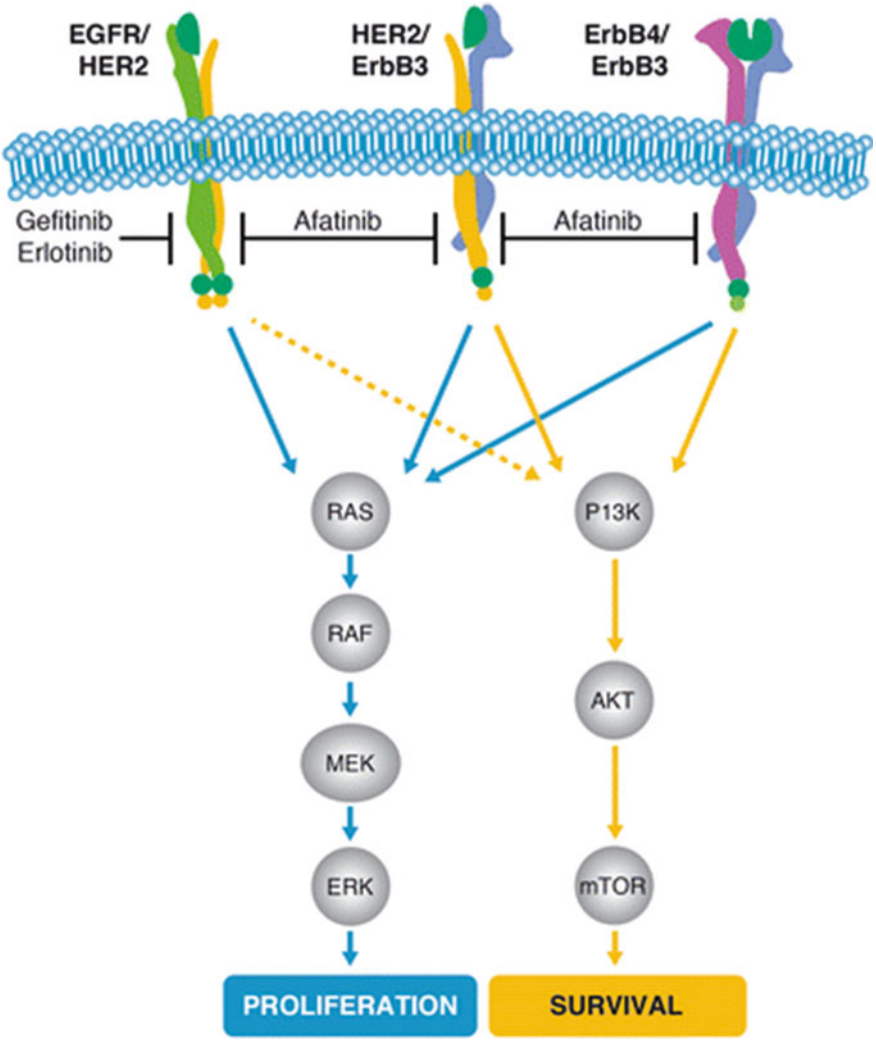

Fig. 1 Irreversible inhibition ErbB receptor family signaling by afatinib. Covalent binding of afatinib to the ErbB family of receptors inhibits downstream signaling of all dimers formed by the receptor (modified from V. Hirsh 2015)

respectively (Solca et al. 2012; Modjtahedi et al. 2014). The inhibitory concentration $(IC)_{50}$ is 0.5 nM for EGFR kinase, 14 nM for HER2 kinase, and 1 nM for HER4 kinase.

The substance leads to reduced auto- and transphosphorylation within the ErbB dimer and inhibition of the consecutive pathways. Afatinib induces apoptosis and reduces tumor cell growth in vitro and in vivo (Minkovsky and Berezov 2008) (Fig. 2).

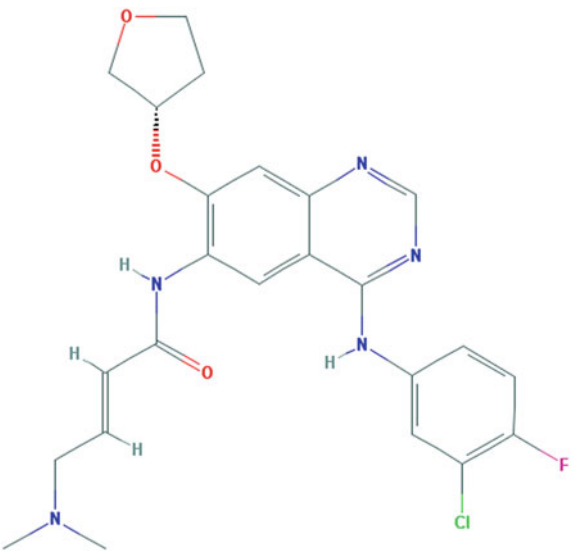

Fig. 2 Structure of afatinib [BIBW 2992; *N*-[4-[(3-chloro-4-fluorophenyl) amino]-7-[[(3*S*)-tetrahydro-3-furanyl]oxy]-6-quinazolinyl]-4-(dimethylamino)-2-butenamide] (Afatinib—Compound Summary for CID 10184653. National Center for Biotechnology Information (2017))

3 Preclinical Data

The in vivo activity of BIBW 2992 was first assessed in a xenograft model of the epidermoid carcinoma cell line A431, expressing wild-type EGFR and HER2 (Fan et al. 1993). Tumor shrinking after administration of afatinib in xenograft and transgenic lung cancer mouse models was found by Li et al. (2008). Mice received oral afatinib once daily for 25 days; the substance was more effective in tumor shrinkage than gefitinib or lapatinib. Tumor regression was also found in xenograft models resistant to erlotinib. By combining afatinib with cetuximab, Regales et al. could treat mice with mutated T790M EGFR effectively (Regales et al. 2009).

Transgenic mice with EGFR exon 19 deletion were treated with afatinib versus gefitinib versus control by Ninomiya et al.; afatinib-treated mice showed significantly less tumor burden. The combination of afatinib with bevacizumab versus afatinib monotherapy could improve the outcome of animals in xenograft tumor models with exon 19 deletion/T790M or L858R/T790M (Ninomiya et al. 2013). For NSCLC, many other transgenic models showed comparable results. For transgenic tumor models besides NSCLC, see Table 1.

4 Clinical Data

4.1 Phase I Clinical Trials with Afatinib

Various trials with continuous or intermittent dosing of afatinib in different indications were conducted. The maximum tolerated dose of continuous afatinib

Table 1 In vivo tumor models

Tumor type	References
HNSCC	Schütze et al. (2007), Young et al. (2015), Dunn et al. (2017)
Gastric cancer	Janjigian et al. (2013)
Pancreatic cancer	Ioannou et al. (2011)
Bladder cancer	Quesnelle and Grandis (2011)
Basal cell carcinoma	Eberl et al. (2012)
Colon cancer	Poindessous et al. (2011)
Esophageal squamous cell carcinoma	Wong et al. (2015)
Ovarian cancer	Wang et al. (2015)
Neuroblastoma	Mao et al. (2017)
Nasopharyngeal carcinoma	Xue et al. (2016)

recommended in the course of these trials was 40–50 mg qd (Agus et al. 2006; Yap et al. 2010). Common side effects of afatinib were rash, diarrhea, acne, and stomatitis. Other phase I trials in various indications and drug combinations are ongoing (Table 2).

5 Afatinib and NSCLC

The clinical trials program LUX-LUNG investigates with eight phase II and phase III studies the effect of afatinib in NSCLC.

5.1 Afatinib as First-Line Therapy

For the approval of the substance in Europe, the LUX-LUNG 3 study was the most relevant of these studies. In this randomized phase III study, $n = 345$ EGFR-mutated patients with advanced or metastatic adenocarcinoma of the lung were included, of whom about 2/3 were of East Asian origin. Patients received either afatinib 40 mg daily or chemotherapy with cisplatin/pemetrexed for up to six cycles. The primary endpoint of this study was PFS. Patients receiving afatinib had a significantly prolonged PFS of 11.1 months while those treated with chemotherapy showed a PFS of 6.9 months (hazard ratio (HR) 0.58 $p = 0.001$). Patients harboring EGFR exon 19 deletion or EGFR L858R mutation demonstrated better PFS than patients with other EGFR mutations (Sequist et al. 2013). Quality of life, as measured by EORTC-QoL C30 and Lung Cancer-13 questionnaires, improved under treatment with afatinib compared to chemotherapy. Furthermore, afatinib improved significantly the time until aggravation of the main symptoms dyspnea and cough. A positive effect of the substance on pain symptoms was found but did not show significance (Yang et al. 2013).

Table 2 Recent phase 1 trials with afatinib

Indication	Afatinib dose	Dose(s) combination partner(s)	References
Solid tumors	30/40/50/60/70 mg qd	Pemetrexed	Chu et al. (2014)
Solid tumors	10/20/30/40 mg qd	Nintedanib	Bahleda et al. (2015)
Solid tumors	20/40 mg qd	Vinorelbine	Mukai et al. (2015)
Solid tumors	30/40 mg qd	Cetuximab	Gazzah et al. (2015)
Solid tumors	20–50 mg qd	Cisplatin/paclitaxel; cisplatin/5-FU	Vermorken et al. (2013)
Solid tumors	20/30/40/50 mg qd	Paclitaxel, bevacizumab, carboplatin	Suder et al. (2015)
NSCLC resistant to erlotinib and gefitinib	40 mg qd	Cetuximab	Mukai et al. (2015)
NSCLC resistant to erlotinib and gefitinib	30/40 mg qd	Sirolimus	Moran et al. (2017)
NSCLC with T790M mutation	90/120/150/160/200 mg qd for 3 days, rep 14 d	–	Results only available on clinicaltrials.gov, NCT01647711, as of 1.12.2017
Breast cancer	20/30 mg qd	Trastuzumab	Ring et al. (2015)
Breast cancer/GI cancer	20/30 mg qd	Trastuzumab	Results only available on clinicaltrials.gov, NCT01649271, as of 1.12.2017

LUX-LUNG 6 evaluated the efficacy of afatinib versus chemotherapy with gemcitabine/cisplatin as first-line therapy in EGFR-mutated Asian patients (Wu et al. 2014). In LUX-LUNG 6, the substance met its primary endpoint by improving PFS.

Pooled overall survival data of LUX-LUNG 3 and LUX-LUNG 6 did not show significant improvement of OS for afatinib patients with common EGFR mutations (exon 19 deletions and L858R mutations) in either trial. However, subgroup analysis of patients with exon 19 deletion showed significantly prolonged OS in the afatinib groups in both studies (see Fig. 3).

Patients harboring L858R did not show a benefit from afatinib over chemotherapy in terms of OS, but suffered less from side effects (Yang et al. 2015b).

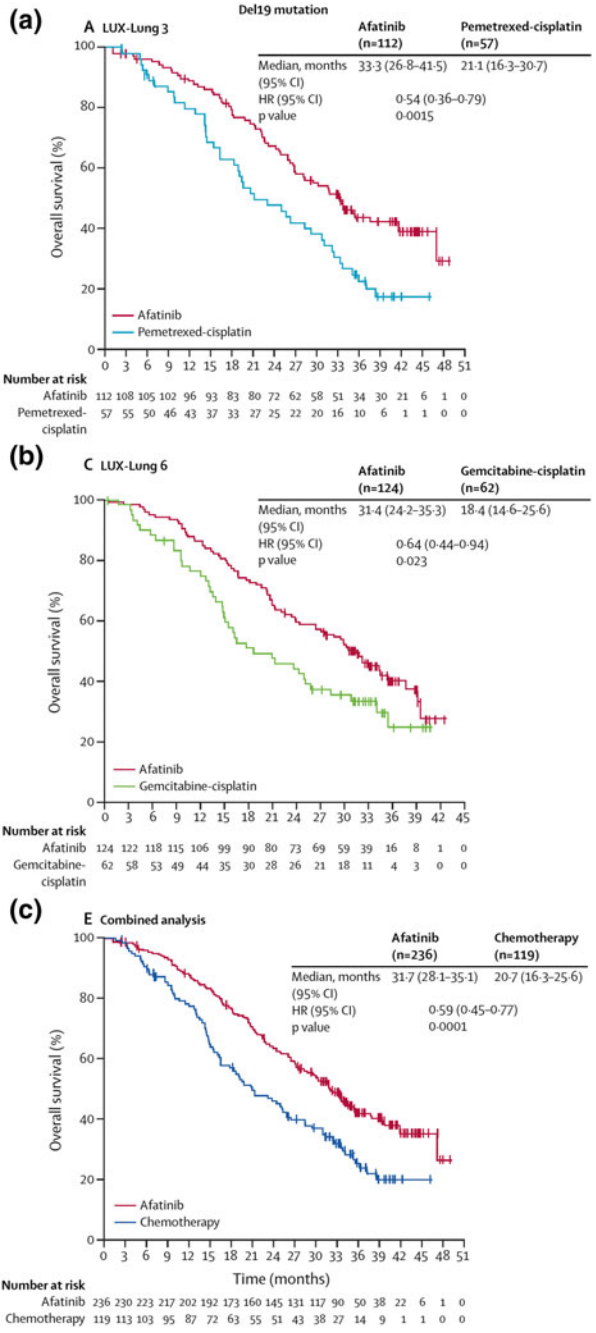

Fig. 3 Overall survival in patients with del19 EGFR mutations. **a** LUX-Lung 3. **b** LUX-Lung 6. **c** Both trials combined. HR = hazard ratio. Del19 = deletion of exon 19

5.2 Afatinib Versus Gefitinib as First-Line Therapy

LUX-LUNG 7 (phase IIb) evaluated the use of afatinib versus gefitinib in treatment-naïve, advanced, and EGFR-mutated NSCLC. The afatinib group showed prolonged PFS, ORR, and TTF, meeting these co-primary endpoints; however, the co-primary endpoint of overall survival was not reached (Paz-Ares et al. 2017).

5.3 Afatinib as Second-Line Treatment in NSCLC with Squamous Histology

In the LUX-LUNG 8 trial, afatinib was compared with erlotinib as second-line treatment after platinum-based chemotherapy in advanced squamous lung cancer. PFS, OS, and RR were significantly prolonged in the afatinib group, but side effects were significantly increased, especially diarrhea and mucositis (Soria et al. 2015). In a meta-analysis, afatinib showed better efficacy than gefitinib or erlotinib in the second-line treatment of advanced squamous NSCLC (Yang et al. 2017).

5.4 Afatinib as Third/Fourth-Line Therapy

In LUX-LUNG 1, a phase IIb/III study, afatinib was used for unselected, pretreated patients with adenocarcinoma of the lung. Two-thirds of all patients were of Asian origin. Patients had progressed under gefitinib or erlotinib and were randomized to receive either afatinib 50 mg/d or placebo. Overall survival, the primary endpoint of this study, could not be reached (Miller et al. 2012).

In the LUX-LUNG 5 phase III trial, afatinib was given to NSCLC patients beyond progression after EGFR inhibition. Patients were pretreated with either afatinib, gefitinib, or erlotinib. In the study, patients received either afatinib + paclitaxel or investigators' choice of chemotherapy following EGFR inhibition. The combination of afatinib + paclitaxel improved PFS, the primary endpoint of the study, compared to monotherapy, but failed in improving OS (Schuler et al. 2016).

5.5 Further Studies in NSCLC Patients

LUX-LUNG 2 evaluated the effect of afatinib in patients with advanced adenocarcinoma harboring EGFR mutations of exons 18–21. In these patients, afatinib was used as first- or second-line therapy (Yang et al. 2012). Patients received 50 and 40 mg afatinib qd, respectively. An objective response could be achieved by 61% of patients with a slight difference between the two patient groups. Median PFS was 10.1 months; patients with common mutations showed a longer PFS than those with uncommon mutations. Median OS was 24.8 months.

LUX-LUNG 4, a single-arm trial, included only Japanese patients with unresectable lung carcinoma resistant to erlotinib and/or gefitinib. Patients received 50 mg afatinib qd. Median PFS was 4.4 months; median OS 19 months (Katakami et al. 2013). The primary endpoint of the study was the objective response rate. 8.2% of patients showed a partial response, and 57.4% showed stable disease. In all phase II studies, diarrhea and skin irritations were the most frequent side effects.

One-hundred NSCLC patients (74% harboring an EGFR mutation) with brain metastases progressing after chemotherapy and EGFR-TKI received afatinib within a compassionate use program. TTF of these patients was 3.6 months, similar to a matched group without CNS metastasis. Cerebral disease control could be shown in 66% of these patients, indicating a positive effect of afatinib in CNS metastasis (Hoffknecht et al. 2015). Further studies support these observations (Li et al. 2017; Hochmair et al. 2016; Sequist et al. 2013).

The presence of rare EGFR mutations leads to lower response rates to TKI and reduced overall survival (Lohinai et al. 2015). A combined analysis of LUX-LUNG 2, LUX-LUNG 3, and LUX-LUNG 6 showed a limited effect of afatinib in patients with de novo T790M or exon 20 insertion. For other uncommon mutations, e.g., G719X, afatinib improved remission rates, and PFS (Yang et al. 2015a) (Table 3).

6 Afatinib in the Treatment of HNSCC

The LUX-Head & Neck studies investigate the potential role of afatinib in HNSCC. In the LUX-Head & Neck 1 trial, afatinib increased significantly PFS (median 2.6 vs. 1.7 months) compared to methotrexate as second-line therapy in recurrent or metastatic HNSCC (Machiels et al. 2015). In the LUX-Head & Neck 2 trial, afatinib was given in locally advanced HNSCC after chemoradiation (Burtness et al. 2014). The study closed early after an interim analysis did not show the potential of reaching a survival benefit for the afatinib group versus placebo. The Asian companion trial LUX-Head & Neck 4 was stopped at the same time. LUX-Head & Neck 3 investigates the use of afatinib versus methotrexate in patients with recurrent and/or metastatic disease after platinum-based chemotherapy; the study is ongoing.

7 Afatinib in the Treatment of Breast Cancer

The LUX-Breast 1 phase III trial compared afatinib + vinorelbine with trastuzumab + vinorelbine in HER2 + metastatic breast cancer after trastuzumab-containing therapy. The afatinib arm showed similar PFS and ORR, but shorter OS compared to the trastuzumab arm (Harbeck et al. 2016). LUX-Breast 3, a phase II trial, evaluated the use of afatinib in progressive, HER2 + stage IV breast cancer. The trial did not show a benefit for the afatinib-containing regimen, neither in primary or

Table 3 Overview LUX-LUNG study program

Study	Patients (*n*)	Conditions (line of tx, histology)	Treatment	RR (%)	PFS (months)	OS (months)	Reference
LUX-LUNG 1	585	Third–fourth line	Afatinib versus placebo	7 versus <1, $p = 0.0071$	3.3 versus 1.1, HR: 0.38, $p < 0.0001$	10.8 versus 12.0, HR: 1.08, $p = 0.74$	Miller et al. (2012)
LUX-LUNG 2	129	First or second line, EGFR mutated	Afatinib 40/50 mg	79/61	10.1 (all patients)	24.8 (all patients)	Yang et al. (2012)
LUX-LUNG 3	345	First line, EGFR mutated	Afatinib versus cisplatin + pemetrexed	69.1 versus 44.3, $p < 0.001$	11.1 versus 6.9, HR: 0.58, $p = 0.0004$	31.6 versus 28.2, HR: 0.78, $p = 0.10$ (Pts. with common mut.)	Sequist et al. (2013)
LUX-LUNG 5	202	Third–fourth line, afatinib beyond progression	Afatinib + paclitaxel versus chemotherapy	32.1 versus 13.2, $p = 0.005$	5.6 versus 2.8, HR: 0.60, $p = 0.0003$	12.2 versus 12.2, HR: 1.0, $p = 0.99$	Schuler et al. (2016)
LUX-LUNG 6	364	First line, EGFR mutated, Asian patients only	Afatinib versus cisplatin + gemcitabine	66.9 versus 23.0, $p < 0.0001$	11.0 versus 5.6, HR 0.28, $p < 0.0001$	23.6 versus 23.5, HR: 0.83, $p = 0.17$ (Pts. with common mutations)	Wu et al. (2014)
LUX-LUNG 7	319	First line, EGFR mutated	Afatinib versus gefitinib	70 versus 56, $p = 0.0083$	11 versus 10.9, $p = 0.017$	27.9 versus 24.5 (prelim.), $p = 0.2580$	Park et al. (2016)
LUX-LUNG 8	795	Second line	Afatinib versus erlotinib	6.0 versus 3.0, $p = 0.055$	2.6 versus 1.9, HR: 0.81, $p = 0.01$	7.9 versus 6.8, HR: 0.81, $p = 0.0077$	Soria et al. (2015)

secondary endpoints (Cortés et al. 2015). Further trials, including treatment in the neoadjuvant setting and the treatment of brain metastases are ongoing.

8 Afatinib in the Treatment of Other Cancer Types

In addition to the indications discussed above, afatinib treatment has been evaluated in various other tumor types. Limited activity of afatinib was seen in unselected refractory prostate cancer patients (Molife et al. 2014). Additional trials in patients with HER2 overexpressing prostate cancer are ongoing. In unselected, refractory glioblastoma patients afatinib showed only modest activity (Reardon et al. 2014). For ongoing trials in various entities, see Table 4.

9 Side Effects/Toxicity

The typical side effects of afatinib are mainly drug class effects. In the LUX-LUNG 3 trial as well as in other trials, the most challenging side effect was diarrhea (grade 3/4: 14.4%). Other grade 3/4 side effects that occurred in >5% of all patients were rash (16.2%), mucositis/stomatitis (11.4%), and nail inflammation (8.7%). In the

Table 4 Recent clinical trials with afatinib (active, not recruiting; recruiting; not yet recruiting), as of 6.12.2017, www.clinicaltrials.gov

Disease	Phase I trial (*n*)	Phase II trial (*n*)	Phase III trial (*n*)	Phase IV trial (*n*)
NSCLC	6	18	3	1
HNSCC	1	2	2	
Breast cancer	1	3	1	
Solid neoplasms	1	4		
Esophageal cancer		4		
Gastric cancer		3		
Brain cancer	2	2		
Pancreatic cancer	2	2		
Intestinal/colorectal neoplasms	1	2		
Urothelial cancer		2		
Neuroectodermal tumor	1	1		
Rhabdomyosarcoma	1	1		
Chordoma		1		
Penile cancer		1		
Uterine sarcoma		1		
Bile duct cancer	1			

LUX-LUNG 6 trial, the rate of diarrhea was only 5.4%, comparable to other TKI. This reduction could possibly be caused by early treatment of stool irregularities. As diarrhea often leads to dose reduction or termination of the therapy, it is recommended to treat first signs of diarrhea with hydration and antidiarrheal medication (Barron et al. 2016).

Skin irritations can vary from mild-to-medium erythematous, acne-like lesions appearing mainly on skin exposed to the sun. Early treatment with skin care and antibiotics is strongly recommended (Aw et al. 2017). Despite the side effects, in the LUX-LUNG 3 and LUX-LUNG 6 trial, the quality of life of patients receiving afatinib was significantly better compared to those receiving chemotherapy (Yang et al. 2013; Geater et al. 2015). In the LUX-LUNG 7 trial, afatinib and gefitinib demonstrated similar improvements in health-related quality of life without significant differences in quality of life. Safety and tolerability analysis did not show significant differences between afatinib and gefitinib (Park et al. 2016).

10 Drug Interactions

Afatinib plasma concentration reaches its maximum 2–5 h after oral administration and declines afterward at least bi-exponentially. Food intake reduces the systemic exposure to afatinib significantly. Afatinib doses over 50 mg per day lead to a slightly enhanced systemic exposure. The effective half-life of the substance is 37 h; the drug is excreted by feces (about 95%) and urine (about 5%) (Wind et al. 2017). The substance has only low potential for drug–drug interaction, but simultaneous treatment with inducers or inhibitors of the *P*-glycoprotein-transporter can lead to different pharmacokinetics of afatinib.

11 Biomarkers

EGFR mutation status in NSCLC can predict response and resistance to EGFR inhibitors. Mutation testing is essential, as afatinib was first approved for EGFR-mutated NSCLC. As afatinib has an effect on rare mutations in EGFR as well, broad mutation tests are needed (Lohinai et al. 2015). In the LUX-Head & Neck 1 trial, patients with prespecified biomarkers (p16-negative, EGFR-amplified, HER3-low, and PTEN-high) showed an increased benefit from afatinib (Cohen et al. 2017).

12 Summary/Perspective

For first-line therapy of patients with EGFR-mutated stage IV NSCLC, the TKIs erlotinib, gefitinib, afatinib, and osimertinib have been approved. Compared to platin-containing chemotherapy, PFS, RR, and quality of life are significantly higher in patients receiving TKI therapy. Most studies did not show an improvement of OS; this might be due to the fact that crossover rates after the failure of chemotherapy to the TKI treatment were high in these trials. Afatinib is effective in terms of OS in NSCLC patients harboring the EGFR exon 19 deletion, as seen in the LUX-Lung 3 and LUX-LUNG 6 trials. The substance appears to penetrate into the CNS and may therefore be a treatment option for pretreated, EGFR-mutated or EGFR-TKI-sensitive NSCLC with brain metastases. The incidence of side effects, such as rash, diarrhea, and stomatitis is higher compared to erlotinib. Direct comparisons of EGFR inhibitors are up to date and only available between afatinib and gefitinib. In the LUX-LUNG 7 study, afatinib could improve PFS, ORR, and TTF, but failed in improving OS. In addition to EGFR-mutated NSCLC, afatinib was approved for squamous cell NSCLC after failure of platinum-containing chemotherapy. For other indications, various studies are ongoing, as well as studies for NSCLC combining afatinib with immunotherapeutics, e.g., pembrolizumab.

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Olaparib

Sylvia Bochum, Stephanie Berger and Uwe M. Martens

Contents

Abstract

Olaparib (Lynparza [AstraZeneca, Cambridge, UK], formerly referred to as AZD2281 or KU0059436) is an oral poly(ADP-ribose) polymerase (PARP) inhibitor. It is rationally designed to act as a competitive inhibitor of NAD^+ at the catalytic site of PARP1 and PARP2, both members of the PARP family of

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enzymes that are central to the repair of DNA single-strand breaks (SSBs) mediated via the base excision repair (BER) pathway. Inhibition of the BER pathway by olaparib leads to the accumulation of unrepaired SSBs, which leads to the formation of deleterious double-strand breaks (DSBs). In cells with an intact homologous recombination (HR) pathway, these DSBs can be repaired effectively. However, in tumors with homologous recombination repair deficiencies, olaparib causes synthetic lethality through the combination of two molecular events that are otherwise nonlethal when occurring in isolation. Olaparib is already approved for the treatment of patients with recurrent ovarian cancer and a BRCA mutation, and it has been shown to provide clinically meaningful benefits among such patients. It has also shown promising activity in patients with metastatic breast or prostate cancer and a germline BRCA mutation. Besides its usage as a single agent, olaparib can also act either as a chemo- and/or radiosensitizer, due to its ability to potentiate the cytotoxic effects of these therapeutic agents. However, a clear patient benefit for the latter application has not been demonstrated yet.

Keywords
Olaparib · PARP inhibitor · DNA repair · BRCA · ATM · Synthetic lethality

1 Introduction

Cells are continually challenged by DNA assaults from endogenous and exogenous sources. The most common forms of DNA damages are single-base lesions and single-strand breaks (SSBs), whereas double-strand breaks (DSBs) constitute the most toxic form of DNA damage (Ciccia and Elledge 2010). Without appropriate repair mechanisms, DNA damage can cause genome instability and, eventually, promote the development of cancer. Different DNA repair pathways repair distinct DNA lesions, although a certain overlap exists. SSBs are normally corrected by the base excision repair (BER) pathway, while DSBs are predominantly repaired by BRCA-dependent homologous recombination (HR) (Dianov and Hübscher 2013; Krokan and Bjørås 2013).

Olaparib represents a novel class of drugs called PARP (Poly ADP-ribose polymerase) inhibitors that primarily interfere with the BER pathway leading to unrepaired SSBs. During DNA replication persistent SSBs are converted to DSBs, which are subsequently repaired by HR. However, in HR-deficient cells (e.g., through loss of function of BRCA1/2), the concurrent inhibition of PARP induces synthetic lethality, a process that is defined by the co-occurrence of two genetic events leading to organismal or cellular death (Bryant et al. 2005).

Currently, several areas of application for olaparib as an anticancer drug are investigated. The majority of clinical trials set focus on the single-agent activity of olaparib in patients with germline or somatic mutations in BRCA or several other genes involved in the HR pathway. The combination with chemotherapeutic agents

or radiotherapy offers the prospect to broaden the clinical benefit of olaparib beyond its use as monotherapy, but predicting the best combination still remains a challenge.

2 Structure and Mechanism of Action

Olaparib (formerly referred to as AZD2281 or KU0059436) is designated chemically as 4-[[3-[4-(cyclopropanecarbonyl)piperazine-1-carbonyl]-4-fluorophenyl]methyl]-2H-phthalazin-1-one. The molecular formula of this orally active small molecule is $C_{24}H_{23}FN_4O_3$ and its relative molecular weight is 435.08 g/mol (Fig. 1). Olaparib acts mainly as a selective and potent inhibitor of the enzymatic activity of the poly(ADP-ribose) polymerases PARP1 and PARP2 with an IC50 of 5 and 1 nM, respectively (Ame et al. 2004).

PARP1 and PARP2 are members of the PARP superfamily, comprising 17 multifunctional enzymes in human that are identified by sequence homology within the conserved catalytic domain (Ashworth 2008). Both have poly ADP-ribosylating activity, with PARP1 carrying out 90% of the overall synthesis of the poly (ADP-ribose) (PAR) biopolymers (Schreiber et al. 2002). This PARylation process represents a reversible posttranslational modification in which target proteins become modified with monomeric, short chains, or long branching chains of ADP-ribose. Olaparib binds to the catalytic domain of PARP1 and PARP2, effectively inhibiting PARylation at low nanomolar concentrations (Rouleau et al. 2010; Javle and Curtin 2011). It also exerts anticancer activity by its ability to trap inactive PARP enzymes on DNA, forming toxic PARP-DNA complexes that cause increased double-strand breaks (DSBs) (Murai et al. 2012; Scott et al. 2015).

As molecular sensors of DNA damage, PARP1 and PARP2 are able to recognize a variety of DNA damage and aberrations, including single-strand breaks (SSBs) and DSBs (Dantzer et al. 2000; Schreiber et al. 2006). Upon binding to the site of damage, which is mediated by their N-terminal DNA-binding domain, both PARPs become activated, subsequently catalyzing the transfer of PAR polymers of varying length and complexity from NAD^+ onto the surface of various nuclear acceptor proteins—including DNA repair factors and the PARPs themselves (automodification) (Ame et al. 2004; Hassa and Hottiger 2008). The synthesis of PAR chains is

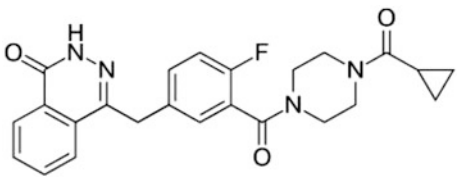

Fig. 1 Molecular structure of olaparib

considered one of the earliest events of cellular DNA damage response as it occurs within seconds (Ciccia and Elledge 2010; Gassman and Wilson 2015).

PARP1 and PARP2 by recruiting PAR-binding proteins to the site of the DNA break are important components of several DNA repair pathways, including base excision repair (BER) and BRCA-dependent homologous recombination (HR) (Rouleau et al. 2010). SSBs are usually repaired via the BER pathway, where PARP1 promotes the recruitment of various DNA repair proteins to the site of damage, including XRCC1 and POLβ (Masson et al. 1998; Campalans et al. 2013).

Inhibition of the BER pathway by Olaparib leads to the accumulation of unrepaired SSBs, which during S phase provoke collapsed replication forks, and eventually the formation of deleterious DSBs. In cells with an intact HR pathway, these DSBs can be repaired effectively (Jackson and Bartek 2009). However, in cells with defective HR, as in the case of BRCA1 or BRCA2 deficiency, the DSBs that result from PARP inhibitor-mediated loss of BER are either repaired by more error-prone DNA repair mechanisms like nonhomologous end joining (NHEJ), single-strand annealing (SSA) and microhomology-mediated end joining (MMEJ), or remain unrepaired, leading to further genomic instability and apoptotic cell death (Fong et al. 2010). This phenomenon is referred to as synthetic lethality, where two defects, which alone are benign, can be lethal when combined (e.g., inhibition of PARP activity and loss of DSB repair by HR) (Bryant et al. 2005; Farmer et al. 2005) (Fig. 2). Besides its usage as monotherapy to induce synthetic lethality, Olaparib can also act either as a chemosensitizer, due to its ability to potentiate cytotoxicity of DNA-damaging chemotherapeutic agents (e.g., alkylators, platinum analogs), or a radiosensitizer by preventing PARP-mediated DNA repair (Ledermann et al. 2012; Rottenberg et al. 2008).

3 Preclinical Data

Preclinical data for olaparib have provided strong evidence for the use of this PARP inhibitor alone or in combination with platinum drugs for the treatment of BRCA-associated cancers and BRCA-like tumors with defective homologous recombination (HR) repair. Olaparib inhibits selectively PARP1 as well as PARP2 activity (Bryant et al. 2005). In contrast to PARP1 single knockout mice, who are viable and fertile suggesting that these are not completely deficient in the repair of single-strand breaks, PARP1/PARP2 double-knockout mice are embryonic lethal, with both enzymes having overlapping and nonredundant functions in the maintenance of genomic stability (Menissier de Murcia et al. 2003; Huber et al. 2004).

It has been demonstrated, that cells containing mutations in BRCA1 or BRCA2 as well as other genes involved in the HR pathway are extremely dependent upon the activity of PARP1 which plays a central role in base excision repair (Bryant et al. 2005; Farmer et al. 2005; Min et al. 2013). When PARP1 is inhibited by

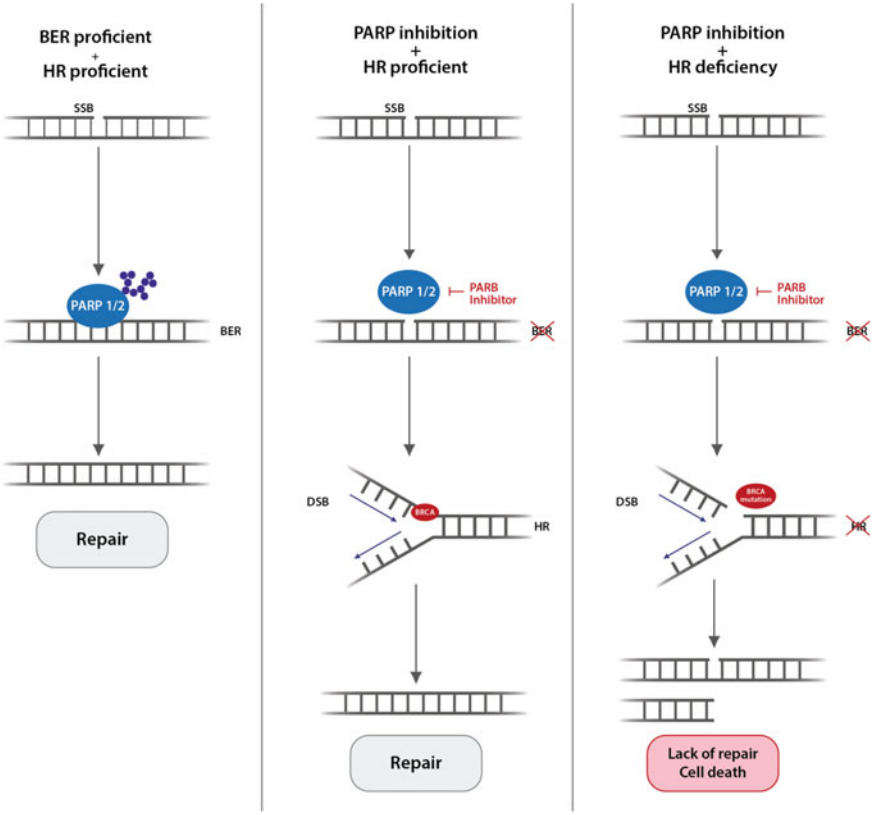

Fig. 2 Concept of synthetic lethality. Left: DNA single-strand breaks (SSB) generated in cells are normally repaired by PARP1/2-mediated base excision repair (BER). Middle: PARP inhibition results in persistence of SSB which are converted to double-strand breaks (DSB) that require a functional homologous recombination (HR) pathway for repair and cell survival. Right: In the absence of functional HR (e.g., BRCA mutation), the treatment with a PARP inhibitor leads to conversion of unrepaired SSBs to DSBs which are left unrepaired and can lead to cell death. In summary, deficiency in either BER or HR alone does not lead to cell death but loss of both functions does. Hence, treatment with PARP inhibitors in HR-defective cells leads to synthetic lethality (adapted with modifications from Dhillon and Taniguchi 2015)

olaparib, single-strand breaks degrade into double-strand breaks that cannot be repaired due to the defect in HR. Therefore, inhibition of PARP1 confers selective cytotoxicity to tumor cells with attenuated HR function, and several in vitro and in vivo data have demonstrated that cell populations with a known defect in homologous recombination (HR) repair are in fact selectively sensitive to single-agent olaparib (McCabe et al. 2006; Min et al. 2013; Kubota et al. 2014). Olaparib has also been investigated in combination regimens, as inhibition of PARP can potentiate the effects of numerous DNA-damaging agents. In a genetically engineered mouse model of *BRCA1*-deficient mammary tumors, treatment with

olaparib and a platinum drug (cisplatin or carboplatin) created potent synergy inducing an increase in overall survival versus either agent alone (Rottenberg et al. 2008).

4 Clinical Data

4.1 Single-Agent Trials

Olaparib entered the clinic in 2005 as a single agent, exploiting the concept of synthetic lethality in defined patient populations, and was the first PARP inhibitor to be approved by the European Medicines Agency (EMA) and the US Food and Drug Administration (FDA).

The FDA granted accelerated approval in 2014 as monotherapy in patients with germline BRCA-mutated advanced ovarian cancer who have been treated with three or more prior lines of chemotherapy (Kim et al. 2015). The same year, olaparib received approval by the EMA as monotherapy in the maintenance treatment of patients with platinum-sensitive, relapsed BRCA-mutated high-grade serous epithelial ovarian, fallopian tube, or primary peritoneal cancer, who are in partial or complete response following platinum-based chemotherapy. This was also acknowledged by the FDA, who in 2017 granted extended approval for the same group of patients regardless of their BRCA mutation status. Furthermore, the FDA warranted Priority Review for the use of olaparib in patients with germline BRCA-mutated, HER2-negative metastatic breast cancer previously treated with chemotherapy, and a Breakthrough Therapy designation for the monotherapy treatment of BRCA1/2 or ATM gene-mutated metastatic castration-resistant prostate cancer (mCRPC) in patients who have received a prior taxane-based chemotherapy and at least one newer hormonal agent.

All current approvals are based on completed clinical studies, where the olaparib dose was 400 mg twice daily using a capsule formulation. However, the capsule formulation has recently been replaced by a 300 mg tablet formulation with improved bioavailability to simplify drug administration (Gupta et al. 2012; Mateo et al. 2016).

4.1.1 Phase I

Olaparib monotherapy was first tested clinically in a phase 1 trial within a BRCA1/2 mutation carrier enriched patient population (Fong et al. 2009). Pharmacokinetics and pharmacodynamics of doses ranging from 10 mg daily for 2 out of 3 weeks to 600 mg twice daily on a continuous schedule were assessed; eventually, a maximum tolerated dose (MTD) at 400 mg twice daily orally was established. Across all delivered doses, an objective response rate (ORR) of 47% and

disease control rate (DCR) of 63% were observed in the group of 19 patients with BRCA mutations and ovarian, breast or prostate cancers. Of note, durable objective antitumor activity was only observed in confirmed carriers of a BRCA1 or BRCA2 mutation and a tumor typically associated with a BRCA-carrier status.

To explore this response in greater detail, a phase I expansion study was added, evaluating olaparib at 200 mg twice daily in 50 patients with BRCA1/2 germline mutation-associated ovarian, primary peritoneal, and fallopian tube tumors. Olaparib results in a high antitumor response and disease control rate in BRCA mutation carriers with advanced ovarian cancer, reporting an ORR of 40% and DCR of 46% with a median response duration of 28 weeks (Fong et al. 2010). Retrospective analyses indicated a strong correlation between prior platinum sensitivity and the extent of olaparib response. Patients with platinum-sensitive disease had an ORR of 69%, while those with platinum-resistant or refractory disease showed less response (ORR of 45 and 23%, respectively), suggesting that platinum and olaparib sensitivity may depend on similar molecular mechanisms.

4.1.2 Phase II

These data were confirmed recently in a phase II study conducted in a group of patients with germline BRCA1/2 mutated advanced ovarian cancer, who were previously treated with three or more lines of chemotherapy (Domchek et al. 2016). The ORR was highest at 46% in patients considered platinum sensitive; for patients designated platinum resistant, it was 30%, and for those designated platinum refractory, it was 14%.

Several previous phase II trials have already reported durable antitumor responses with olaparib monotherapy in advanced ovarian and breast cancer patients with BRCA1/2 mutations, with ORRs in the 400 mg twice daily dosing cohorts of 33 and 41% respectively (Audeh et al. 2010; Tutt et al. 2010). However, in patients with high-grade serous ovarian cancer with or without *BRCA1/2* mutations an ORR of 41% for patients with BRCA1/2 mutations and 24% for those without such mutations was found, suggesting that tumors susceptible to PARP inhibition may also harbor defects in DNA repair that are unrelated to BRCA mutations (Gelmon et al. 2011). In a randomized Phase II study targeting patients highly enriched for deficiency in homologous recombination (HR), olaparib maintenance treatment significantly improved progression-free survival (PFS) in patients with platinum-sensitive, relapsed, high-grade serous ovarian cancer compared to placebo (8.4 vs. 4.8 months) (Ledermann et al. 2012). Further encouraging results with a significant improvement in PFS of 6.9 months were reported in a subgroup analysis of BRCA-mutated ovarian cancer patients (either germline or somatic), when olaparib was used as maintenance following response to platinum-based chemotherapy (Ledermann et al. 2014).

Besides efficacy in ovarian and breast cancer, olaparib monotherapy showed also response across several other tumor types associated with germline BRCA1/2 mutations. A phase II trial (TOPARP-A) in patients with metastatic, castration-resistant prostate cancer whose disease had progressed following chemotherapy, showed an ORR of 33% (Mateo et al. 2015). Of note, ORR was

increased to 88% in those patients with deleterious mutations in DNA damage repair genes—including BRCA1/2, ATM, Fanconi anemia pathway genes, and *CHEK2,* all of which exhibit synthetic lethal interaction in combination with concurrent PARP inhibition (McCabe et al. 2006; Murai et al. 2012).

4.1.3 Phase III

Several phase III trials are currently recruiting patients to study safety and effectiveness of olaparib monotherapy in different clinical settings and cancer types. Data of one randomized phase III trial (OlympiAD) have been reported so far. This trial compared olaparib monotherapy with standard therapy in patients with a germline BRCA mutation and human epidermal growth factor receptor type 2 (HER2)-negative metastatic breast cancer who had received no more than two previous chemotherapy regimens for metastatic disease (Robson et al. 2017). The response rate in the olaparib group was approximately double the rate in the standard-therapy group (59.9 vs. 28.8%). PFS was also significantly longer in the olaparib group (7.0 vs. 4.2 months), whereas overall survival (OAS) did not differ significantly between the two groups. The latter is likely confounded by subsequent treatment after disease progression that included PARP inhibitors, platinum-based therapy, and cytotoxic chemotherapy. Based on this trial, olaparib was granted Priority Review by the FDA. If the drug will be approved in this setting, metastatic breast cancer would be the third indication for olaparib in the United States.

4.2 Combination with Chemotherapy

PARP inhibitors are also considered as sensitizers of tumor cells to anticancer agents that induce DNA damage and strand breaks, requiring repair by the base excision repair (BER) pathway. Olaparib is currently under investigation in combination with several chemotherapeutic agents in an attempt to enhance their antitumor activity. However, to assess the specific benefit of olaparib in combination with chemotherapy can be challenging, especially since increased marrow suppression is a common dose-limiting toxicity, often requiring significant dose modifications of either the PARP inhibitor or the cytotoxic drug.

4.2.1 Platinum Agents

In the preclinical setting, the combination of olaparib and a platinum drug (cisplatin or carboplatin) has already demonstrated synergistic cytotoxicity in BRCA-deficient cell lines and tumors by suppressing DNA damage repair.

In a phase I study, olaparib was combined with cisplatin in patients with histologically confirmed metastatic cancer that had progressed on standard treatment (Balmaña et al. 2014). Promising antitumor activity was observed in these heavily pretreated patients, but particularly in patients with germline BRCA1/2 mutations. The ORR in the overall population was 41%, and 43% and 71% among patients with a BRCA1/2 mutation who had ovarian or breast cancer, respectively. Continuous olaparib monotherapy after combination treatment provided durable

responses, especially among breast cancer patients, supporting the use of olaparib as maintenance therapy in this setting.

A phase I/Ib trial examined the combination of olaparib and carboplatin in the treatment of breast or ovarian cancer patients harboring BRCA1/2 mutations who have received multiple prior chemotherapy regimens (Lee et al. 2014). The 45 patients received up to eight cycles of combination therapy before being transferred onto a daily maintenance olaparib regimen until disease progression. More than 85% of the patients had clinical benefit by prolonged stable disease and/or reduction in tumor size, with response rates of 44% in ovarian cancer and 87% in breast cancer, suggesting at least additive effects by these agents. Interestingly, two-thirds of the ovarian cancer patients were either platinum resistant or refractory, yet had a response rate of 25% and a clinical benefit rate of 70%, supporting the hypothesis that tumors with DNA repair defects may still be sensitive to PARP inhibition based therapy, even after acquiring platinum resistance (Lee et al. 2014). In contrast, a phase I/Ib study of olaparib and carboplatin in 28 women with sporadic triple negative breast cancer without apparent germline BRCA mutation, showed only modest activity with a response rate of less than 20% (Lee et al. 2017).

4.2.2 Paclitaxel

In a phase I/II trial olaparib (200 mg twice daily) was combined with paclitaxel as a first/second-line therapy for metastatic triple negative breast cancer. Partial response was confirmed in 7 of 19 patients (37%) (Dent et al. 2013). However, even though this patient cohort was not heavily pretreated and was not having excessive bone marrow involvement, the treatment combination was associated with a greater than expected incidence and severity of neutropenia, which resulted in the delivery of a lower paclitaxel dose intensity than planned and treatment delays in a number of patients.

In a randomized phase II study, patients with platinum-sensitive, recurrent ovarian cancer received either olaparib (200 mg twice daily) plus paclitaxel/carboplatin followed by olaparib monotherapy (400 mg twice daily), or paclitaxel/carboplatin alone followed by no further treatment in the maintenance phase. PFS was significantly improved for those patients receiving olaparib in addition to chemotherapy (12.2 vs. 9.6 months) with a manageable toxicity profile, while OAS data have not been reported yet (Oza et al. 2015).

Gastric cancer cell lines, particularly those with low ataxia telangiectasia mutated (ATM) protein expression, have been sensitive to olaparib in preclinical studies (Kubota et al. 2014). Since ATM loss results in a deficient HR pathway, PARP inhibition in theory seems to be an attractive target in gastric cancer. In a randomized, double-blinded phase II trial, single-agent paclitaxel was compared against the combination of olaparib and paclitaxel as a second-line therapy in patients with recurrent or metastatic gastric cancer followed by maintenance monotherapy with olaparib (200 mg twice per day) or placebo (Bang et al. 2015). The study population was enriched for patients with tumors exhibiting low or undetectable ATM levels. Interestingly, there was no significant increase in PFS observed between the two treatments in the overall population, whereas the

combination of olaparib and paclitaxel was associated with a significant improvement in OAS versus paclitaxel plus placebo (13.1 vs. 8.3 months). This benefit was even more pronounced in patients with low ATM levels. However, a subsequent double-blind, randomized, placebo-controlled phase III trial (GOLD) in 525 patients with advanced gastric cancer that had progressed following, or during, first-line chemotherapy, failed to show a significant improvement in OAS in both the overall and the ATM-negative patient populations (Bang et al. 2017).

4.2.3 Topoisomerase Inhibitors

The safety and tolerability of combining olaparib with the topoisomerase I inhibitor topotecan has been examined in an open-label phase I trial involving patients with advanced solid malignancies (Samol et al. 2012). However, due to the high incidence of hematological adverse events the trial had to be terminated prematurely.

An interim review of a phase I trial investigated the combination of olaparib with pegylated liposomal doxorubicin (PLD) in 44 patients with advanced solid tumors. Doxorubicin acts on topoisomerase IIA, causing the accumulation of cytotoxic double-strand breaks (DSBs). Therefore, the combination of PARP inhibition with PLD may provide a synergistic effect in patients, especially those with a deficient HR pathway. The ORR was 33%, with complete responses in three patients. A total of 13 responders had ovarian cancer, of these 10 were platinum sensitive, 11 had a germline BRCA mutation (Del Conte et al. 2014).

4.2.4 Novel Targeted Agents

Several clinical trials are currently exploring the combination of olaparib with the vascular endothelial growth factor receptor (VEGFR) multikinase inhibitor, cediranib. The rationale behind this combination is based on the observation that VEGFR inhibition may lead to tumor hypoxia that results in impairment of homologous recombination (HR) repair through downregulation of a number of HR proteins including RAD15 and BRCA1, eventually increasing the sensitivity of tumor cells to PARP inhibitors (Bindra et al. 2005; Chan and Bristow 2010).

In a randomized, open-label phase II study, patients with recurrent platinum-sensitive high-grade serous ovarian cancer received either cediranib plus olaparib (200 mg twice daily) or olaparib monotherapy (400 mg twice daily). Patients taking the drug combination had a PFS of 17.7 months, compared with 9 months among those given olaparib alone (Liu et al. 2014). Approximately 53% of patients were BRCA mutation carriers. However, in this molecular subgroup, only a slight trend toward increased activity of the cediranib/olaparib combination was seen (PFS of 19.4 vs. 16.5 months), whereas patients without BRCA mutation showed a significant increase in PFS from 5.7 months with olaparib alone to 16.7 months with the combination cediranib/olaparib. This outcome was startling as BRCA mutation carriers were expected to benefit more from the combination with a PARP inhibitor than BRCA-negative patients. Currently, nine clinical trials are ongoing elucidating safety and efficacy of this combination.

4.3 Combination with Radiotherapy

Ionizing radiation is capable of directly damaging DNA and regulatory proteins. Radiosensitization of solid tumors by the application of drugs that interfere with DNA damage repair pathways offers a promising opportunity to increase the effectiveness of radiotherapy. PARP-inhibitors like olaparib are not only capable to impair the base excision repair pathway, but also have only minimal systemic toxicity as single agents, therefore, representing ideal candidates to act as radiosensitizers.

The radiosensitizing effects of PARP inhibitors observed in vitro have been confirmed in various in vivo studies in several tumor types including lung, glioblastoma, colon, and head and neck cancer (Russo et al. 2009; Khan et al. 2010). However, clinical trials have only recently been initiated. Currently, eight phase I trials combining olaparib and radiotherapy with or without concurrent chemotherapy are actively recruiting patients, but no data have been reported yet.

5 Toxicity

At the maximum tolerated dose of 400 mg twice daily, olaparib is comparably well tolerable, especially in single-agent studies (Fong et al. 2009). Across several clinical trials olaparib monotherapy has been associated with adverse events (AE) of mostly mild or moderate severity (grade 1/2), generally not requiring treatment discontinuation. In two phase II studies of patients with chemotherapy-refractory breast and ovarian cancer a rather favorable toxicity profile was found, with fatigue, nausea and vomiting being the most common AEs (Audeh et al. 2010; Tutt et al. 2010). Approval of olaparib by the FDA was primarily based on an open-label, nonrandomized clinical trial in 298 patients with deleterious or suspected deleterious germline *BRCA* mutation-associated cancer, including 193 patients with ovarian cancer. Grade $\geq$ 3 AEs were reported for 54% of patients with about half of them considered causally related to olaparib. Anemia was the most common AE (17%). Approximately 40% of patients experienced AEs that led to dose modification (interruption and/or reduction), but less than 4% of patients required discontinuation of study treatment (Kaufman et al. 2015).

Dose-limiting toxicities of olaparib are usually myelosuppression and central nervous system side effects (Fong et al. 2009). The incidence of severe hematologic toxicities varied widely across clinical trials. Severe neutropenia was more common with olaparib plus cytotoxic chemotherapy than with chemotherapy alone, suggesting that the combination therapy might intensify chemotherapy-induced toxicities (Oza et al. 2015; Bang et al. 2015). A rare, but serious complication in patients who received olaparib represents treatment-related myelodysplastic syndrome/acute myeloid leukemia (MDS/AML) (Ricks et al. 2015). Among the 2618 patients exposed to olaparib at the time of the FDA review, 22 cases of MDS/AML were reported (0.8%), with 17 cases resulting in death. Most of these

patients had previously received multiple lines of DNA-damaging, platinum-containing chemotherapies, which may have contributed to this AE. However, given the mechanism of action and increased rates of MDS/AML seen in randomized studies, the risk of developing MDS/AML in patients with germline or somatic DNA repair deficiencies receiving olaparib warrants a high level of awareness (Friedenson 2007; Kim et al. 2015).

6 Drug Interactions

Olaparib is primarily metabolized in the liver by the isozymes CYP3A4/5, which can result in drug interactions with CYP3A inhibitors (e.g., macrolide antibiotics, azole antifungals), resulting in increased plasma levels of olaparib. Dose reductions to 150 mg twice daily are recommended for concomitant use of a strong CYP3A inhibitor and to 200 mg for concomitant use of a moderate CYP3A inhibitor (EMA 2015).

Concomitant use of a strong or moderate CYP3A inducer (e.g., carbamazepine, phenobarbital, rifampicin) should be avoided. If a CYP3A inducer must be co-administered, there is a potential for reduced efficacy of olaparib (EMA 2015).

Olaparib itself may inhibit CYP3A4 in vitro and it cannot be excluded that olaparib may increase the exposures to substrates of this enzyme in vivo. Therefore, caution should be exercised when substrates of CYP3A4 are combined with olaparib, in particular, those with a narrow therapeutic margin (e.g., simvastatin, cisapride, cyclosporine, tacrolimus) (EMA 2015).

7 Biomarker

Currently, BRCA mutation status is the only validated predictive biomarker for PARP inhibitor (PARPi) sensitivity in breast and ovarian cancer patients. Several trials have confirmed the efficacy of olaparib in patients with germline BRCA1/2 mutations, who have advanced breast cancer or ovarian cancer (Audeh et al. 2010; Tutt et al. 2010). Improved response rates in cancers with somatic BRCA1/2 mutations have been reported, too, supporting the hypothesis that the majority of somatic *BRCA1/2* mutated cases have a biological phenotype similar to germline *BRCA1/2* mutated tumors (Dougherty et al. 2017).

Responses to PARPi have been observed in BRCA1/2 wild-type patients as well, suggesting that alterations in genes associated with homologous recombination (HR) other than BRCA1/2 may also confer sensitivity. HR is a multifactorial process with many gene products involved in the signaling and repair of DNA damage, and defects in any component (e.g., ATM, RAD51) can compromise the entire HR pathway (McCabe et al. 2006).

Of note, all HR mutations seem highly predictive of platinum sensitivity and improved overall survival, with non-BRCA mutations having a similar impact as BRCA mutations (Pennington et al. 2014). Therefore, prior sensitivity to platinum-based chemotherapy represents a useful clinical surrogate for HR deficiency and thus a potential antitumor response to olaparib (Fong et al. 2010). However, platinum sensitivity and PARPi responsiveness are not always concordant. The complex crosstalk between different DNA repair pathways may underlie this finding. For example, nucleotide excision repair (NER) gene mutations are associated with platinum sensitivity in ovarian cancer patients, but convey resistance to PARPi treatment (Ceccaldi et al. 2015). Otherwise, antitumor activity of olaparib can be observed in platinum-refractory and resistant tumors as well (Fong et al. 2010).

With decreasing costs of next-generation sequencing, routine testing for germline and somatic mutations in DNA damage repair genes will become available in the near future, facilitating the identification of molecular subgroups sensitive to PARP inhibition. However, to comprehensively identify potential responders to treatment with PARP inhibitors like olaparib, data from functional assays deciphering epigenetic and post-transcriptional modifications will be necessary, too.

8 Summary and Perspectives

Several inhibitors of poly(ADP-ribose) polymerases (PARPs), which play a key role in DNA damage repair pathways, have been developed in the past as antitumor agents based on the concept of synthetic lethality. Olaparib was the first PARP inhibitor to be approved in advanced ovarian cancer therapy for those with germline BRCA1/2 mutations. In the meantime, further PARP inhibitors have been approved in Europe and the US, mainly for the treatment of BRCA-mutant ovarian cancer.

However, a broader range of applications of PARP inhibitors is highly anticipated with regard to tumor entities and their molecular phenotype. In 2016, based on results of a compelling phase II trial, olaparib received a FDA breakthrough therapy designation for the treatment of patients not only with BRCA1/2 but also with ATM gene-mutated metastatic castration-resistant prostate cancer (mCRPC). Currently, a multitude of clinical trials in different malignancies are ongoing with olaparib or other PARP inhibitors as single agent or in combination with various cytotoxic or antiangiogenic agents. Challenges for the future will remain the selection of the best agent in each clinical context and the identification of suitable biomarkers for predicting efficacy and mechanisms of clinical resistance.

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Gefitinib

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Abstract

Gefitinib is an orally active selective inhibitor epidermal growth factor receptor (EGFR). The large randomised phase III IPASS study (gefitinib 250 mg, daily vs carboplatin and paclitaxel) showed a beneficial effect on progression-free survival (PFS) and quality of life in selected patient populations under the treatment with gefitinib (HR for TKI 0.74; 95% CI: 0.65–0.85). In the subgroup of patients with EGFR mutation the effect of gefitinib on PFS was notably, PFS HR 0.48; 95% CI: 0.36–0.64, $p < 0.001$) and the objective response rate (RR) was 71.2% with gefitinib versus 47.3% with chemotherapy. However no significant difference of overall survival was found. Based on this study gefitinib was approved for the first-line treatment of the patients with non-small cell lung cancer (NSCLC) with sensitising EGFR mutations (exon 19 deletion or L858R point mutation). Gefitinib is metabolized in the liver. Most of the adverse effects of gefitinib, such as rash, dry skin and diarrhoe, are mild to moderate in severity and are reversible.

Keywords

Non-small cell lung cancer · Epidermal growth factor receptor (EGFR) · EGFR mutation · Tyrosine kinase inhibitor (TKI) · Gefitinib

1 Introduction

Gefitinib (originally coded ZD1839) is an orally bioavailable, competitive, reversible inhibitor of epidermal growth factor receptor's (EGFR) tyrosine kinase domain, which interrupts signaling in target cancer cells with mutated and overactive EGFR. EGFR (HER-1/ErbB1) is a receptor tyrosine kinase of the ErbB family, which also includes Erb2 (HER2), ErbB3 (HER3), and ErbB4 (HER4). EGFR is overexpressed in many human epithelial malignancies including non-small cell lung cancer (NSCLC) (Hirsch et al. 2003). It is linked to multiple signaling pathways involved in tumor growth and angiogenesis such as the Ras/Raf pathway and the PI3K/Akt pathways (Bronte et al. 2014). These pathways ultimately lead to the activation of transcription factors such as Jun, Fos, and Myc, as well as cyclin D1, which stimulate cell growth and mitosis. Uncontrolled cell growth and mitosis lead to cancer. The activating mutations cause ligand-independent activity of receptor tyrosine kinases and occur in 8–15% of patients with NSCLC worldwide (Shigematsu et al. 2005; Pao et al. 2004). These mutations cause structural alterations in the ATP-binding site of the intracellular domain of EGFR as demonstrated by biochemical and crystallographic analyses. Specific missense and deletion mutations in the tyrosine kinase domain of the EGFR genes are most often located in exon 19 as a base pair deletion (delE746_A750; del19) or a substitution of arginine for leucine at position 858 in

exon 21 (L858R). The mutants possess increased affinity for tyrosine kinase inhibitors (TKI) such as gefitinib, erlotinib, afatinib, and osimertinib and lead to clinical response (Arteaga and Engelman 2014). These EGFR mutations are more seen in the patients' subgroup of adenocarcinoma histology, female gender, Asian ethnicity and never-smoking status, stage IV disease at diagnosis, the presence of bone metastases, and the absence of adrenal metastases ($p \leq 0.03$). EGFR mutations occur at about 47.9% Asian patients with adenocarcinoma compared with 15% in Caucasian/European patients (Sholl et al. 2015).

2 Structure and Mechanism of Action

Gefitinib is a low-molecular-weight 4-(3′-chloro-4′-Fluoroanilino)-7-methoxy-6-(3-morpholinopropoxy) quinazoline, $C_{22}H_{24}C1FN_4O_3$, a synthetic anilinoquinazoline compound (Fig. 1).

Gefitinib selectively binds to the adenosine triphosphate (ATP)-binding site of the EGFR tyrosine kinase domain. Thus, the autophosphorylation of the EGFR is inhibited, which results in inhibition of the Ras signal transduction pathway. Gefitinib is a selective inhibitor of the EGFR tyrosine kinase which is also referred to as HER1 or ErbB-1 (Lynch et al. 2004). Thus, the activation of the EGFR tyrosine kinase by the anti-apoptotic Ras signal transduction cascade is inhibited interrupting the uncontrolled cell proliferation leading to induction of apoptosis in cancer cells.

Research on gefitinib-sensitive non-small cell lung cancers has shown that a mutation in the EGFR tyrosine kinase domain is responsible for activating anti-apoptotic pathways (Sordella et al. 2004; Arteaga and Engelman 2014) (Table 1).

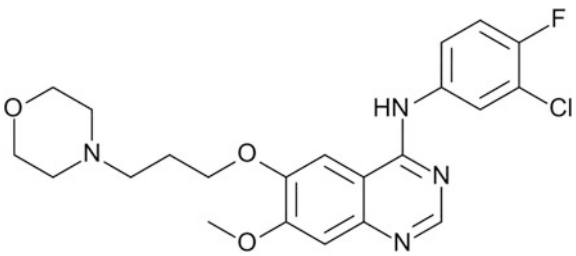

Fig. 1 Structure of gefitinib (*N*-(3-Chloro-4-fluorophenyl)-7-methoxy-6-[3-(morpholin-4-yl)propoxy]chinazolin-4-amin)

Table 1 Efficacy (IC_{50} values) of the EGFR kinase inhibitors gefitinib and afatinib in *EGFR* mutant Ba/F3 cells

EGFR Genotype	Gefitinib (nM)	Afatinib (nM)
L718Q	513	2.76
L844V	154	3.3
ΔE746_A750	43.8	0.52
ΔE746_A750/L718Q	61	2
ΔE746_A750/C797S	12.6	15.7
ΔE746_A750/L844V	24.36	0.66
ΔE746_A750/T790M	>3300	232
ΔE746_A750/T790M/L718Q	>3300	2115
ΔE746_A750/T790M/L844V	>3300	877
ΔE746_A750/T790M/C797S	>3300	678
L858R	49.84	0.51
L858R/L718Q	1117	7.94
L858R/C797S	72.7	185
L858R/L844V	147	1.02
L858R/T790M	>3300	1250
L858R/T790M/L718Q	>3300	1209
L858R/T790M/L844V	>3300	436
L858R/T790M/C797S	>3300	>3300

Modified from Ercan et al. (2015)

3 Pharmacology

Gefitinib is absorbed slowly after oral administration with a mean bioavailability of 60%. Peak plasma levels occur 3–7 h post administration. The mean elimination half-life is 48 h.

The bioavailability of gefitinib is not significantly altered by food intake. In the blood, gefitinib is bound to 90% to serum albumin and alpha 1-acid glycoproteins (independent of drug concentrations). Gefitinib is eliminated by hepatic metabolism, primarily via cytochrome P450 isoenzyme 3A4 (CYP3A4) and much less by CYP3A5 and CYP2D6. Three sites of biotransformation have been identified: metabolism of the N-propoxymorpholino group, demethylation of the methoxy substituent on the quinazoline, and oxidative defluorination of the halogenated phenyl group. Excretion is predominantly via the feces (86%), with renal elimination of drug and metabolites accounting for less than 4% of the administered dose (Campbell et al. 2010). The very high distribution volume of gefitinib (1400 l) indicates that gefitinib is extensively distributed throughout the body in tissues such as liver, kidney, gastrointestinal tract, lung, and in tumors. In nonclinical studies, a single dose of 12,000 mg/m^2 (about 80 times the recommended clinical dose on an mg/m^2 basis) was lethal to rats. Half of this dose did not cause mortality in mice.

4 Clinical Data

4.1 Phase I

In several phase-I studies, the maximum tolerated doses (MTDs) were 800 and 1000 mg/day, respectively (Baselga et al. 2002; Herbst et al. 2002). The antitumor activity was apparent at much lower doses, in particular, in patients with EGFR mutations. The acute toxicity of gefitinib up to 500 mg in clinical studies has been low. Symptoms of overdose include diarrhea and skin rash. The recommended dose of gefitinib is 250 mg per day as a single dose with or without food.

4.2 Phase II/III

The first trials with gefitinib were for unselected populations of patients with advanced NSCLC.

On the basis of encouraging results emerging from phase-II studies (The IDEAL trial), which showed a good activity profile of gefitinib as second/third-line treatment in terms of response rate (RR), a multicentre randomized phase-III trial (ISEL) was conducted comparing the efficacy of this drug versus placebo (Fukuoka et al. 2003). The ISEL study failed to demonstrate an overall survival (the primary endpoint) benefit for gefitinib in an unselected population of predominantly refractory patients with advanced NSCLC (hazard ratio [HR] 0.89; 95% confidence interval [CI] 0.77–1.02; $p = 0.087$; median survival 5.6 vs. 5.1 months). However, gefitinib prolonged median overall survival in never smokers (8.9 vs. 6.1 months; HR = 0.67; 95% CI 0.49–0.92; $p = 0.012$) and the Asian population (9.5 vs. 5.5 months; HR = 0.66; 95% CI 0.48–0.91; $p = 0.01$). The rate of responses was 8% (Thatcher et al. 2005).

In the Phase-III INTEREST (IRESSA NSCLC trial evaluating response and survival against Taxotere) study, gefitinib was compared with docetaxel in the second-line and third-line setting in patients with NSCLC not selected on the basis of clinical or molecular characteristics. The overall survival with gefitinib in unselected patients was not inferior to docetaxel. Median overall survival was 7.6 months in the gefitinib group and 8.0 months in the docetaxel group (HR = 1.020; 96% CI 0.905–1.150). Progression-free survival was similar for gefitinib and docetaxel (593 [90.0%] vs. 544 [82.8%] events; HR 1.04, 95% CI 0.93–1.18; $p = 0.47$; median progression-free survival (PFS) 2.2 vs. 2.7 months). Objective response rates were similar in both treatment groups (9.1% vs. 7.6%; OR 1.22, 95% CI 0.82–1.84; $p = 0.33$). This study reported efficacy in symptom improvement and a better toxicity profile leading to a better quality of life associated with gefitinib treatment (Kim et al. 2008; Douillard et al. 2009).

Further investigations identified that the occurrence of EGFR gene mutations in the kinase domain in specific patient types is strongly associated with response to gefitinib (Lynch et al. 2004).

The first randomized trial to specifically compare gefitinib with chemotherapy in clinically preselected patients with a preplanned subgroup analysis of EGFR-mutated patients was the IPASS (Iressa Pan-Asia Study) trial. The phase-III IPASS trial improved PFS with gefitinib compared with paclitaxel–carboplatin chemotherapy in chemotherapy-naive, never or light smokers with adenocarcinoma histology. The primary outcome of interest was PFS, and the trial was designed to show noninferiority. Participants were not randomly assigned by marker status (presence of EGFR mutation), although the marker analysis was preplanned. The study used the amplification refractory mutation system and EGFR 29-mutation detection testing. Of patients whose tissue was evaluable, almost 60% tested positive for the mutation (primarily exon 21 L858R mutations and exon 19 deletions). In the subset of EGFR mutation-positive patients, PFS was significantly prolonged with gefitinib compared with chemotherapy (HR, 0.74; 95% CI, 0.65–0.85). The study demonstrated the benefit of first-line EGFR TKI over platinum-based combination chemotherapy in patients with EGFR mutation prospectively.

This biomarker translational study of 447 patients with tumor samples available for EGFR mutation analysis confirmed that the benefit is confined to patients with activating mutation (HR for PFS, 0.48; 95% CI, 0.36–0.64), while EGFR mutation-negative patients had a significantly better PFS if treated with chemotherapy (see Fig. 2).

The tumor response rate was significantly higher in patients with activating mutation if they were treated with first-line gefitinib.

Gefitinib showed similar overall survival to chemotherapy with no significant difference in the overall population or in patients with EGFR mutation. However, 64.3% of patients in the chemotherapy arm with activating EGFR mutation received gefitinib as salvage therapy on disease progression post-chemotherapy. This crossover treatment explains the similarity in OS rates between the two treatment arms in the subgroup of patients with activating EGFR mutation (HR, 1.00; 95% CI, 0.76–1.33). Gefitinib improved significantly symptoms related to lung cancer (75.6% vs. 53.9%; OR, 2.70; 95% CI, 1.58–4.62; $p < 0.001$) (Mok et al. 2009; Yang et al. 2008). The IPASS trial was a milestone for the understanding of the activity of gefitinib in patients with EGFR-mutated lung cancer.

The First-SIGNAL [First-Line Single-Agent Iressa Versus Gemcitabine and Cisplatin Trial in Never Smokers with Adenocarcinoma of the Lung] was conducted exclusively in Korea. The study design was similar to that of IPASS, but the primary endpoint was OS. Gefitinib as a first-line therapy did not demonstrate superiority in OS compared with chemotherapy in these clinically selected Korean patients (in never smokers with lung adenocarcinoma at stage IIIb/IV) (HR, 0.932; 95% CI, 0.716–1.213; $p = 0.604$; median OS, 22.3 vs. 22.9 months, respectively). The 1-year PFS rates were 16.7% with gefitinib and 2.8% with chemotherapy (HR, 1.198; 95% CI, 0.944–1.520). Response rates were 55% with gefitinib and 46% with chemotherapy ($p = 0.10$). Only 14% patients had EGFR-mutated lung cancer,

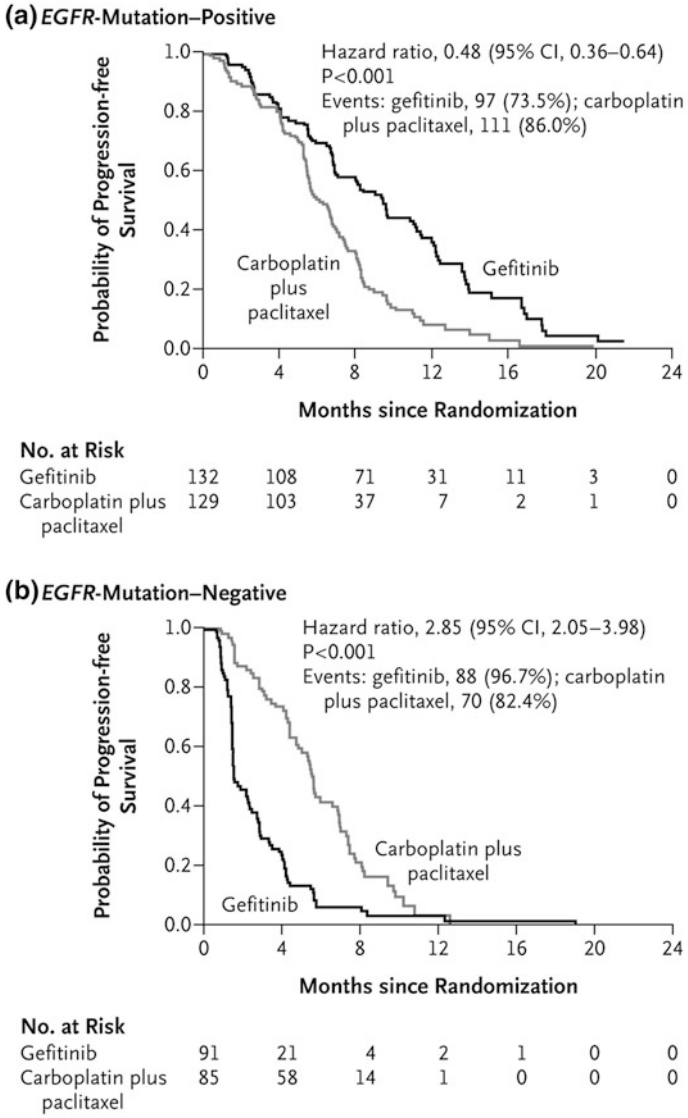

Fig. 2 Progression-free survival curves in EGFR-positive (**a**) and EGFR-negative (**b**) patients (adapted from Mok et al. NEJM 2009)

and the results of the EGFR mutation-positive subgroup for PFS and overall response were limited in number and were in contrast to other trials (Han et al. 2012).

Two randomized Japanese trials, NEJ002 and WJTOG, compared first-line treatment of EGFR-mutated NSCLC with gefitinib versus carboplatin–paclitaxel

and gefitinib versus cisplatin–docetaxel, respectively (Maemondo et al. 2010; Mitsudomi et al. 2010).

The results of the NEJ002 study showed the clear superiority of gefitinib in patients with EGFR-mutated NSCLC with significant improvement in PFS (median PFS 10.8 vs. 5.4 months HR: 0.30, $p < 0.001$) compared with paclitaxel–carboplatin in chemotherapy-naive patients (Maemodo et al. 2010).

The WJTOG3405 phase-III trial supports the IPASS results by showing that Japanese patients with EGFR-mutated NSCLC who received gefitinib had a median progression-free survival time of 9.2 (95% CI, 8.0–13.9) versus 6.3 months (95% CI, 5.8–7.8; HR, 0.489; 95% CI, 0.336–0.710; $p < 0.0001$) for those treated with cisplatin plus docetaxel. The objective response rate was significantly higher among patients receiving gefitinib (62.1%) versus patients receiving chemotherapy (32.2%) (Mitsudomi et al. 2010).

In all these trials, almost all patients who progressed after first-line chemotherapy received gefitinib as second-line treatment. Because of the high crossover rate, the OS was similar in both arms.

A metaanalysis of the IPASS, North-East Japan, West Japan, and first-SIGNAL trials confirms the results of studies comparing chemotherapy and gefitinib in first-line treatment. A higher RR (72% vs. 38% OR: 4.04) and a statistically significant increase in PFS (HR: 0.45) in patients with EGFR-mutated NSCLC treated with gefitinib could be demonstrated (Ku et al. 2011).

In 2009 on the basis of IPASS study, EMA approved gefitinib for the treatment of locally advanced or metastatic NSCLC in all treatment lines limited to patients bearing activating mutations of the EGFR gene (Table 2).

Gefitinib was used as control arm in three phase-IIb or phase-III studies investigating the efficacy of the second- and third-generation EGFR TKIs afatinib (phase-IIb:LUX-LUNG 7), dacomitinib (phase-III: ARCHER), and osimertinib (phase-III:FLAURA) in advanced or metastatic EGFR-mutated NSCLC, respectively (Paz-Ares et al. 2017; Wu et al. 2017; Soria et al. 2018).

Key results are shown in Table 3.

Table 2 First-line treatment of EGFR mutant NSCLC: Gefitinib versus Chemotherapy (CT)

Trial	TKI	Patient group	PFS (month)			OS
			TKI	Chemo	HR (95% CI)	HR (95% CI)
IPASS	Gefitinib	Asian	9.5	6.3	0.48 (0.36–0.64)	0.78 (0.50–1.20)
First signal	Gefitinib	Korean	8.4	6.7	0.61 (0.31–1.22)	0.82 (0.352–1.922)
NEJ002	Gefitinib	Japanese	10.8	5.4	0.322 (0.236–0.438)	0.88 (0.634–1.241)
WJTOG3405	Gefitinib	Japanese	9.6	6.6	0.52 (0.378–0.715)	1.185 (0.767–1.829)

Table 3 First-line treatment of EGFR mutant NSCLC: Gefitinib versus second- or third-generation EGFR TKI

Trial	TKI	Patient group	PFS (month)			OS
			2nd/3rd Gen TKI	Gefitinib	HR (95% CI)	HR (95% CI)
Lux-Lung 7	Afatinib versus Gefitinib	Asian and Non-Asian	11.0	10.9	0.73 (057–0.95) $p = 0.0165$	0.86 (0.86–1.12) $p = 0.2850$
ARCHER	Dacomitinib versus Gefitinib	Asian and Non-Asian	14.7	9.2	0.59 (0.47–0.74) $p < 0.0001$	*
FLAURA	Osimertinib versus Gefitinib or erlotinib	Asian and Caucasian	18.9	10.2	0.46 (0.37–0.57) $p < 0.001$	0.63 (0.45–0.88) $p = 0.007$

*OS at 18 months

4.3 Gefitinib in Combination with Chemotherapy

The concomitant administration of gefitinib and chemotherapy was investigated in a randomized, placebo-controlled trial, The Iressa NSCLC Trial Assessing Combination Treatment (INTACT 2). In this phase-III study, combining gefitinib with paclitaxel and carboplatin in chemotherapy-naive patients with advanced NSCLC did not show a survival benefit for the combination compared to chemotherapy alone (Herbst et al. 2004).

4.4 Resistance to EGFR TKI

The main limitation of the widespread benefits of first- and second-generation EGFR TKIs is the development of acquired resistance in patients with EGFR-mutated NSCLC treated with these drugs. Resistance mutations, e.g., EGFR-T790M, are located at the gatekeeper amino acid residue. This genomic event is present in 60–65% of cases with acquired resistance but recent studies using highly sensitive methods suggest a frequency of up to 35% detection in pretreatment biopsies. T790M abrogates the inhibitor effects of first-generation EGFR TKI by increasing the affinity of the receptor for ATP, leading to disruption of kinase–drug binding and activation of downstream signaling pathways. Other mechanisms of acquired resistance include bypass mechanisms comprising the hepatocyte growth factor receptor (MET), ERBB2, and others. These changes are detected in <15% and can be co-identified with EGFR-T790M in the same tumour sample. Furthermore, cases of tumour transformation to small-cell lung cancer has been seen (for review, see Arteaga and Engelman 2014).

5 Toxicity

The analysis of data from IPASS trial about toxic effects of gefitinib shows a good tolerability profile, with an incidence of adverse events significantly lower compared to chemotherapy (61–13% for chemotherapy vs. gefitinib $p < 0.001$; dose reduction of 35% vs. 16% for gefitinib).

The most frequently reported adverse events (AE) were skin rash (acneiform eruption), diarrhea, and nausea. These were observed within the first month of therapy and generally reversible. Most of the side effects of gefitinib were mild to moderate (grade 1/2). Hepatotoxicity (asymptomatic hypertransaminasemia) occurs rarely and recovered upon discontinuation of therapy. In addition, clinical trials have reported adverse pulmonary events related to gefitinib including interstitial lung disease (ILD) (serious adverse effect in 1% patients worldwide). The incidence is highest in patients of Asian origin, more frequently in Japanese patients (4–6%) than in Caucasian (0.2–0.3%).

Health-related quality of life (QoL) is an important end point for anticancer therapy. Four of the six randomized studies have captured QoL as a secondary end point. Given its lower toxicity profile and higher efficacy, QoL of patients receiving first-line EGFR TKI is better than that of patients receiving first-line chemotherapy. In the IPASS study, improvement in QoL was significantly greater in the gefitinib arm in patients with known activating EGFR mutation, while the opposite was observed in patients with EGFR wild type. A sustained, clinically relevant improvement in global QoL by Functional Assessment of Cancer Therapy-Lung (FACT-L) was observed in 70.2% of EGFR mutation-positive patients treated with gefitinib compared with 44.5% of patients treated with chemotherapy (odds ratio [OR], 3.01; 95% CI, 1.79–5.07; $p < 0.001$).

Compared to the second-generation EGFR TKIs afatinib and dacomitinib with irreversible ATP competition gefitinib has a favorable toxicity profile (Paz-Ares et al. 2017; Wu et al. 2017), while in the FLAURA study osimertinib had also few side effects (see regarding chapters in this book).

6 Drug Interactions

Drugs that induce CYP3A4 activity increase the metabolism of gefitinib. In patients receiving a potent CYP3A4 inducer such as rifampicin or phenytoin, a dose of gefitinib can be increased to 500 mg/day but only in the absence of severe adverse drug reaction (more rash and diarrhea). Patients taking warfarin should have their International Normalized Ratio (INR) monitored. INR elevations and bleeding events have been reported in patients taking both gefitinib and warfarin. Drugs that inhibit CYP3A4 activity (ketoconazole, itraconazole, and others) can lead to higher gefitinib plasma concentrations. H2-receptor antagonists such as ranitidine or cimetidine may reduce plasma concentrations of gefitinib by causing sustained elevations in gastric pH (Shah et al. 2005).

7 Summary and Perspective

For first-line therapy of patients with EGFR-mutated stage IV NSCLC the TKIs erlotinib, gefitinib, afatinib, and osimertinib have been approved. Compared to platinum-containing chemotherapy, PFS, RR, and quality of life are significantly higher in patients treated with EGFR TKIs. Gefitinib was one of the first TKI to be introduced. Gefitinib is effective in terms of PFS and RR in NSCLC patients harboring an EGFR exon 19 deletion or L858R mutation, as seen in the trials mentioned above. The substance has a good toxicity profile. In a direct comparison of gefitinib with afatinib, the side effects were favorable for gefitinib. Comparison with osimertinib showed better efficacy of the latter with regard to PFS, and OS data are awaited and comparable toxicity.

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Alectinib

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Abstract

Alectinib is an ATP-competitive small molecule and a second-generation inhibitor of ALK. EML4-ALK rearrangement is found in 3–5% of patients with NSCLC. The first-generation inhibitor crizotinib has changed the treatment dramatically, though most of the patients show disease progression within one year. Extra-thoracic progress, i.e., CNS metastases is common. The second-generation inhibitor alectinib has shown significant improvement in PFS and a remarkable prolongation of time to CNS progression. Alectinib has received approval as first-line therapy as well as second-line therapy after crizotinib failure. The toxicity profile is favorable compared to crizotinib and chemotherapy.

Keywords

Alectinib · EML4-ALK · ALK-inhibitor · NSCLC · CNS metasteses

1 Introduction

Chromosomal rearrangement of ALK defines a distinct group of about 5% of patients with non-small-cell lung cancer (Soda et al. 2007). Crizotinib, a specific inhibitor of ALK improved progression free survival compared to chemotherapy (Shaw et al. 2013; Solomon et al. 2014). It achieved approval as second-line therapy in 2012 and was FDA approved as first-line therapy in 2016 and has been standard of care in first-line treatment of ALK+ NSCLC. Despite the highly effective therapy with crizotinib, most of the patients relapse within 1 year, the median PFS with crizotinib is 8–10 months. Resistance can be due to acquired ALK mutations or activation of alternative pathways (Muller et al. 2016; Song et al. 2015; Gainor et al. 2017).

A common site of relapse or progress is the CNS in about 50% of the patients (Costa et al. 2015), which reflects a low penetration through the blood–brain barrier for crizotinib (Yoshida et al. 2016). Extra-thoracic progression, especially of the CNS occurs frequently in ALK+ NSCLC and more often than in ROS1+ or other entities (Gainor et al. 2017). Therefore, second-and third-generation ALK inhibitors are important subjects of current research. Ceritinib, brigatinib, and alectinib are second-generation ALK inhibitors, meanwhile also third-generation ALK inhibitors such as lorlatinib are available (Awad and Shaw 2014).

Alectinib (Chugai/F. Hoffmann-La Roche, Basel, Switzerland), formerly referred to as CH5424602 is a second-generation anaplastic lymphoma kinase inhibitor designed to inhibit ALK more specifically and potent than crizotinib (Kinoshita et al. 2012; Sakamoto et al. 2011; Kodama et al. 2014a, b, c).

It is an orally active ATP-competitive small molecule that has shown high enzymatic activity against ALK (Kinoshita et al. 2012), furthermore it has shown activity in RET (Kodama et al. 2014a, b, c) and LTK and GAK (Sullivan and Planchard 2016).

Preclinical studies suggest inhibition of most observed acquired ALK resistance mutations after crizotinib such as L1196M, C1156Y, and F1174L. In comparison to crizotinib, alectinib shows no activity in MET and ROS1 (Kinoshita et al. 2012; Sakamoto et al. 2011).

Comparable free levels of alectinib are found in plasma and CSF, which can explain the high effect on CNS metastasis.

Two phase III studies have shown improved PFS after crizotinib failure in second-line therapy, a first-line study compared to crizotinib shown improved PFS, ORR and time to CNS progression (Peters et al. 2017; Hida et al. 2017).

Alectinib has been approved as second-line therapy after crizotinib failure in ALK+ advanced NSCLC. FDA approval was granted as first-line therapy in November 2017, approval in Europe was granted in December 2017.

2 Structure and Mechanism of Action

Alectinib (Fig. 1) is designated chemically as 9-Ethyl-6,6-dimethyl-8-(4-morpholin-4-ylpiperidin-1-yl)-11-oxo-5*H*-benzo[*b*]carbazol-3-carbonitril.

Its molecular formula is $C_{30}H_{34}N_4O_2$ and its relatively molecular mass is 482, 628.

Alectinib functions as a specific competitive inhibitor of ATP of receptor tyrosine kinases. Due to blocking of the ATP binding site, phosphorylation is disrupted, consequently there is no activation in down streaming pathways, i.e., STAT 3 and PI3K/AKT which leads to induction of apoptosis of the cell (Webb et al. 2009).

The IC50 values in vitro are as follows (Fig. 2):

Preclinical studies showed a high brain to plasma ratio of alectinib in rats. In vitro studies confirmed the penetration of the blood–brain barrier. No transportation via the P-glycoprotein efflux or BCRP transporter was evident (Kodama et al. 2014a, b, c).

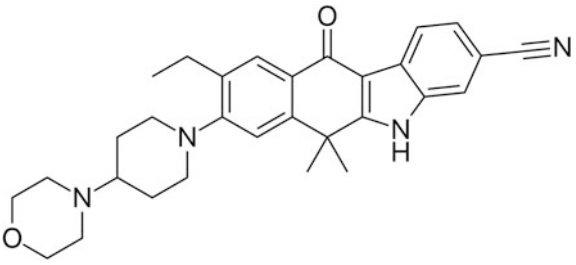

Fig. 1 Structure of alectinib

Mean IC 50 values of cellular ALK phosphorylation (nmol/L)

Mutation status	Crizotinib	Alectinib	Ceritinib	Brigatinib	Lorlatinib
Parental Ba/F3	763.9	890.1	885.7	2774.0	11293.8
EML4-ALK V1	38.6	11.4	4.9	10.7	2.3
EML4-ALK C1156Y	61.9	11.6	5.3	4.5	4.6
EML4-ALK I1171N	130.1	397.7	8.2	26.1	49.0
EML4-ALK I1171S	94.1	177.0	3.8	17.8	30.4
EML4-ALK I1171T	51.4	33.6	1.7	6.1	11.5
EML4-ALK F1174C	115.0	27.0	38.0	18.0	8.0
EML4-ALK L1196M	339.0	117.6	9.3	26.5	34.0
EML4-ALK L1198F	0.4	42.3	196.2	13.9	14.8
EML4-ALK G1202R	381.6	706.6	124.4	129.5	49.9
EML4-ALK G1202del	58.4	58.8	50.1	95.8	5.2
EML4-ALK D1203N	116.3	27.9	35.3	34.6	11.1
EML4-ALK E1210K	42.8	31.6	5.8	24.0	1.7
EML4-ALK G1269A	117.0	25.0	0.4	ND	10.0
EML4-ALK D1203N+F1174C	338.8	75.1	237.8	123.4	69.8
EML4-ALK D1203N+E1210K	153.0	82.8	97.8	136.0	25.6

Fig. 2 Modified acc. Gainor et al. (2016)

3 Preclinical Data

In 2011 Kinoshita et al. first described 9-substituted 6,6-dimethyl-11-oxo-6,11-dihydro-5H-benzo[b]carbazoles as highly selective and potent ALK inhibitors.

The high selectivity was achieved by altering the amino acid sequence close to the E_0 region of the ATP binding site. Furthermore, high antitumor efficacy was seen in a xenograft mouse model (Kinoshita et al. 2011, 2012).

Efficacy of alectinib in intracranial tumors was tested by implantation of EML4-ALK+ positive NSCLC cells in mouse models, where a tumor regression as well as a survival benefit was shown. To evaluate plasma and brain distribution ^{14}C labeled alectinib was used in rats, a high brain to plasma ratio was measured. Furthermore, in vitro drug permeability tests showed no transport by P-glycoprotein efflux transporter (Kodama et al. 2014a, b, c).

Nanjo et al. (2015) established an in vivo imaging model to investigate efficacy of ALK inhibitors in bone and brain metastases as well as pleural carcinomatosis. An EML4-ALK+ cell line from a male patient was injected in brain, bone and pleura of immunodeficient mice, tumor size were analyzed luminescence. Alectinib showed high efficacy in all three sites, whereas crizotinib shrank the pleural carcinomatosis, but not brain or bone metastases.

4 Clinical Data

4.1 Phase I

In the AF-001JO study 24 japanese treatment-naïve ALK+ NSCLC patients were treated with alectinib 20–300 mg twice daily, no dose-limiting toxicities or adverse event of grade 4 were evident (Seto et al. 2013).

In the AF-002JG study, 47 patients were enrolled after progression or intolerance to crizotinib. Dose escalation was performed from 300 to 900 mg twice daily. Alectinib was well tolerated with an investigator-assessed objective response rate of 55% of the patients, 36% showed a stable disease and 9% had progressive disease. Dose-limiting toxic effects in patients receiving 900 mg twice daily were grade 3 headache and grade 3 neutropenia. The recommended dose for the following phase II study was, therefore, chosen at 600 mg twice daily (Gadgeel et al. 2014).

4.2 Phase II

In the two studies NP28673 (global) and NP28761 (North America), 138 patients and 87 patients were included, respectively. Alectinib was administered orally, 600 mg twice daily until progression or dose-limiting toxicities. Both studies showed that patients who previously had no options for further therapy had a PFS of a median of 8.9 and 8.2 months and an OS of 26 and 22.7 months, respectively. The median response time was 15.2 and 14.9 months, respectively. Both studies showed furthermore, that alectinib shrank measurable CNS lesions in 64% of the patients with CNS metastases, (95% CI: 49.2%, 77.1%), in 22% (n = 29) a complete response of the CNS was achieved (Shaw et al. 2016; Ou et al. 2016).

4.3 Phase III

The Japanese J-ALEX Trial compared alectinib 600 mg administered twice daily to crizotinib 250 mg daily as first-line treatment for ALK+ NSCLC in 207 patients. Results showed a PFS of 10.2 months 95% CI (8.2–12) for crizotinib and not reached 95% CI (20.3-NR) for alectinib. Discontinuation due to AE occurred in 8.7% of the patients treated with alectinib and in 20.2% treated with crizotinib.

Dose interruptions occurred in 29.1% versus 74% of the patients, respectively (Hida et al. 2017).

The ALEX trial was a subsequent global phase III study that compared alectinib and crizotinib as first-line therapy in treatment—naïve Caucasian and Asian patients with advanced ALK+ NSCLC. Patients were randomized for either alectinib 600 mg twice daily, or crizotinib 250 mg twice daily, crossover was not possible. The primary endpoint was PFS measured by RECIST criteria; Secondary endpoints included PFS by IRC, time to CNS progression, ORR, OS, safety, and tolerability. The results were similar to those of the J-ALEX study. The median PFS for crizotinib was 11.1 months 95% CI (9.1–13.1) and not reached for alectinib 95% CI (17.7 months-NR). After 12 months of treatment 41.4% 95% CI (33.2–49.4) of the crizotinib group and 9.4% 95 CI (5.4–14.7) in the alectinib group showed CNS progression. The objective response rate for crizotinib was 76% 95% CI (68–82) and 83% 95 CI (76–89) for alectinib (Peters et al. 2017). The cumulative incidence of CNS progression after twelve months of treatment was 41.4% (95% CI 33.2–49.4) for crizotinib and 9.4% (95% CI 5.4–14.7) (Fig. 3).

The ALUR trial was a randomized Phase III study that included patients with advanced or metastatic ALK+ NSCLC that progressed after first-line therapy with platin-based chemotherapy or crizotinib. It compared second-line therapy with alectinib to second-line chemotherapy of current standard (pemetrexed 500 mg/m^2 q3w or docetaxel 75 mg/m^2 d3w). The primary endpoint was the investigator assessed PFS.

Median PFS was significantly improved for alectinib with 9.6 months, 95% CI (6.9–12.2) compared to chemotherapy with 1.4 months, 95% CI (1.3–1.6).

The ORR of CNS metastasis was 54.2% for alectinib and 0% for chemotherapy (Wolf et al. 2016).

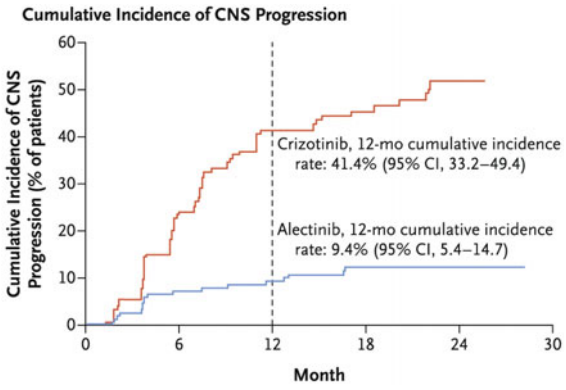

Fig. 3 Aus Peters et al. (2017), NEJM. Cumulative incidence of CNS progression Peters et al. (2017)

5 Toxicity

Alectinib is generally well tolerated and showed less toxicity compared to crizotinib.

In the phase II studies NP28673 (global) and NP28761 (North America), the most common adverse events were constipation, nausea, vomiting and diarrhea, as well as myalgia and peripheral edema, mainly grade 1 or 2. The most common grade 3 or 4 adverse events were elevated liver enzymes in about 3% of the patients, elevated blood bilirubin in 3.2%, and anemia in 2%.

The ALEX Study showed a similar profile of adverse events for alectinib. Compared to crizotinib it has a favorable profile, the most common adverse events were nausea 14%, diarrhea 12%, vomiting 7%, ALT 15% and AST 14%, elevation and peripheral edema 17%. Alectinib showed more adverse events of anemia 20%, increased blood bilirubin 15%, and myalgia 16% compared to crizotinib.

The most common side effects of the phase III studies were fatigue, constipation, edema, myalgia, and anemia. Most of them grade 1 or 2. There was no evidence of grade 3 or more diarrhea, vomiting, peripheral edema, or dysgeusia.

6 Drug Interactions

Alectinib and its main metabolite M4 are metabolized in the liver by cytochrome P 450 (CYP3A). It was shown that multiple enzymes are involved in the metabolism, which is likely to reduce the risk of drug interactions. CYP3A induction with rifampicin and CYP3A inhibition with posaconazole showed minor effects without clinical relevance, furthermore alectinib had no effect on the pharmacokinetic of midazolam, a sensitive substrate of CYP3A so that no dose adjustments are required (Nakagawa et al. 2017; Morcos et al. 2017).

Alectinib and M4 are in vitro inhibitors of the efflux transporters P-gp and BCRP, so that clinical monitoring is recommended when P-gp substrates (i.e., digoxin, dabigatranetexilat, topotecan, sirolimus, everolimus, nilotinib, or lapatinib) or BRCP substrates (i.e., methotrexate, mitoxantron, topotecan, and lapatinib) are taken (Yang et al. 2017).

7 Biomarkers

The efficacy of alectinib was tested in ALK+ NSCLC patients. Patients with newly diagnosed NSCLC need to be tested for ALK, ROS1, and EGFR mutation prior to first-line treatment initiation. The gold standard to detect ALK rearrangement is fluorescence is situ hybridization (FISH). To be classified as ALK+, the rearrangement has to be found in at least 15% of the tumor cells. Immunohistochemistry can also detect overexpression of ALK. Park et al. (2012) could show that

patients IHC is a sensitive and specific as well as cost-effective method, patients with an IHC score of 3 were all FISH positive while a patients with a score 0 were all FISH negative. In the future also, next-generation sequencing will play an important role for detecting ALK rearrangement, especially in patients with high likelihood of kinase alterations that were not found by other methods (Dacic et al. 2016; Peled et al. 2012).

8 Summary and Perspective

Treatment and outcome for NSCLC patients has changed and improved dramatically after the discovery of driver mutations such as ALK, EGFR, and ROS1.

Alectinib has shown promising results in first- and second-line treatment of advanced ALK+ NSCLC. It showed increased response rates as first-line therapy as well as favorable outcome with less toxicity compared to standard chemotherapy in second line after crizotinib failure. Comparable free levels of alectinib in plasma and CSF can explain the efficacy in CNS metastases.

Alectinib is generally well tolerated; the profile of adverse events is favorable compared to chemotherapy or crizotinib.

The main adverse events are grade 1 or 2, while grade 3 or 4 are very rare.

Based on the J-ALEX and ALEX trial, Alectinib will become the new standard for treatment-naive patients with advanced ALK+ NSCLC. The choice of therapy should be based on the presence of resistance mutations, side effects, and tolerability.

Furthermore, alectinib might become the standard in second-line therapy after crizotinib failure, chemotherapy can be postponed, and overall survival is improved.

Future studies will help to investigate the ideal sequence of different TKI, chemotherapy, and immunotherapy in order to optimize patients' outcome and their quality of life. Furthermore ongoing studies are under way, that combine TKI and immunotherapy approaches.

Distinction between disease progression of the primary tumor and progression of single extra-thoracic sites, for example, brain lesions is recommended, as for example stereotactic radiation of CNS lesions might be indicated, while TKI therapy can be continued until systematic progression in order to postpone chemotherapy or a following TKI. In patients with oligo-metastatic disease similar approaches, i.e., surgery or radiotherapy of extra-thoracic progression while continuation of TKI therapy might be rational.

Different mechanisms of resistance are found so far, few patients show initial TKI resistance. More common is the activation of alternative pathways and acquired ALK mutations that lead to changes in the ATP binding pocket. Therefore, frequent re-biopsies in order to detect resistance mutations and patterns of resistance early are essential. In the future liquid biopsies of circulating free tumor DNA might be a minimally invasive procedure that could replace tissue biopsy.

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Osimertinib

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Abstract

Epidermal growth factor receptor (*EGFR*)-mutated (exons 18–21) advanced non-small cell lung cancers (NSCLCs) are generally characterized by exquisite sensitivity to treatment with an EGFR-tyrosine kinase inhibitor (-TKI). First-generation or reversible EGFR-TKIs include gefitinib and erlotinib, while, more recently, second-generation or irreversible EGFR-TKIs have been developed, namely afatinib and dacomitinib, with the aim of overcoming/delaying acquired resistance to treatment. Nevertheless, clinical trials have shown that resistance eventually emerges after a median time of slightly less than one year, regardless of whether first- or second-generation EGFR-TKIs are used. In this context, a secondary *EGFR* mutation in exon 20, namely T790M, has been found to be responsible for approximately 60% of cases of acquired resistance. Alternatively, T790M resistance mutation can be found de novo, in which case it limits the antitumor activity of both first- or second-generation EGFR-TKIs. Osimertinib is an orally bioavailable, third-generation EGFR-TKI that acts by irreversibly binding both *EGFR* activating mutations and T790M, while sparing wild-type *EGFR*. On this basis, osimertinib has proven more efficacious than platinum-based chemotherapy in the setting of *EGFR* T790M-positive NSCLCs pretreated with a first- or second-generation EGFR-TKI. More recently, in another phase 3 trial, osimertinib outperformed gefitinib or erlotinib as first-line treatment of *EGFR*-mutated (ex19del or L858R) advanced NSCLCs, thus emerging as a new standard of care in this setting. In the present review, we will discuss the preclinical and clinical development of osimertinib, briefly touching upon its activity in special populations and biomarkers of sensitivity to treatment.

Keywords

EGFR mutation · EGFR-TKI · Non-small cell lung cancer · Osimertinib · T790M mutation

1 Introduction

Advanced non-small cell lung cancer (NSCLC) represents a heterogeneous disease, for which molecular profiling of the tumor is essential in order to guide optimal decision on systemic treatment (Novello et al. 2016). Currently, the most established therapeutic target is the epidermal growth factor receptor (EGFR), a member of a family of structurally related transmembrane receptors that include also HER2, HER3, and HER4 (Metro and Crinò 2012). Activating somatic gene mutations in the tyrosine kinase (TK) domain of *EGFR* (exons 18–21) has been reported in approximately 50% of Asian patients and 10–15% of Caucasian patients with lung adenocarcinoma, with exon 19 deletion (ex19del) and L858R point mutation (L858R) being the two more commonly identified mutations. Importantly, these activating *EGFR* mutations results into a phenomenon known as "oncogene addiction", which implies dependency on EGFR signaling pathways for cancer growth and survival (Metro and Crinò 2012).

Four EGFR-TK inhibitors (EGFR-TKIs) (gefitinib, erlotinib, afatinib, and icotinib, the latter to be used China only) have consistently demonstrated superior efficacy as compared with platinum-based chemotherapy in phase 3 trials of *EGFR* mutation positive advanced NSCLC, thus emerging as standard first-line treatment in this context (Liang et al. 2014; Shi et al. 2017). Gefitinib and erlotinib were the earliest EGFR-TKIs to be developed for clinical use, and are generally referred to as first-generation or reversible EGFR-TKIs (Metro and Crinò 2017). Afatinib is a second-generation EGFR-TKI, which blocks EGFR in an irreversible manner, also inhibiting other members of the EGFR family (HER2 and HER4), thus acting as a pan-HER inhibitor (Metro and Crinò 2011; Yonesaka et al. 2015). Other second-generation pan-HER EGFR-TKIs include dacomitinib, which has recently terminated its phase 3 stage of clinical development (Wu et al. 2017).

Despite first- or second-generation EGFR-TKIs produce prolonged responses in the majority of *EGFR*-mutated patients, the disease will eventually relapse, usually after a median time of one year (Metro and Crinò 2012). Among the mechanisms that sustain acquired resistance, there is the occurrence of a secondary mutation in exon 20 of *EGFR*, namely T790M. This mutation leads to enhanced affinity for ATP, thus reducing the ability of first-generation EGFR-TKIs such as gefitinib and erlotinib to bind the TK domain of EGFR. Clinically, the T790M-mediated resistance mechanism is not even overcome by second-generation EGFR-TKIs such as afatinib and dacomitinib, despite they have been shown to be active against *EGFR* T790M-positive NSCLC in preclinical models (Dong et al. 2017). In fact, the potent inhibition of wild-type EGFR by these agents prevents the inhibition of *EGFR* T790M-positive NSCLC at clinically achievable doses. Of note, T790M resistance mutation develops in roughly 60% of cases, so that third-generation irreversible EGFR-TKIs have been developed, all being characterized by low selectivity for wild-type EGFR and high potency towards NSCLCs with activating *EGFR* mutations and T790M resistance mutations (Wang et al. 2016). Among them, osimertinib (AZD9291, TAGRISSO™, AstraZeneca) will be discussed in detail, being the topic of this review.

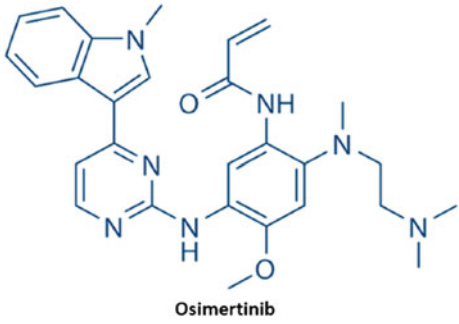

Fig. 1 Chemical structure of osimertinib

2 Structure and Mechanism of Action

Osimertinib mesylate molecular formula is C28H33N7O2·CH4O3S and chemically belongs to mono-anilino-pyrimidine small molecule (Zhang et al. 2016). Its molecular weight is 596 g/mol, and its name is N-(2-{2-dimethylaminoethyl-methylamino}-4-methoxy-5-{[4-(1-methylindol-3-yl)pyrimidin-2yl]amino}phenyl) prop-2-enamidemesylate salt (Fig. 1). Osimertinib's mechanism of action against EGFR is determined by irreversible binding through a covalent bond with the C797 amino acid of the EGFR ATP binding site in the presence of specific *EGFR* alterations (in particular L858R, ex19del, and double mutation which include T790M), at approximately ninefold lower concentrations than wild-type EGFR (Cross et al. 2014; Zhang 2016). As a consequence of EGFR inhibition, different pathways, in particular RAS/RAF/MAPK and PI3 K/AKT, involved in DNA synthesis and proliferation, are inhibited (Zhang 2016). Compared with other third-generation EGFR-TKIs, osimertinib features a unique and characteristic chemical structure (Cross et al. 2014; Santarpia et al. 2017). Moreover, osimertinib has a low activity against other kinases (Cross et al. 2014; Santarpia et al. 2017).

3 Pharmacodynamics and Preclinical Data

Similarly to the first-generation EGFR-TKIs, osimertinib is able to inhibit EGFR phosphorylation in EGFR cell lines harboring activating *EGFR* mutations (Cross et al. 2014; Santarpia et al. 2017). However, different from first-generation EGFR-TKIs, osimertinib induces an important inhibition of the process of phosphorylation of EGFR in T790M mutant cell lines (H1975: L858R/T790M and PC-9VanR: ex19del/T790M) with an increased power (mean IC50 <15 nM) compared with wild-type EGFR (mean IC50: 480–1865 nM). Thus, osimertinib has the capacity to inhibit the phosphorylated forms of EGFR of L858R, ex19del, and double mutants containing T790M to a greater extent than the wild-type form of EGFR (Cross et al. 2014; Santarpia et al. 2017; Zhang 2016). In a preclinical study

on murine models, osimertinib showed two different metabolites: AZ5104 and AZ7550. In particular, AZ7550 highlighted the same characteristic of inhibition on EGFR compared with osimertinib, while AZ5104 had an increased power against the EGFR wild-type form with a minor selectivity (Cross et al. 2014; Santarpia et al. 2017). In preclinical studies, it also showed at the concentration of 1 μM, the capacity to inhibit ACK1, ALK, BLK, BRK, ErbB2, ErbB4, MLK1, and MNK2 while it did not have the ability to act against the IGF-1R and insulin receptor, characterized by a methionine gatekeeper in their kinase domains (Cross et al. 2014; Santarpia et al. 2017). When administered once daily, in xenograft models harboring an activating mutation and T790M in the *EGFR*-TK domain, osimertinib featured an important dose-dependent regression of the tumoral mass (Cross et al. 2014; Santarpia et al. 2017). On the other hand, continuous administration produced a complete response which lasted over time (Cross et al. 2014; Santarpia et al. 2017). In a preclinical study for the evaluation of brain activity of osimertinib, the latter showed at the clinical relevant dose a greater penetration of the mouse blood–brain barrier as compared with other TKIs (Ballard et al. 2016). In another preclinical experience, osimertinib was able to sensitize both ABCB1-transfected and drug-selected cell lines to different drugs, for example colchicine, paclitaxel, and vincristine. This finding indicates the possibility to adopt osimertinib in association with other drugs in ABCB1-positive drug-resistant cancers (Zhang et al. 2016).

4 Pharmacokinetic Profile and Metabolism

In in vivo xenograft models, osimertinib showed good bioavailability, was widely distributed in tissues, and had a moderate clearance resulting in a 3 h half-life, which was similar to the half-life of its active metabolites, AZ7550 and AZ5104 (Cross et al. 2014). Early pharmacokinetic (PK) studies in two patients enrolled in the phase 1 "AURA" trial demonstrated that osimertinib and its active metabolites have a longer half-life of approximately 50 h, which results in a flat PK profile after multiple once-daily dosing (Cross et al. 2014). Following oral administration, osimertinib shows a linear PK profile, with the area under the plasma concentration time curve (AUC), and maximal plasma concentration (Cmax) increasing in a dose proportional manner across the 20–240 mg dose range (Planchard et al. 2016; Tagrisso—European Medicines Agency—Europa EU; TAGRISSO (osimertinib) tablets, for oral use—FDA). At the recommended dose of 80 mg once daily, osimertinib exhibits a threefold increase in accumulation with steady-state exposures achieved after 15 days of dosing, and can be administered regardless of food. Osimertinib is principally degraded in the liver via oxidation (CYP3A) and dealkylation, with AZ7550 and AZ5104 as pharmacologically active metabolites with AUC of approximately 10% of the osimertinib exposure at steady state (Planchard et al. 2016; Tagrisso—European Medicines Agency—Europa EU; TAGRISSO (osimertinib) tablets, for oral use—FDA). Elimination occurs primarily via feces (68%) and in a lower degree by urine. Overall, PKs in healthy

volunteers were similar to those in patients, and did not show clinically significant differences across different variables, including sex, age, ethnicity, smoking status, mild (CLcr 60–89 mL/min) or moderate (CLcr 30–59 mL/min) renal impairment, mild (total bilirubin $\leq$ upper limit of normal (ULN) and AST > ULN or total bilirubin > 1.0–1.5 times ULN and any AST), or moderate hepatic impairment (total bilirubin between 1.5 to 3 times ULN and any AST). The PK profile of osimertinib in patients with end-stage renal disease (CLcr <15 mL/min) or with severe hepatic impairment is unknown (Planchard et al. 2016; Tagrisso—European Medicines Agency—Europa EU; TAGRISSO (osimertinib) tablets, for oral use—FDA).

5 Clinical Data

5.1 Phase 1

Clinical evaluation of osimertinib was initially performed in the "AURA" phase 1/2 study (Table 1) (Jänne et al. 2015). The phase 1 study consisted of an escalation and expansion parts. It included patients who had a known activating *EGFR* mutation or had clinically acquired resistance from treatment with an EGFR-TKI, all having radiologically documented disease progression while still receiving such treatment. In the escalation part, patients received a single dose of osimertinib (in capsule form) followed by a PK evaluation period; after 7 days, they received the same oral dose once daily for the remainder of the study. The five predefined escalating doses of osimertinib were: 20, 40, 80, 160, and 240 mg. In the expansion part, five additional cohorts of patients received osimertinib once daily at each of

Table 1 Activity of osimertinib in *EGFR* T790M-positive advanced non-small cell lung cancers pretreated with a n EGFR-TKI

Variable	"AURA" phase 1	"AURA" extension	"AURA2"	"AURA3"
No. of pts	138	201	210	279
Phase	1	2	2	3
Osimertinib dose (mg)	20–240	80	80	80
Line of treatment	≥ 2	≥ 2	≥ 2	2
ORR (%)	61	62	70	71
DCR (%)	95	90	92	93
DoR	88% of pts had an estimated DoR $\geq$ 6 months	15.2 months	11.4 months	9.7 months
PFS (months)	9.6	12.3	9.9	10.1

DCR disease control rate; *DoR* duration of response; *No*. number; *NR* not reported; *ORR* overall response rate; *PFS* progression-free survival; *pts* patients

the previously mentioned dose. Re-biopsy for central confirmation of T790M mutation was mandatory prior to enrollment in the expansion part. Two-hundred and fifty-three patients were enrolled, 31 in the escalation part and 222 in the expansion part. No dose-limiting toxic effects were seen in the escalation part during the first 28-day evaluation period at any dose level, so that a maximum tolerated dose could not be assessed. Overall, the any grade incidences of adverse events occurring in $\geq 10\%$ of patients were diarrhea (47%), rash (40%), nausea (22%), and decreased appetite (21%). Diarrhea and rash increased in frequency in a dose-dependent manner. Of the 239 patients who could be evaluated for response across all dose levels, the overall response rate (ORR) was 51% (n = 123). Of the 222 patients enrolled in the expansion part, 138 were confirmed as T790M-positive, 62 were T790M-negative, and 22 had unknown T790M status. The ORR was 61% in T790M-positive patients (78/127 evaluable for response), and 21% (13/61 evaluable for response) in those who were T790M-negative. Median progression-free survival (PFS) in the expansion part was 8.2 months, being 9.6 months and 2.8 months in T790M-positive and T790M-negative patients, respectively. Importantly, responses in T790M-positive patients were similar across all dose levels, while there was an increase in diarrhea and rash at the 160 and 240-mg dose levels (any grade diarrhea 68 and 76% for 160 and 240 mg, respectively; any grade rash 63 and 71% for 160 and 240 mg, respectively). On this basis, the 80-mg dose was selected for further clinical testing. A recent update of patients with centrally confirmed T790M-positive NSCLC from the 80 mg daily expansion cohort showed consistent result, with an ORR of 71% (43/61 evaluable for response), and a median PFS of 9.7 months (Yang et al. 2016a). The "AURA" trial enrolled two additional cohorts of *EGFR*-mutated treatment-naïve NSCLCs as well as an extension cohort of T790M-positive patients. The results of both cohorts will be reported in the next paragraph.

5.2 Phase 2

In the phase 2 extension component of "AURA", 201 patients with *EGFR* T790M-positive (as defined according to the results obtained on tumor tissue from a re-biopsy prior to study entry) advanced NSCLC, who had progressed after at least one EGFR-TKI, received oral osimertinib at the recommended dose of 80 mg once daily (Table 1) (Yang et al. 2017a). The primary end-point was ORR by blinded independent central review (BIRC) according to RECIST 1.1. Study results showed an ORR of 62% (122/198 evaluable for response), and a disease control rate of 90% (179/198). The median duration of response was 15.2 months. Tumor shrinkage was seen in 94% of patients, with a mean best percentage change in target lesion size from baseline of −42.7%. Overall, the median PFS by BIRC was 12.3 months, while 1-year overall survival (OS) rate was 79%.

"AURA2" was a multicentre, open-label, single-arm, phase 2 study in which patients with *EGFR* T790M-positive (as defined according to the results obtained on tumor tissue from a re-biopsy prior to study entry) advanced NSCLC who had

progressed after an EGFR-TKI were treated with osimertinib 80 mg once daily (Table 1) (Goss et al. 2016). The primary end-point was ORR by BIRC according to RECIST 1.1. Two-hundred and ten patients were enrolled in approximately 5 months, all of whom received at least one dose of osimertinib. The ORR was 70% (140/199 evaluable for response), with a disease control rate of 92% (182/199). The median duration of response was 11.4 months, while tumor shrinkage was seen in 94% of patients, the mean best percentage change in target lesion size from baseline being −52%. Overall, at a median follow-up of 13 months (7.6–14.2), the median PFS by BIRC was 9.9 months, while 1-year OS was 81%. Importantly, the activity of osimertinib reported in "AURA2" resembled pretty much of that observed in the phase 2 extension component of "AURA".

On the basis of similar inclusion criteria (progression on a prior EGFR-TKI, central confirmation of T790 M mutation on tumor tissue prior to study entry, no restriction based on the number of previous lines of therapy), a pooled analysis gathered together the two populations from "AURA2" and from the phase 2 extension cohort of "AURA", for a target population of 411 patients (Yang et al. 2016a). The ORR by BIRC was 66% (262/397 evaluable for response), with a median duration of response was 12.5 months. Overall, the median PFS was 11.0 months, with 48% of patients being progression free at 12 months.

The "AURA" study also included two expansion parts investigating two doses of osimertinib, either 80 mg or 160 mg once daily, in untreated patients with *EGFR*-mutated advanced NSCLC (Ramalingam et al. 2018). Thirty patients each received one of the two doses of osimertinib with the following outcomes for 80 and 160 mg: ORR was 67 and 87%, disease control rate 93 and 100%, median duration of response 19.3 and 16.7 months, and median PFS was 22.1 and 19.3 months, respectively. These impressive results suggested that osimertinib is far more efficacious when used upfront rather than after progression on an EGFR-TKI.

5.3 Phase 3

Osimertinib has been tested in two randomized phase 3 trials. "AURA3" was a study in which 419 patients with *EGFR* T790M-positive (as defined according to the results obtained on tumor tissue from a re-biopsy prior to study entry) advanced NSCLC who had progressed after a first-line EGFR-TKI (gefitinib, erlotinib, or afatinib) were allocated in a 2:1 ratio to either osimertinib (n = 279) or standard platinum-pemetrexed chemotherapy (n = 140) with maintenance pemetrexed allowed (Table 1) (Mok et al. 2017a). Demographic and clinical characteristics were well balanced between the two arms, with 33 and 36% of patients having central nervous system (CNS) metastases at study entry in the osimertinib and platinum-pemetrexed groups, respectively. Importantly, the primary end-point of the study, which was investigator-assessed PFS according to RECIST 1.1, was met, being significantly superior for osimertinib as compared with chemotherapy (10.1 vs. 4.4 months, respectively; HR = 0.30; 95% CI 0.23–0.41; $P < 0.001$). Of note,

the HR for PFS favored osimertinib across all predefined subgroups that were assessed (Asian vs. non-Asian, smokers vs. never smokers, ex19del vs. L858R, the presence vs. absence of CNS metastases). Among key secondary end-points, investigator-assessed ORR significantly favored osimertinib as compared with chemotherapy (71 vs. 31%, respectively; $P < 0.001$). Consistently, also patients reported outcomes were far better in the osimertinib group than in the platinum-pemetrexed group across five prespecified symptoms ($P = 0.001$ each for appetite loss, cough, chest pain, dyspnea, and fatigue) during the overall period from randomization until 6 months. Overall survival data were not mature after a median follow-up of 8.3 months. However, it should be pointed out that as much as 60% of the patients who were treated with platinum-pemetrexed crossed over to receive osimertinib, which will likely represent a bias when interpreting final OS results.

The second phase 3 trial was the "FLAURA" study, in which osimertinib was compared with a first-line EGFR-TKI (gefitinib or erlotinib) in patients with previously untreated, *EGFR*-mutated (ex19del or L858R), advanced NSCLC, with investigator-assessed PFS according to RECIST 1.1 being the primary end-point (Soria et al. 2018). Five hundred and fifty-six patients were randomized in a 1:1 ratio to trial treatments (279 to osimertinib and 277 to standard EGFR-TKI) over a period of slightly more than 1 year. Overall, no significant differences in terms of baseline characteristics were noted between the two trial groups. Similarly to "AURA3" a relevant proportion of patients had CNS metastases prior to treatment, namely 19% (53/279) and 23% (63/277) of patients in the osimertinib and standard EGFR-TKI groups, respectively. At a median follow-up of 15.0 months (0–25.1) in the osimertinib group, and 9.7 months (0–26.1) in the standard EGFR-TKI group, the investigator-assessed median PFS was 18.9 months for osimertinib vs. 10.2 months for standard EGFR-TKI, which was highly statistically significant (HR = 0.46; 95% CI 0.37–0.57; $P < 0.001$). A similar benefit for PFS was noted in the assessment by BIRC (17.7 vs. 9.7 months, respectively; HR = 0.45; 95% CI 0.36–0.57; $P < 0.001$). Importantly, PFS was consistently improved in all predefined subgroups, including race (Asian vs. non-Asian), *EGFR* mutation type (ex19del vs. L858R), and CNS involvement (present vs. absent). Among secondary end-points, a superimposable ORR as assessed by the investigator was observed (80 vs. 76%, respectively; $P = 0.24$), while a statistically significant improvement in favor of osimertinib was reported for median best percentage change in target lesion size (−54.7 vs. −48.5%, respectively; $P = 0.003$) and median duration of response (17.2 months (95% CI, 13.8–22.0) vs. 8.5 months (95% CI, 7.3–9.8), respectively). Data on OS were immature at the interim analysis (25% of maturity). However, the survival rate at 18 months favored osimertinib (83 vs. 71%, respectively; $P = 0.007$), despite the fact that a lower proportion of patients randomized in the osimertinib arm received a first post-treatment anticancer therapy upon discontinuation of trial treatment as compared with patients allocated in the standard EGFR-TKI group (29 vs. 47%, respectively).

5.4 Special Populations

5.4.1 Patients with CNS and Leptomeningeal Metastases

The CNS is a common site of progression in *EGFR*-mutated advanced NSCLC patients pretreated with a first-line EGFR-TKI, regardless of whether brain metastases are present prior to treatment (Metro et al. 2015). This phenomenon likely reflects the longer survival obtained by these patients, being particularly frequent in case of prolonged clinical benefit during treatment with an EGFR-TKI. However, CNS progression occurs despite the fact that first- and second-generation EGFR-TKIs have demonstrated to be active against brain metastases from *EGFR*-mutated NSCLC, with intracranial responses occurring in up 80% of patients treated with upfront gefitinib or erlotinib (Jamal-Hanjani and Spicer 2012). Nevertheless, more than 30% of patients who experience disease progression during treatment with first-line EGFR-TKI have CNS progression (Heon et al. 2010). In this context, the use of a drug such as osimertinib is very attractive, as it showed in a preclinical study greater penetration of the mouse blood–brain barrier as compared with gefitinib, rociletinib (another third-generation EGFR-TKI), or afatinib, and at clinically relevant doses induced a sustained tumor regression in an *EGFR*-mutant PC9 mouse model with brain metastases (Ballard et al. 2016).

A relevant activity against CNS metastases was initially observed in *EGFR* T790M-positive patients pretreated with an EGFR-TKI. A pooled analysis of the phase 2 extension component of "AURA" and "AURA2" trials was conducted in order to assess CNS response to osimertinib, as both studies allowed patients with stable, asymptomatic CNS metastases who were off steroids for at least 4 weeks before the first dose of osimertinib (Goss et al. 2018). Fifty evaluable patients with ≥ 1 measurable lesion in the CNS were identified by BIRC according to RECIST 1.1. The CNS ORR was 54%, with 12% of complete CNS response, and a CNS disease control rate of 92%.

The phase 3 "AURA3" trial allowed patients with stable, asymptomatic brain metastases off steroids for at least 4 weeks (Mok et al. 2017b). The investigator-assessed PFS showed a similar benefit in patients with (HR = 0.32) or without (HR = 0.40) CNS metastases. A subgroup analysis was conducted in patients with baseline CNS metastases as assessed by BIRC to define efficacy outcomes according to RECIST 1.1. The CNS full analysis set comprised patients with ≥ 1 measurable and/or nonmeasurable CNS disease, being 27% in the osimertinib (75/279) and 29% in the chemotherapy arms (41/140), respectively, while the CNS evaluable for response set included only patients with ≥ 1 measurable CNS metastases, which were 11% each in the osimertinib (30/279) and chemotherapy arms (16/140), respectively. The results significantly favored osimertinib in terms of both CNS PFS (11.7 vs. 5.6 months, respectively) and CNS ORR (70 vs. 31%, respectively). Interestingly, seven patients in the osimertinib group were identified as having leptomeningeal metastases, and four of them experienced a response to treatment (two complete responses and two partial responses) as per RANO-leptomeningeal metastases criteria. Although these data

are retrospective and obtained on a small sample size, they reinforce the superiority of osimertinib in the setting of *EGFR* T790M-positive advanced NSCLC pretreated with a first-line EGFR-TKI as compared with platinum-based chemotherapy.

Data on the CNS activity of osimertinib in untreated, *EGFR*-mutated, advanced NSCLC were retrospectively retrieved from the phase 3 "FLAURA" trial, as this study allowed patients with asymptomatic or symptomatic brain metastases stable for at least 4 weeks and off steroids (Soria et al. 2018). Patients who had CNS involvement at baseline benefitted from osimertinib in terms of investigator-assessed PFS to a similar extent (HR = 0.47) than patients without CNS metastases (HR = 0.46). Also, CNS progression was less frequent in the osimertinib group as compared with platinum-pemetrexed regardless of whether brain metastases were present at baseline (19 vs. 43%, respectively, in patients with CNS metastases and 3 vs. 7%, respectively, in patients without CNS metastases). Importantly, although, these data suggest that osimertinib is active in the CNS also in untreated patients, it should be noted that baseline brain imaging was mandated only in patients with known or suspected CNS metastases, with follow-up imaging only in patients with confirmed CNS metastases.

The ongoing phase 1 "BLOOM" is testing osimertinib at a dose of 160 mg q.d. in *EGFR* mutation positive patients with leptomeningeal carcinomatosis by positive cerebrospinal fluid (CSF) cytology after exposure to an EGFR-TKI (Yang et al. 2016b, 2017b).Two cohorts were included based on the presence of T790M mutation. Preliminary data from the 21 patients who were not selected for T790M showed a 100% clearance of tumor cells from CSF in 6 patients, thus suggesting a potential role for osimertinib in the treatment of this poor prognosis population (Yang et al. 2017b). Also, osimertinib demonstrated a good CSF penetration rate in this cohort, with a CSF-to-plasma ratio of 2.3%.

Additional clinical cases have confirmed a significant activity of osimertinib in patients with *EGFR* T790M-positive advanced NSCLC and CNS involvement (Koba et al. 2017; Reichegger et al. 2016; Ricciuti et al. 2016; Uemura et al. 2017). Overall, they reinforce the notion that osimertinib is clinically active against CNS disease. In addition, they suggest that osimertinib could delay the use of brain radiotherapy in a *EGFR* T790M-positive patient with asymptomatic CNS progression on first-line EGFR-TKI, with a potential advantage in terms of long-term cognitive function (Ricciuti et al. 2016).

5.4.2 Elderly and Poor Performance Status Patients

Results from both prospective trials and retrospective studies have documented that elderly patients with *EGFR*-mutated advanced NSCLC treated with a first- or second-generation EGFR-TKI such as gefitinib, erlotinib, and afatinib benefit from treatment to a similar extent than the overall population (Losanno and Gridelli 2017). However, data on the efficacy of osimertinib in the elderly population are lacking. Nevertheless, subgroup analyses from both "AURA" and "FLAURA" phase 3 trials showed a similarly favorable HR in the osimertinib-treated patients regardless of age (<65 years or $\geq$ 65 years) (Mok et al. 2017a; Soria et al. 2018).

In addition, the same trials reported low rates of adverse events of grade 3 or higher, which suggests that osimertinib is a valuable treatment option also for elderly patients.

On the other hand, "real-life" data on the efficacy of osimertinib outside clinical trials are very limited, as phase 2 and registrative trials included only patients in good clinical conditions, namely with performance status (PS) of 0 or 1 (Mok et al. 2017a; Soria et al. 2018). Therefore, the safety profile and the activity of osimertinib in patients with PS $\geq$ 2 still need to be addressed. Sonoda et al. have recently conducted a retrospective analysis on 30 patients treated in a "real-life" scenario. Nine out of 30 patients matched the AURA3 eligibility criteria (PS $\leq$ 1 and one prior EGFR-TKI) while 21 patients did not (PS $\geq$ 2 and/or two or more prior EGFR-TKIs and/or symptomatic CNS metastases) (Sonoda et al. 2017). The ORR with osimertinib was 78 and 67% for the matched and unmatched cohorts, with a similar safety profile between the two groups. "ASTRIS" was a single-arm "real-life" study evaluating the efficacy and safety of osimertinib in patients with *EGFR* T790M-positive advanced NSCLC pretreated with an EGFR-TKI. Out of 1217 patients enrolled, 86% had a PS 0–1, with the remaining 14% having PS 2 (De Marinis et al. 2017). Preliminary results from the 886 patients who were evaluable for response showed an ORR of 64%. Overall, only 4% of patients had an adverse event leading to treatment discontinuation.

5.4.3 Patients with EGFR Rare Mutations

EGFR non-T790M rare mutations include mutations other than ex19del and L858R, and consist of approximately 15% of all *EGFR* mutations involving exon 18–21, often being found as compound mutations (Fig. 2) (Kobayashi and Mitsudomi 2016). Among them, the most frequently reported are ins20, G719X, S768I, and L861Q. Owing to their rarity, data on clinical the activity of first- or second-generation EGFR-TKIs in this context are mostly retrospective. Nevertheless, EGFR-TKIs have demonstrated antitumor activity in tumors bearing any of the

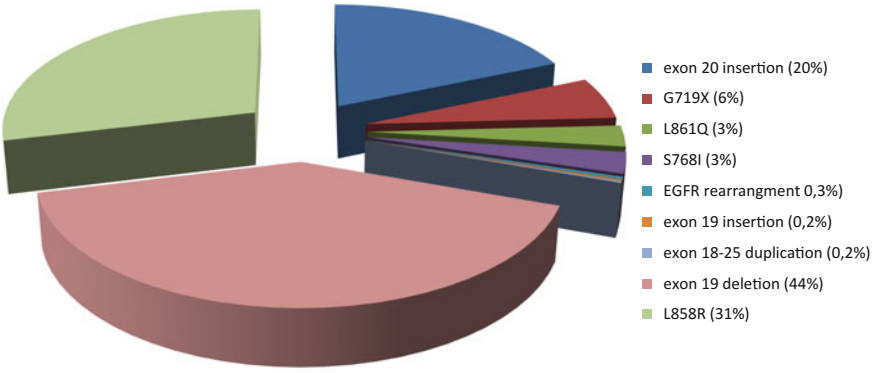

Fig. 2 Graphical representation of *EGFR* genomic aberrations and each corresponding percentage

aforementioned *EGFR* rare mutation, the lowest activity being reported in patients with ins20 mutation. On the other hand, relatively higher ORR for afatinib compared with first-generation EGFR-TKIs was observed in tumors bearing G719X, S768I, and L861Q mutations (Kobayashi and Mitsudomi 2016). In addition, cases with de novo T790M mutation seem to respond poorly to both first- and second-generation EGFR-TKIs.

Data on the activity of osimertinib against *EGFR* rare mutations are scarce, as the "FLAURA" study enrolled only *EGFR* mutation positive patients with ex19del or L858R (Mok et al. 2017a). On the other hand, only five of the untreated patients enrolled in the dose expansion cohorts of "AURA" trial had rare mutations ($n = 3$ G719X, $n = 1$ G719X/S768I, $n = 1$ L861Q), for which a poorer outcome was observed, the median PFS being 8.3 months (Ramalingam et al. 2018). Therefore, more data are needed in order to define the activity of osimertinib in patients with *EGFR* rare mutations. In the same dose expansion cohort of "AURA" trial, there were also seven patients who had a de novo T790 M mutation (coexistent with L858R in all cases), for which a response of 85.7% was reported (6/7) (Ramalingam et al. 2018). However, based on its mechanism of action, it can be anticipated that osimertinib is highly active in untreated patients with de novo *EGFR* 790M mutation.

6 Toxicity

Osimertinib showed a very manageable safety profile across phase 1/2, 2, and 3 trials in patients with *EGFR*-mutated advanced NSCLC with or without T790M-positive NSCLC (Jänne et al. 2015; Goss et al. 2016; Yang et al. 2016a, 2017a; Mok et al. 2017a; Soria et al. 2018). Table 2 lists the most common $\geq$ grade 3 adverse events (AEs) occurring in the "AURA" and "FLAURA" trials. In the phase 1 study, no dose-limiting toxicities were observed across the 20–240-mg dose range (Jänne et al. 2015). The most commonly reported any grade AEs were diarrhea (47%), rash (40%), nausea (22%), and decreased appetite (21%). Worthy of note, diarrhea and rash increased in frequency and severity in a dose-dependent manner at 160 and 240-mg doses, likely because of the inhibition of wild-type *EGFR*. Any grade $\geq$ 3 AEs occurred in 32% of the patients, and dose reduction or drug discontinuation were observed in 7 and 6% of cases, respectively. Serious AEs judged by investigators to be possibly treatment-related were reported in 6% of patients. It should be highlighted that 6 patients (2%) developed pneumonitis-like events. All of them discontinued osimertinib and at the study cutoff data these events had resolved or were resolving. QTc prolongation was observed in 4.5%, while 6.5% of patients developed hyperglycemia, though it did not lead to dose reduction or discontinuation (Jänne et al. 2015).

Recent data from the extension cohort of the phase 1/2 "AURA" study confirmed a favorable safety profile of osimertinib, with most AEs being grade 1 or 2 in severity, determining discontinuation of treatment only in 3% of cases (Yang et al. 2017a).

Table 2 Adverse events ≥ grade 3 (%) across the AURA and FLAURA trials

Adverse events	AURA phase 1	AURA phase 2 extension cohort	AURA 2	AURA 3	FLAURA
Number of patients enrolled	253	201	210	279	279
Diarrhea	2	1	1	1	2
Skin rash	1	1	1	1	1
Decreased appetite	1	1	0	1	3
Fatigue	1	0	0	1	1
Anemia	2	0	1	1	1
Thrombocytopenia	0	1	1	1	0
Dyspnea	2	0	0	1	1
ILD	2	4	2	4	2
Discontinuation due to AEs	6	3	5	7	13
Number of toxic deaths	1	3	7	4	2
Reference	Jänne et al. (2015)	Yang et al. (2017a, b)	Goss et al. (2016)	Mok et al. (2017a, b)	Soria et al. (2018)

In "AURA2", osimertinib confirmed a manageable safety profile (Goss et al. 2016). Among 210 patients with *EGFR* T790M-positive NSCLC, 207 reported at least one AE, with 179 (85%) reporting a possibly treatment-related AE as assessed by investigators. Nonetheless, only 3% of patients had the dose reduced, and 5% of them discontinued osimertinib because of adverse events. The most common grade ≥ 3 AEs included pulmonary embolism (3%), prolonged QTc (2%), neutropenia (2%), anemia, dyspnoea, hyponatraemia, increased alanine aminotransferase, and thrombocytopenia (1% each). Investigator-assessed treatment-related serious AEs were reported in 5% of patients (interstitial lung disease (1%), and lung infection, thrombocytopenia, dehydration, cerebral infarction, pleurisy, pneumonitis, pulmonary embolism, drug-induced liver injury, jaundice, and pyrexia (<1% each)). Overall, 3.5% of deaths occurred due to adverse events. However, the only fatal event assessed as possibly osimertinib-related by the investigator was a case of interstitial lung disease (Goss et al. 2016).

The osimertinib phase 3 trials confirmed an acceptable safety profile of this drug (Mok et al. 2016; Soria et al. 2018). In "AURA3", the most commonly reported any grade AEs were diarrhea (41%), rash (34%), dry skin (23%) and paronychia (22%). The corresponding rates in "FLAURA" were 58, 58, 36, and, 35%, respectively (Soria et al. 2018). A high rate of stomatitis (29%), and decreased appetite were also seen in "FLAURA".

Finally, in the real-world "ASTRIS" study, the preliminary safety data showed the discontinuation of osimertinib in 4% of patients, with AEs leading to death occurring in 2% of cases. Interstitial lung disease was reported in 25 patients (2%), and QTc prolongation in 9 patients (1%) (De Marinis et al. 2017). In summary,

osimertinib is well tolerated and seems to have a better safety profile if compared with first- and second-generation EGFR-TKIs. However, real-life data and long-term follow-up from clinical trials are required for an accurate assessment of its safety and delayed toxicities.

7 Drug Interactions

Osimertinib is primarily metabolized in the liver by CYP3A4 and CYP3A5, and in vitro studies have demonstrated that osimertinib is a competitive inhibitor of CYP3A (Tagrisso—European Medicines Agency—Europa EU; TAGRISSO (osimertinib) tablets, for oral use—FDA). Accordingly, strong CYP3A inducers (e.g., rifampin, phenytoin, carbamazepine) are expected to decrease the exposure of osimertinib, thus leading to reduced efficacy. On this basis, if concurrent administration of strong CYP3A is unavoidable, the dose od osimertinib should be increased to 160 mg once daily (TAGRISSO (osimertinib) tablets, for oral use—FDA). In vitro studies have also shown that osimertinib is a substrate of breast cancer resistant protein (BCRP), and coadministration of osimertinib with another BCRP substrate (e.g., rosuvastatin) leads to augmented risk of exposure-related toxicities (Tagrisso—European Medicines Agency—Europa EU; TAGRISSO (osimertinib) tablets, for oral use—FDA). Finally, the exposure of osimertinib is not affected by concurrent administration of gastric acid reducing agents (Vishwanathan et al. 2018).

8 Biomarkers

EGFR features different types of mutations, the most common are deletions/indels of exon 19 (45%, and the most common is represented by the delE746_A750), the exon 21 point mutation L858R mutation (35%), and mutations of exon 20 (7%, in particular in-frame insertions and indels) (Fig. 2) (Costa 2016). Instead, after treatment with first- or second-generation EGFR-TKIs, the most common mutation is T790M in exon 20 (Costa 2016). Osimertinib has showed activity against different kinases. In particular, it has shown the capacity to inhibit the phosphorylated forms of EGFR of L858R, ex19del, and double mutants containing T790M as compared with EGFR wild-type forms. Moreover, this molecule had the ability to inhibit also ACK1, ALK, BLK, BRK, ErbB2, ErbB4, MLK1 and MNK2 (Cross et al. 2014; Santarpia et al. 2017). To date, another diagnostic possibility is represented by the ability to analyze *EGFR* mutations for the administration of different EGFR-TKIs in patients without tissue availability at first diagnosis (for *EGFR* activating mutations) and at progression (for the identification of T790M resistance mutation) on cell-free DNA (cfDNA) extracted from liquid biopsy samples and, in particular, from plasma (Malapelle et al. 2016; Pisapia et al. 2017).

In a proof of concept study, Malapelle et al. showed a high sensitivity (90.5%) and analytical specificity (100%) on cfDNA by adopting a next generation sequencing narrow panel that analyze 568 mutations in six genes (*EGFR, KRAS, NRAS, BRAF, cKIT* and *PDGFRα*) (Malapelle et al. 2017).

9 Summary and Perspectives

Osimertinib has led to a paradigm shift in *EGFR*-mutated advanced NSCLCs, as it proved superior to platinum-based chemotherapy in the setting of *EGFR* T790M-positive disease upon progression on first- or second-generation EGFR-TKIs (Mok et al. 2017a). In this context, approximately 60% of patients are candidate to osimertinib based on the presence of T790M in either plasma or tissue. Importantly, retrospective data from the "AURA" trial suggest that patients positive for T790M in plasma derive similar benefit from osimertinib as those who are T790M in tissue (Oxnard et al. 2016). In addition, owing to the relatively low sensitivity for the methods of detection of T790M in plasma, tumor biopsy could still be needed for patients with T790M-negative results in plasma. On this basis, guidelines have been developed for the detection of T790M, which suggest the possibility to assess T790M mutation in plasma first, while reserving tissue biopsy for patients who test negative for T790M in plasma.

More recently, osimertinib has demonstrated to be superior over a first-generation EGFR-TKI as upfront treatment of *EGFR*-mutated NSCLC, thus potentially establishing a new first-line treatment option in this setting (Soria et al. 2018). In fact, first-line osimertinib could allow access to this drug to all *EGFR*-mutated patients, not only the approximately half of them who develop acquired resistance mediated by T790M on progression on a first- or second-generation EGFR-TKI.

Osimertinib has also shown outstanding activity against CNS metastases from *EGFR*-mutated NSCLC, including leptomeningeal metastases (Yang et al. 2016b, 2017b). This ability renders osimertinib an important treatment strategy capable of delaying or even sparing whole brain radiotherapy, which could have a negative impact on cognitive function and quality of life.

Given the above mentioned achievements of osimertinib, several other third-generation EGFR-TKIs are in clinical development (Santarpia et al. 2017). However, a number of issues still need to be answered with regard to osimertinib. First, whether osimertinib could have a role in the adjuvant setting for *EGFR*-mutated patients who receive surgery for early stage disease. With regard to this, the "ADAURA" trial is currently randomizing patients with pathological stage Ib→IIIA to osimertinib or placebo for 2 years with standard adjuvant platinum-based chemotherapy allowed (Tazza and Metro 2017). Second, osimertinib combination strategies hold promise in the treatment of *EGFR*-mutated advanced NSCLC. Among others, the appealing combination of osimertinib with the anti-VEGF monoclonal antibody bevacizumab is being tested in clinical studies

in either first- or second-line setting (NCT02803203, NCT03133546). On the other hand, although very attractive, the combination of osimertinib with immune checkpoints inhibitors has raised safety concerns based on the preliminary results of the phase 1b "TATOON" trial. This is an ongoing multi-arm study that evaluates different schedules of osimertinib in combination with other investigational agents, which revealed an unexpectedly high rate of interstitial lung disease events for the osimertinib/durvalumab combination (Ahn et al. 2016). Finally, a challenge ahead consists on what treatment should be offered in patients who progress on osimertinib. Recent evidence suggests that one mechanism of resistance to osimertinib is the acquisition of a tertiary *EGFR* mutation, namely C797S. In a study by Thress et al. (2015), *EGFR* C797S mutation was detected in 6 of 15 patients with osimertinib-resistant T790M-positive tumors. However, despite other less common mechanism of resistance to osimertinib have been identified (i.e., *EGFR* L718Q mutation, *BRAF* mutation, small-cell transformation, and *HER2* or *MET* amplification), the cause of resistance to osimertinib in many cases remains unknown (Bersanelli et al. 2016; Ho et al. 2017; Li et al. 2017; Planchard et al. 2015). Therefore, a number of other resistance mechanisms, possibly independent of *EGFR*, remain to be identified. Clearly, the detection of new mechanisms of resistance through either liquid biopsy techniques or tumor re-biopsy will give an insight into the dynamic evolution of oncogene-addicted tumor cells during treatment with osimertinib, thus helping establish novel, potentially targetable molecular alterations.

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