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Exploiting Kinase Inhibitors for Cancer Treatment: An Overview of Clinical Results and Outlook

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Abstract Mutated or dysregulated protein kinases represent major oncogenic drivers in cancer. Due to the general druggability of these potential oncoproteins, protein kinases have been regarded the most significant drug targets in cancer cells for the past three decades. Starting with the approval of imatinib for targeting BCR-ABL in leukemia positive for Philadelphia chromosome, a multitude of different kinase inhibitors have been developed and approved for the market so far. Additionally, many new compounds with increased efficacy and target specificity are under development and clinical testing. While several of these compounds allow for an efficient temporary treatment success in different tumor entities, long-term cancer control is often limited due to the development of therapy resistance. Thus, overcoming drug resistance in tumors represents a major challenge for successful cancer therapies in the future.

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Keywords Cancer therapeutics, Kinase inhibitors, Protein kinases, Therapy resistance

Abbreviations

ALL	Acute lymphoblastic leukemia
CEL	Chronic eosinophilic leukemia
CLL	Chronic lymphoblastic leukemia
CML	Chronic myeloid leukemia
CNS	Central nervous system
CRC	Colorectal cancer
DFSP	Dermatofibrosarcoma protuberans
ER	Estrogen receptor
FDA	Food and Drug Administration
GI	Gastrointestinal
GIST	Gastrointestinal stromal tumor
HCC	Hepatocellular carcinoma
HES	Hypereosinophilic syndrome
MDS/MDP	Myelodysplastic/myeloproliferative diseases
NRY	Non-receptor protein-tyrosine kinase
NSCLC	Non-small cell lung carcinoma
PDAC	Pancreatic ductal adenocarcinoma
PH	Philadelphia chromosome
PNET	Primitive neuroectodermal tumor
RCC	Renal cell carcinoma
RY	Receptor protein-tyrosine kinase
S/T	Protein-serine/threonine protein kinase
SEGA	Subependymal giant cell astrocytoma
shRNA	Short hairpin RNA
T/Y	Threonine/tyrosine dual specificity protein kinase

1 Introduction

Over the past 20 years, research revealed that many diseases emerge from impairments in signal transduction. This insight has been used by scientists to unravel molecular mechanisms that drive complex diseases such as solid tumors, leukemias, systemic autoimmune diseases, and inflammatory diseases. Hence, biologists, chemists, physicians, and pharmacologists have focused their clinical research toward development of specific molecules targeting key signaling cascades of these diseases. To this point, most molecules aiming to this direction of treatment represented protein kinase inhibitors. Kinases are proteins that play a critical role in cellular signal transduction by phosphorylating downstream targets. Because dysregulation and mutations of protein kinases play major roles in human diseases, this family of

enzymes has become one of the most significant drug targets over the past three decades. It all started in 1978, when the protein kinase c-SRC was found to share high similarity to a protein from sarcoma virus and act as an oncogene [1]. In addition, studies in the early 1980s pointed out that hyperactivation of a protein kinase (protein kinase C) represents a key mechanism for tumor promotion [2]. The idea to target this group of enzymes therapeutically was also fueled by findings showing that naphthalene-sulphonamides were able to block kinases [3]. These molecules were used as a starting point to further synthetize drugs that inhibit protein kinases.

One of the key experiments for the development of kinase inhibitors was the crystallization of protein kinase A in 1991. Susan Taylor and colleagues revealed the structure of the kinase core for the very first time, giving insight into a key element of all kinases in the genome. This study demonstrated that residues involved in the binding of ATP were conserved among kinases [4, 5]. The crystal structure of PKA gave valuable information for the structural function of these enzymes. However, since core domains of kinases are highly conserved, the idea of selective inhibition of a protein kinase was also considered to be a major challenge.

Starting from the late 1980s, molecules targeting more than one kinase with different efficacies were developed. Some years before that, the only purely isolated tyrosine protein kinases were epidermal growth factor receptor (EGFR) and insulin receptor. The new molecules were 1,000-fold more potent against EGFR than against insulin receptor kinase. Interestingly, these drugs were found to be inactive against serine/threonine kinases [6]. Based on this evidence, scientists could then develop more inhibitors against these kinases that show structure/activity relationships.

Later on, new findings strengthened the idea of targeted kinase drug development. In particular, ATP mimics were found to selectively inhibit platelet-derived growth factor receptor (PDGFR), while they were not potent against other protein kinases. In addition, a study in the mid-1990s showed that quinoxalines are potent inhibitors of PDGFR though not able to interact with EGFR. Accordingly, quinazolines showed the opposite effect [7, 8]. Based on this finding, years later, Zeneca developed the inhibitor gefitinib that targets EGFR. Since 1988, when the first study showing targeted inhibition of the catalytic activity of EGFR was published, the number of protein kinase inhibitor agents developed climbed steadily. It is interesting to note that although EGFR and receptor tyrosine-protein kinase erbB-2 (HER2) share high homology, scientists were able to develop selective molecules against these targets with low cross-reaction already since 1993 [9].

A breakthrough was the first approval of a protein kinase inhibitor by the FDA (2001). This molecule was imatinib, firstly developed by Zeneca as a PDGFR inhibitor. Interestingly, it was later shown that the drug had also high efficacy against BCR-ABL, making it suitable for treatment of chronic myelogenous leukemia (CML) and acute lymphocytic leukemia (ALL) patients positive for Philadelphia chromosome [10, 11]. Since 2001, 48 kinase inhibitors have been approved to the market (Table 1) [12]. The vast majority are drugs against tyrosine protein kinases and receptors for the treatment of cancer. Only a few, ten of them, target serine/threonine kinases. The main difficulty of developing selective agents against serine/threonine kinases is the high similarity of the ATP-binding domain of these

Table 1 Approved protein kinase inhibitors (adapted from http://www.brimr.org/PKI/PKIs.htm)

Drug	Company	Known targets	Class	Disease	Year approved
Abemaciclib	Lilly	CDK4/6	S/T	Breast cancer	2017
Acalabrutinib	Acerta Pharma	BTK	NRY	Mantle cell lymphoma	2017
Afatinib	Boehringer Ingelheim	EGFR, ErbB2, ErbB4	RY	NSCLC	2013
Alectinib	Hoffmann-La Roche	ALK, RET	RY	NSCLC (ALK^+)	2015
Axitinib	Pfizer	VEGFR1/2/3, PDGFRβ	RY	RCC	2012
Baricitinib	Lilly	JAK1/2	NRY	Rheumatoid arthritis	2018
Binimetinib	Array	MEK1/2	S/T	Melanoma	2018
Bosutinib	Pfizer	BCR-Abl, SRC, LYN, and HCK	NRY	CML	2012
Brigatinib	Ariad	ALK, ROS1, IGF-1R, Flt3, EGFR	RY	NSCLC (ALK^+)	2017
Cabozantinib	Exelixis	RET, MET, VEGFR1/2/3, KIT, TrkB, FLT3, AXL, TIE2	RY	RCC, HCC, medullary thyroid cancer	2012
Ceritinib	Novartis	ALK, IGF-1R, InsR, ROS1	RY	NSCLC, ALK^+ after crizotinib resistance	2014
Cobimetinib	Genentech	MEK1/2	T/Y	Melanoma with BRAF mutations together with vemurafenib	2015
Crizotinib	Pfizer	ALK, MET (HGFR), ROS1, MST1R	RY	NSCLC (ALK^+ or $ROS1^+$)	2011
Dabrafenib	GSK	BRAF, $BRAF^{V600E}$, CRAF	S/T	Melanoma, NSCLC ($BRAF^{V600+}$)	2013
Dacomitinib	Pfizer	EGFR family	RY	EGFR-mutant NSCLC	2018
Dasatinib	Bristol-Myers Squibb	BCR-ABL, EGFR, SRC, LCK, YES, FYN, KIT, EphA2, PDGFRβ	NRY	CML	2006
Encorafenib	Array	BRAF	S/T	Melanoma	2018
Erlotinib	Genentech	EGFR	RY	NSCLC, pancreatic cancer	2004
Everolimus	Novartis	FKBP12/mTOR	S/T	Breast cancer ($HER2^-$), PNET, RCC, angiomyolipoma, SEGA	2009
Fostamatinib	Rigel	SYK, spleen tyrosine kinase	RY	Thrombocytopenia	2018

Gefitinib	AstraZeneca	EGFR	RY	NSCLC	2003–2005, 2015
Gilteritinib	Astellas	FLT3	RY	AML	2018
Ibrutinib	Pharmacyclics and J&J	Bruton tyrosine kinase	NRY	Mantle cell lymphoma, CLL, lymphoplasmacytic lymphoma, marginal zone B-cell lymphoma	2013
Imatinib	Novartis	BCR-ABL, KIT, PDGFR	NRY	CML, ALL (Ph^+), aggressive systemic mastocytosis, CEL, DFSP, HES, GIST, MDS/MDP	2001
Lapatinib	GSK	EGFR, ErbB2	RY	Breast cancer	2007
Larotrectinib	Bayer	TRK	RY	Solid tumors with NTRK gene fusion proteins	2018
Lenvatinib	Eisai	VEGFR1/2/3, PDGFR, FGFR, KIT, RET	RY	Differentiated thyroid cancer	2015
Lorlatinib	Pfizer	ALK	RY	NSCLC (ALK^+)	2018
Midostaurin	Novartis	FLT3, PDGFR, VEGFR2, PKC	RY	AML, mastocytosis, mast cell leukemia	2017
Neratinib	Puma	ErbB2/HER2	RY	Breast cancer ($HER2^+$)	2017
Netarsudil	Aerie Pharm.	RRH kinase	NRS/T	Glaucoma	2018
Nilotinib	Novartis	BCR-ABL, PDGFR, DDR1	NRY	CML (Ph^+)	2007
Nintedanib	Boehringer Ingelheim	FGFR1/2/3, PDGFRα/β, VEGFR1/2/3, Flt3	RY	Pulmonary fibrosis, idiopathic	2014
Osimertinib	AstraZeneca	EGFR T970M	RY	NSCLC	2015
Palbociclib	Parke-Davis	CDK4/6	S/T	Breast cancer (ER^+/ $HER2^+$)	2015
Pazopanib	GSK	VEGFR1/2/3, PDGFRα/β, FGFR1/3, KIT, LCK, FMS, LTK	RY	RCC, soft tissue sarcomas	2009
Ponatinib	Ariad	BCR-Abl, BCR-ABL^{T315I}, VEGFR, PDGFR, FGFR, EphR, SRC family kinases, kit, RET, TIE2, Flt3	NRY	CML, ALL (Ph+)	2012
Regorafenib	Bayer	VEGFR1/2/3, BCR-Abl, BRAF, BRAF (V600E), KIT, PDGFRα/β, RET, FGFR1/2, TIE2, EPH2A	RY	CRC, HCC, GIST	2012

(continued)

Table 1 (continued)

Drug	Company	Known targets	Class	Disease	Year approved
Ribociclib	Novartis	CDK4/6	S/T	Breast cancer	2017
Ruxolitinib	Incyte	JAK1/2	NRY	Myelofibrosis, polycythemia vera	2011
Sirolimus	Wyeth	FKBP/mTOR	S/T	Renal transplant, lymphangioleiomyomatosis	1999
Sorafenib	Onyx	VEGFR1/2/3, B-/RAF1, $BRAF^{V600E}$, KIT, FLT3, RET, p38, and PDGFRß	RY	Thyroid cancer, differentiated HCC, RCC	2005
Sunitinib	Pfizer	PDGFRα/β, VEGFR1/2/3, KIT, FLT3, CSF-1R, AXL, RET	RY	RCC, GIST, pancreatic neuroendocrine tumors	2006
Temsirolimus	Wyeth	FKBP12/mTOR	S/T	RCC	2007
Tofacitinib	Pfizer	JAK3	NRY	Rheumatoid arthritis, psoriatic arthritis, ulcerative colitis	2012
Trametinib	GSK	MEK1/2	T/Y	Melanoma	2013
Vandetanib	AstraZeneca	RET, EGFRs, VEGFRs, BEK, TIE2, EPHRs, SRC family kinases	RY	Thyroid cancer, medullary	2011
Vemurafenib	Genentech	$BRAF^{V600E}$, RAF1	S/T	Melanoma ($BRAF^{V600+}$)	2011

enzymes. In addition, more serine/threonine kinases (420) than tyrosine kinases (90) were found in the human kinome. In the future, the use of different development strategies along with alternative targeting domains might extend the numbers of FDA-approved inhibitors [13, 14].

2 EGFR Inhibitors

The epidermal growth factor receptor (EGFR) is a transmembrane protein that acts as a receptor for ligands of the epidermal growth factor (EGF) family [15]. The *EGFR* gene is located at chromosome 7, and the encoding protein product encompasses 1,210 amino acids. EGFR is a member of the ErbB family, which is a family of four similar receptors with tyrosine kinase activity. The family consists of EGFR or HER1, HER2, HER3, and HER4. EGFR is a cell surface receptor and represents the starting point of signal transduction mechanisms controlling diverse cellular responses such as cell proliferation, migration, survival, and apoptosis [16]. Mutations and amplification of the *EGFR* gene can lead to overexpression of the receptor. This results in constant kinase activity and uncontrolled activation of downstream pathways. In breast cancer patients, the incidence of overexpressed *EGFR* is approximately 10–30% [17]. Apart from breast cancer, upregulated EGFR can also be found in several other epithelial tumor entities such as lung cancer, prostate cancer, and squamous carcinomas of head and neck [17–19] (Fig. 1).

In addition, deletion of *EGFR* can also be found in several malignancies. One of the most common deletions in the *EGFR* locus is EGFRvIII, where exons 2–7 of *EGFR* are deleted giving rise to a receptor lacking ligand-binding domain but remaining constantly active [20, 21]. Amplification of this mutant is present in gliomas such as glioblastomas with a frequency of 64% (grade IV) but also in head and neck squamous carcinomas and medulloblastomas [21, 22]. High expression of EGFR has been also correlated with short survival time of cancer patients [23].

Lapatinib is an inhibitor of both EGFR and Her2 receptor tyrosine kinases. It was approved in 2007 for treatment of breast cancer, non-small cell lung cancer (NSCLC), head and neck cancer, as well as gastric cancer. The use of lapatinib

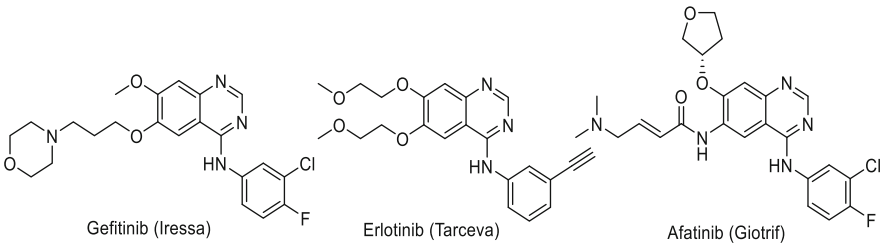

Fig. 1 Chemical structures of EGFR inhibitors

can inhibit the signaling of MAPK and PI3K pathways in patients with overexpressing EGFR and HER2. In particular, the response to lapatinib is linked to HER2 overexpression. The dual specificity of this drug results in inhibition of phosphorylation of AKT, RAF, and ERK. Interestingly, breast cancer patients positive for HER2 amplification with brain metastases are treated with lapatinib in combination with capecitabine for improvement of survival rates [24–27]. Gefitinib is an inhibitor targeting selectively EGFR (Fig. 1). Patients with locally advanced or metastatic NSCLC experienced beneficial outcome when treated with gefitinib [28]. In addition, treatment of EGFR mutation-positive NSCLC patients with gefitinib improved progression-free survival in comparison with chemotherapy. This was the first study that showed longer progression-free survival of patients treated with selective therapy compared to classic chemotherapy [29, 30]. Erlotinib (another kinase inhibitor targeting EGFR) is used for treatment of locally advanced or metastatic NSCLC (Fig. 1). A clinical study published in 2011 revealed that use of erlotinib prolongs survival of NSCLC patients, previously treated with first-line chemotherapy, leading to its approval for this use [31]. In addition, erlotinib combined with gemcitabine increases overall survival of patients with unresectable pancreatic cancer positive for mutant EGFR [32]. Despite providing therapeutic benefit, use of erlotinib has severe side effects such as breathing abnormalities, skin rush, diarrhea, and cough, and the recommended dosage is close to the maximum tolerated dose [33].

Afatinib is an irreversible inhibitor of ErbB family of kinase receptors (Fig. 1). As a first-line treatment of patients with lung adenocarcinoma carrying activating mutations in EGFR, afatinib increased progression-free survival but not overall survival, when compared to gefitinib [34]. In addition, the LUX-Lung 6 trial revealed that patients with advanced lung adenocarcinoma treated with afatinib had prolonged progression-free survival and time to treatment failure in comparison with those treated with gemcitabine in combination with cisplatin [35].

3 ALK Inhibitors

Anaplastic lymphoma kinase (ALK) is a tyrosine kinase receptor. In 1994, ALK was described for the first time as a component of a fusion protein derived from translocation t2;5 in anaplastic large cell lymphoma [36]. Several years later, the full length of ALK receptor tyrosine kinase was characterized. It consists of an extracellular ligand-binding domain, a transmembrane domain, and an intracellular kinase domain that shares high similarity with the insulin receptor (ER) [37, 38]. Although the physiological function of ALK is not completely revealed, it has been described to play a critical role in early embryo development and neural system development [38–41]. Furthermore, activation of ALK is involved in activation of PI3K-AKT, CRKL-C3G, MEKK2/3-MEK5-ERK5, JAK-STAT, and MAPK signaling pathways [38, 42–45]. Around 3–7% of NSCLC patients (usually non-smokers) have a particular mutation, where

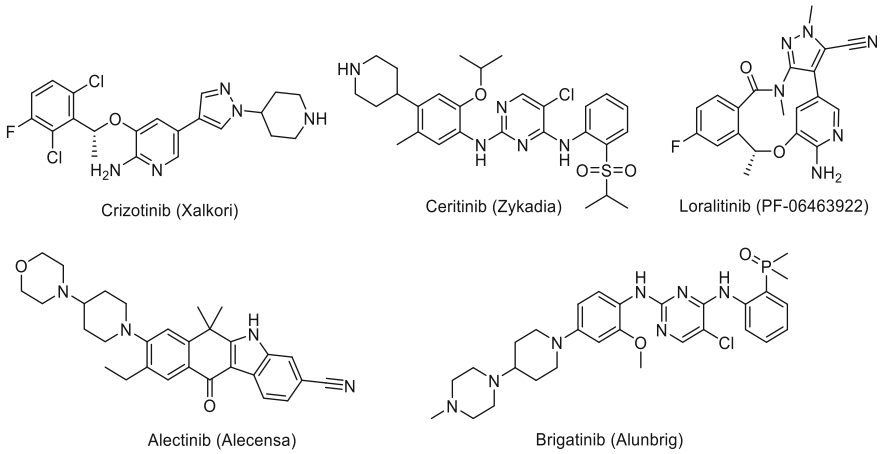

Fig. 2 Chemical structures of ALK inhibitors

echinoderm microtubule-associated protein-like 4 (EML4) gene is fused to ALK gene. This inversion of chromosome 2 results in the expression of the fusion protein EML4-ALK consisting of the N-terminal region of EML4 and the intracellular/kinase region of ALK [46, 47] (Fig. 2).

The first inhibitor of this category approved by FDA was crizotinib (2011) Fig. 2. Initially, it was developed as a c-Met inhibitor but is also able to target ALK, proto-oncogene tyrosine-protein kinase (ROS1), and hepatocyte growth factor receptor (HGFR) [48]. Profile 10,019 phase-I clinical trial and profile 100,513 showed significant objective response rates, prolonged progression-free survival, and median progression-free survival in pretreated ALK-positive (EML4-ALK) NSCLC patients [49, 50]. Based on this evidence, crizotinib received conditional approval for use also in Canada, in 2012. Subsequently, phase-III trials revealed superior median progression-free survival and greater reduction in symptoms related with lung cancer when treated with crizotinib compared to chemotherapy [51, 52]. The results from these studies led to full approval of crizotinib marking it as the "gold standard" of ALK-positive NSCLC.

Ceritinib represents a next-generation ALK inhibitor and showed a higher potency than first-generation inhibitors such as crizotinib (Fig. 2). Ceritinib showed a potency in inhibiting ALK-positive NSCLC that were previously treated and resistant to crizotinib [53]. This finding suggested potency in treatment of mutated and therapy-resistant ALK tumors. Indeed, phase-I ASCEND-1 trial resulted in significant overall response rates in patients pre-acquired with both identified and non-identified resistance mechanisms to crizotinib [54]. An ASCEND-2 phase-II trial showed beneficial response of ceritinib in patients pretreated with crizotinib or chemotherapy and with or without brain metastases [55]. These data resulted in the approval of ceritinib as the first-choice treatment for crizotinib-resistant, ALK-positive NSCLC patients in 2014. In addition, an ASCEND-5 phase-III trial confirmed superior response of ceritinib. In this trial, NSCLC patients with brain

metastases that were previously treated either with crizotinib or platinum-based chemotherapy were treated with ceritinib or chemotherapy [56]. Finally, ceritinib demonstrated potency against naive ALK-inhibitor NSCLC patients. The ASCEND-4 study revealed a median progression-free survival of 16.6 months of patients with advanced ALK-positive NSCLC treated with ceritinib versus 8.1 months of the chemotherapy-treated group [57]. The results from this study led to approval of ceritinib in 2017 by FDA as first-line treatment for patients with ALK-positive NSCLC [58]. Alectinib is another selective ALK inhibitor (Fig. 2). It was approved by FDA in 2015 for the treatment of NSCLC patients with acquired resistance to crizotinib (NP28673 and NP28761 phase-II clinical trials) [59, 60]. Later, the randomized phase-III clinical trial ALEX showed extended beneficial activity of alectinib in ALK-positive NSCLC patients. In particular, results demonstrated a superior progression-free survival rate of alectinib compared to crizotinib in naive ALK-inhibitor patients. In addition, alectinib was found to be less toxic and more active toward CNS. Only 12% of patients in the alectinib group showed a CNS progression event compared to 45% of the crizotinib group. Taking into account the previous results, FDA approved alectinib as first-line treatment of ALK-positive, metastatic NSCLC in 2017 [58, 61]. Latest additions to the ALK inhibitors' list include brigatinib and lorlatinib. Brigatinib is an ALK inhibitor used for NSCLC resistant to crizotinib (Fig. 2). It has received accelerated approval by the FDA in 2017 after phase-II clinical trial ALTA demonstrated significant results for the treatment of patients with progressed NSCLC [62, 63]. Lorlatinib was accepted for the same treatment in 2018 (Fig. 2). Both drugs demonstrate significant intracranial activity making them very potent in decreasing the formation of brain metastasis [63, 64]. Phase-III clinical trial CROWN is currently ongoing for comparison of lorlatinib with crizotinib as first-line treatments [58, 65].

4 VEGFR Inhibitors

More than 40 years ago, the hypothesis of targeting angiogenesis as a tumor therapy was established [66]. Although many factors are involved in mechanisms leading to blood vessel formation, activation of vascular endothelial growth factor (VEGF) pathways was described to be critical in pro-angiogenic signaling. Several types of solid cancers overexpress VEGF-A, making initially this protein to a highly relevant target for selective antiangiogenic therapeutic strategy [67, 68]. Another strategy for inhibiting angiogenesis is the blockage of tyrosine kinase activity of the corresponding receptors VEGFR1, VEGFR2, and VEGFR3. Many receptor tyrosine kinase inhibitors targeting VEGFR have been approved so far. Sorafenib, sunitinib, axitinib, regorafenib, pazopanib, vandetanib, cabozantinib, and lenvatinib are used for treatment of different solid carcinomas such as renal cell carcinoma (RCC), hepatocellular carcinoma (HCC), thyroid cancer, pancreatic neuroendocrine tumor, gastrointestinal stromal tumor (GIST), and metastatic colorectal cancer (CRC) (Fig. 3). Sorafenib and sunitinib represent pioneer kinase inhibitors for the inhibition

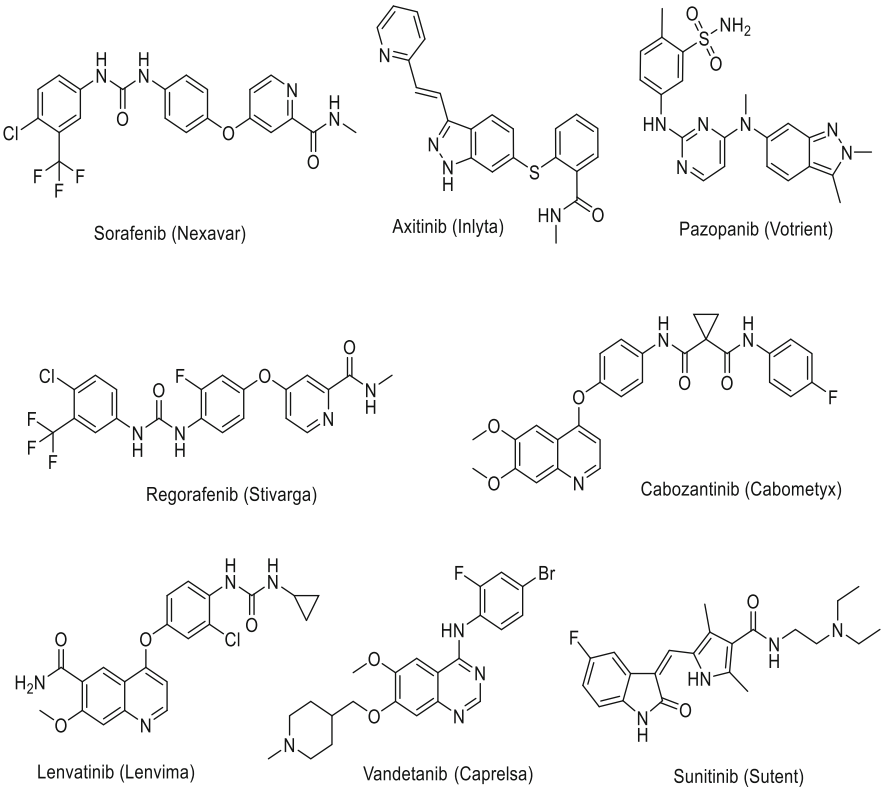

Fig. 3 Chemical structures of VEGFR inhibitors

of angiogenic signaling in cancer. These multikinase inhibitors were initially approved for the treatment of advanced renal cell carcinoma. The pivotal phase-III study TARGET resulted in significantly prolonged progression-free survival time of patients with resistant, advanced renal cell carcinoma when treated with sorafenib. Sunitinib showed similar results in the randomized phase-III trial, where it was compared as first-line treatment to subcutaneous injection of interferon-α for treatment of metastatic RCC. Patients of sunitinib group showed improvement in median progression-free survival and objective response rate [69–72]. Sorafenib and sunitinib have been also accepted by FDA for treatment of HCC and advanced pancreatic neuroendocrine tumors, respectively [73, 74] (Fig. 3).

Regorafenib was the first therapeutic agent to show improvement in the overall survival of patients with metastatic CRC, previously progressed on classic therapies [75]. Based on these results, regorafenib was approved by FDA in 2012 for treatment of metastatic CRC. Half a year later, regorafenib was also accepted for the treatment of advanced GIST [76]. Regorafenib also shows beneficial outcome when used as treatment of HCC, significantly longer overall survival in second-line HCC patients, leading to its approval by the FDA for this use in 2017 [77].

Second-generation VEGFR/multikinase inhibitors include pazopanib, cabozantinib, lenvatinib, axitinib, and vandetanib (Fig. 3). All of them have been approved by the FDA for the treatment of one or several cancer types including thyroid cancer, RCC, soft tissue sarcoma, and medullary thyroid cancer [78].

5 BCR-ABL Inhibitors

The ABL protein family consists of two members: c-ABL and ARG. Physiologically, c-ABL is involved in actin remodeling, cell adhesion, motility, DNA damage response, and microbial pathogen response. In several types of cancer, deregulation and uncontrolled expression of c-ABL kinase has been described [79, 80]. When phosphorylated, c-ABL induces activity of downstream targets, activating ERK5, RAC/JNK, and STAT 1/3 pathways. C-ABL is also a molecular component driving CML. Translocation of part of chromosome 9 to chromosome 22 (Philadelphia chromosome) leads to the expression of oncogenic fusion protein BCR-ABL [81] highlighting ABL is an important target for the development of selective inhibitors. Imatinib was the first kinase inhibitor to be approved by FDA (2001) (Fig. 4). It is an inhibitor of three different targets: ABL, mast/stem cell growth factor receptor (tyrosine kinase KIT or CD117), and PDGFR [82]. After phase-III clinical trial showed improved cytogenetic response rates of CML patients treated with imatinib, the drug was accepted for treatment of CML in blast, accelerated, and chronic phases [83]. Later, in 2002 and 2008, imatinib was approved also for treatment of GIST both for advanced, metastatic tumors and previously resected tumors [84, 85]. Unfortunately, imatinib treatment is not successful in around 30% of patients [86]. The reason is acquired resistance, based on either a reduced cellular uptake of the drug, an increased activity of efflux transporters, or point mutations leading to conformational changes of BCR-ABL and therefore to a reduced binding to imatinib. In addition, resistance is acquired by amplification and overexpression of *BCR-ABL* gene [87]. Second-generation ABL inhibitors such as nilotinib, dasatinib, and bosutinib were developed, in order to overcome mutation-related resistance (Fig. 4). They were all approved for the treatment of CML: nilotinib and dasatinib as first- or second-line treatment and bosutinib as second-line therapy [88]. Nilotinib showed highly promising results because it was potent against almost all mutations resulting in BCR-ABL-dependent resistance [89]. Dasatinib showed high potency in patients with chronic phase CML and a faster treatment response when it was compared to imatinib [90]. Due to its unique structure, dasatinib is also potent against some conformation-altering mutations of BCR-ABL [91, 92]. Bosutinib has a much different structure. It was initially designed as a SRC inhibitor but found to have activity against ABL [93]. Although bosutinib is not potent against major resistant mutants and does not have high selectivity for BCR-ABL, it has the benefit to be not sensitive to resistance efflux transporters and remains in the cells [94, 95]. Therefore, bosutinib is approved for second-line treatment of CML, while trials that test it as first-line treatment are ongoing [88, 96] (Fig. 4).

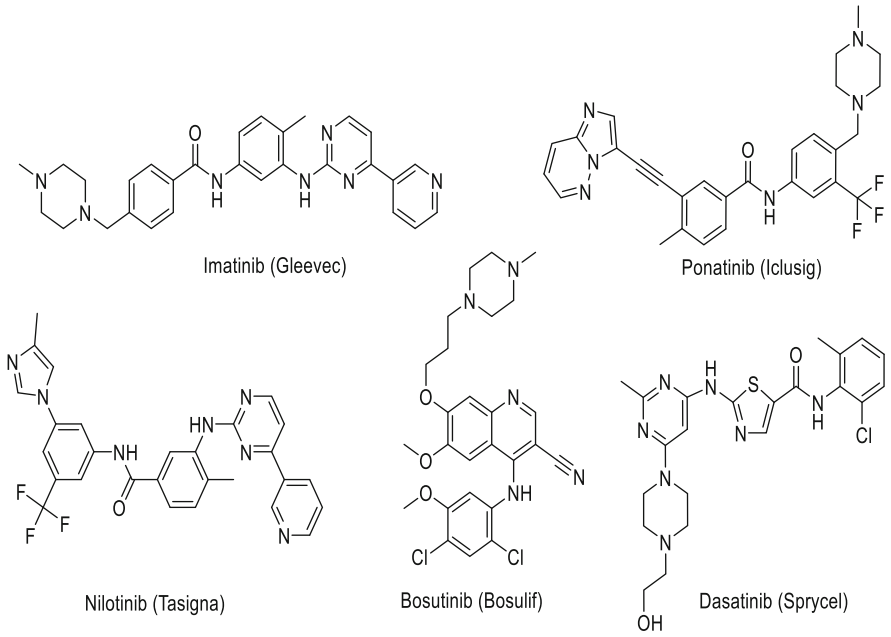

Fig. 4 Chemical structures of ABL inhibitors

The only approved third-generation inhibitor against ABL is ponatinib (Fig. 4). It is a dual SRC/ABL inhibitor that is accepted for the treatment of CML and Ph+ ALL. The structure of ponatinib was modified accordingly so that is highly potent against resistant mutants [97]. Clinically, it shows potency in the treatment of progressed and pretreated Ph+ leukemias. In addition, patients with resistant mutations also benefit from ponatinib treatment. In the corresponding study with 43 patients harboring the abovementioned characteristics, 98% showed a complete hematologic response and 72% a major cytogenetic response [98]. Finally, ponatinib has proven to be a valuable alternative to stem cell transplantation in patients with mutant, advance CML and Ph+ ALL [88, 99].

6 RAF Inhibitors

The RAS/MAPK pathway controls cell growth, proliferation, and survival in a broad range of different tumor entities. Activation of membrane-associated RAS proteins (KRAS, NRAS, HRAS) results in a recruitment of RAF proteins (ARAF, BRAF, and RAF1) leading to a phosphorylation of MEK1 and MEK2 which in turn phosphorylate and activate extracellular signal-regulated kinase (ERK1 and ERK2) (Fig. 5).

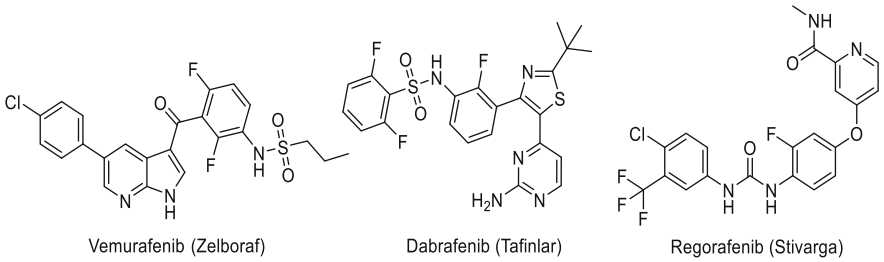

Fig. 5 Chemical structures of RAF inhibitors

RAS represents an important proto-oncogene and a major oncogenic driver. It was found mutated in around 30% of all human cancer entities [100]. Due to the fact that RAS proteins do not harbor any cavities for small molecule interaction, approaches to directly inhibit the function of RAS have not been successful so far. Therefore, the inhibition of RAS downstream factors such as RAF, MEK, and ERK has gained interest for the treatment of cancer [100].

RAF monomers are usually inactive, since the N-terminal domain of BRAF triggers autoinhibition [101, 102]. Upon activation, RAF forms homo- and heterodimers which induces downstream signaling to MEK. While physiological RAS activation induces MEK activation mainly via the formation of BRAF dimers [103], oncogenic RAS often triggers the formation of BRAF-RAF1 heterodimers [101, 104, 105].

BRAF mutations are present in 8% of all human tumors [106]. They were found in more than 50% of melanoma patients and were also identified in CRC (5–10%), hairy cell leukemia (~100%), thyroid carcinomas (25–45%), and, as a rare event, ovarian and lung cancer [106, 107]. Ninety percent of all BRAF mutations account for a substitution of valine with glutamic acid at position 600 (V600E) [100]. This mutation results in a constitutive kinase activity of BRAF monomers and protects BRAF from ERK-mediated negative feedback signaling [102].

The identification of BRAF mutations as oncogenic drivers led to intensified efforts in order to develop more selective and potent BRAF inhibitors. This work yielded in the development of vemurafenib (Zelboraf) and dabrafenib (Tafinlar) as FDA-approved drugs for the treatment of $BRAF^{V600E}$-mutated advanced melanoma [108–110] (Fig. 5).

Vemurafenib is a $BRAF^{V600E}$ inhibitor with an IC_{50} of 31 nM, which inhibits also BRAF proteins with other mutations (V600D, V600K, and V600R) as well as RAF1 (IC_{50} = 48 nM) [109]. It shows only a low affinity to wild-type BRAF (IC_{50} = 100 nM) [111]. In preclinical treatment studies, vemurafenib was effective in xenograft models of $BRAF^{V600E}$-mutated melanoma [108] and showed efficacy against BRAF-mutated melanoma cell lines [112].

Upon successful phase-I and phase-II clinical trials, a phase-III clinical trial of vemurafenib was initiated on 675 patients suffering from metastatic, $BRAF^{V600E}$-mutated melanomas (who did not receive any treatment before). This trial showed a median overall survival of 13.2 months for patients under vemurafenib treatment,

while patients under dacarbazine (as a control) showed only a survival of 9.9 months. Furthermore, vemurafenib resulted in a significant response in 48.4% of patients (in comparison with 5.5% in the dacarbazine-treated group) [113, 114].

Although vemurafenib was in general well tolerated by patients, several adverse symptoms were found upon treatment such as fatigue, nausea, alopecia, lymphopenia, neutropenia, headache, and diarrhea [113, 115–118].

Dabrafenib was shown to be in general a more potent Raf inhibitor than vemurafenib. It inhibits $BRAF^{V600E}$ with an IC_{50} of 0.8 nM, wild-type BRAF with an IC_{50} of 3.2 nM, and RAF1 with an IC_{50} of 5 nM [109, 119]. In preclinical assays, dabrafenib also showed efficacy against BRAF-mutated cell lines and reduced tumor development in xenograft melanoma mouse models [119]. A phase-III clinical trial of dabrafenib was performed in a total of 250 patients suffering from $BRAF^{V600E}$-mutated metastatic melanoma. While 187 patients received 150 mg dabrafenib twice per day, 63 patients received dacarbazine treatment [120]. In the dabrafenib-treated group, 6 patients (3%) showed a complete and 87 patients (47%) partial response, while 78 patients (42%) displayed a stable disease. In the dacarbazine group, one patient (2%) showed a complete and three patients (5%) partial response. A stable disease was seen in 30 patients (48%). The median progression-free survival in the dabrafenib group was 5.1 months, while dacarbazine-treated patients showed a progression-free survival rate of 2.7 months.

Side effects found often associated with dabrafenib treatment were pyrexia, headache, neutropenia, fatigue, thrombocytopenia, leukopenia, asthenia, hyponatremia, arthralgia, nausea, chills, myalgia, vomiting, diarrhea, and hair loss [108, 109, 117, 118, 120].

Overall, vemurafenib and dabrafenib induced initial therapeutic effects against $BRAF^{V600}$ mutant melanomas. However, the long-term treatment success is limited due to the development of secondary resistance. Thus, most patients relapse after 1 year of treatment [121]. In addition, other tumor entities with $BRAF^{V600E}$ mutation, such as colorectal, pancreatic, and thyroid cancer, mostly show a primary resistance to these drugs [122, 123]. It was hypothesized that long-term control of tumor development by these inhibitors is limited by the fact that they do not efficiently inhibit the dimerization of RAF and are partly unsuccessful in targeting BRAF and RAF1 dimers. Thus, BRAF homodimeric or BRAF-RAF1 heterodimeric signaling can trigger therapy resistance [100–102]. In addition, it was shown that therapy resistance of RAF inhibition can be also induced by the formation of different $BRAF^{V600E}$ splice variants which can form resistant dimers [124].

Of note, RAF inhibitors were also applied in tumors without BRAF mutation. For example, the previously mentioned multikinase inhibitor sorafenib represents a RAF inhibitor and showed certain efficacy in the treatment of patients with HCC and RCC. Sorafenib was approved by the FDA for the treatment of these tumor types [125–129]. However, development of resistance against sorafenib is a frequent incident in treated patients.

Sorafenib inhibits RAF1 with an IC_{50} of 6 nM, wild-type BRAF with an IC_{50} of 25 nM, and $BRAF^{V600E}$ with an IC_{50} of 38 nM. Of note, it is hypothesized that the effect of sorafenib is based on a combined inhibition of RAF and other kinases such

as VEGFR. Sorafenib inhibits VEGFR1 with an IC_{50} of 26 nM and VEGFR2 with an IC_{50} = 90 nM. Other kinases that were found to be influenced by sorafenib are FLT-3 (IC_{50} = 33 nM), p38 (IC_{50} = 38 nM), RET (IC_{50} = 47 nM), c-KIT (IC_{50} = 68 nM), and FGFR1 (IC_{50} = 580 nM) [109, 126, 130]. A phase-III clinical trial testing sorafenib in advanced HCC (SHARP trial) was performed with 602 patients that were treated either with 400 mg sorafenib twice daily (299 patients) or with placebo (303 patients) [74, 129]. In that trial, 7 patients (2%) showed partial response, and 211 patients (71%) had a stable disease in the sorafenib group, whereas 2 patients (1%) showed partial response and 204 patients (67%) had a stable disease in placebo group. The median overall survival was 10.7 months upon sorafenib and 7.9 months upon placebo treatment. Median time to symptomatic progression was 4.1 months in the sorafenib group and 4.9 months in the placebo group. In addition, median time to radiological progression was 5.5 months in the sorafenib group and 2.8 months in the placebo group [109].

As mentioned before, phase-III clinical trials of sorafenib were also conducted against other tumor entities such as RCC and thyroid carcinoma [74, 128, 131]. In a trial against radioactive iodine refractory thyroid cancer (DECISION), 417 patients were treated either with 400 mg sorafenib twice per day (207 patients) or with placebo (210 patients) [132]. Sorafenib triggered a partial response in 12.2% of patients which was seen in only 0.5% of patients treated with the placebo. Furthermore, the sorafenib-treated group showed a median overall survival of 10.8 months compared to 5.8 months in the placebo-treated group.

Adverse effects associated with sorafenib were fatigue, anorexia, hypertension, nausea, vomiting, alopecia, flushing, constipation, voice change, diarrhea, headache, joint pain, pruritus, weight loss, hemorrhage (upper GI), neuropathy, stomatitis, hypophosphatemia, musculoskeletal pain, and abdominal pain [74, 109, 118, 125, 129, 132].

The molecular mechanisms for resistance against sorafenib are complex. It was shown that activation of p38alpha signaling during sorafenib therapy circumvents sorafenib-mediated inhibition of Raf in HCC. In line with this, inhibition of p38alpha improved the outcome of sorafenib in HCC mouse models [133].

For patients that show a progressive disease under sorafenib, recently the RAF inhibitor regorafenib (which targets also several other kinases, such as VEGFR, PDGFR) was approved by the FDA [77] (Fig. 5). In the RESORCE trial, 573 patients that progressed under sorafenib received either 160 mg regorafenib or placebo. The median survival under regorafenib was 10.6 months compared to 7.8 months under placebo treatment [77]. Furthermore, regorafenib was approved by the FDA for the treatment of metastatic colorectal cancer and advanced gastrointestinal stromal tumor (GIST) [75, 76].

7 MEK Inhibitors

The observation of therapy resistance and paradoxical ERK activation upon RAF inhibition resulted in increased effort to develop inhibitors targeting MEK. Several MEK inhibitors, such as refametinib, selumetinib, cobimetinib, and trametinib, have been tested in clinical trials for different tumor entities, e.g., NSCLC and melanoma [134, 135] (Fig. 6).

Also combination therapies of RAF and MEK inhibitors were tested and FDA approved and were able to further increase the treatment responses in melanoma patients [136]. In patients with metastatic melanoma, two phase-III trials were performed in order to test the combination of BRAF inhibitors and MEK inhibitors. In the COMBI-d trial, 423 patients with $BRAF^{V600}$ mutations, which were not treated before, received either dabrafenib in combination with trametinib or dabrafenib alone [137]. The application of the combination therapy resulted in a 3-year overall survival of 44% compared to 32% in the group that received a dabrafenib monotherapy. Adverse effects of the trametinib and dabrafenib combination were pyrexia, fatigue, nausea, headache, diarrhea, rash, and arthralgia [137, 138].

In addition, also a combination of cobimetinib and vemurafenib was tested in 495 patients with untreated advanced $BRAF^{V600}$-mutated melanoma (coBRIM trial) [139]. In this trial, the 3-year rate of relapse-free survival was 58% in the group that received the combination therapy compared to 39% in the placebo group. The 3-year overall survival rate was 86% in the combination-therapy group compared to 77% in the placebo group [139].

In addition, different studies tested also the combination of RAF/MEK inhibitors with immunotherapies [140].

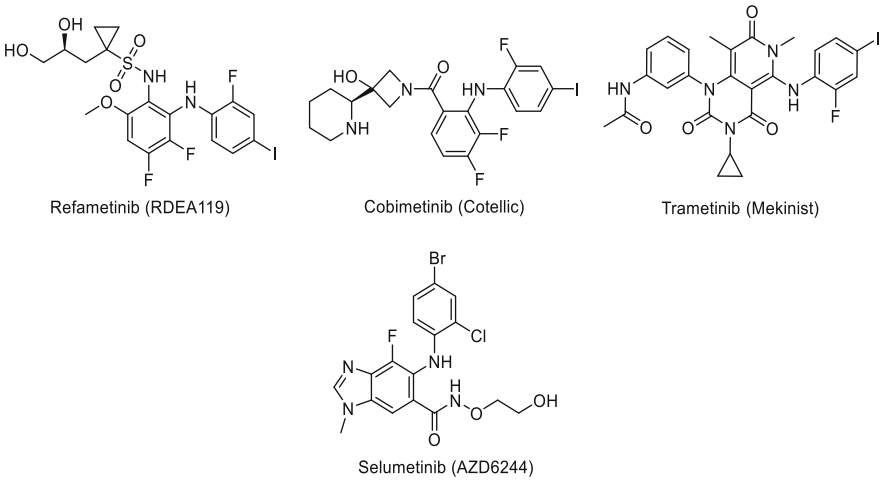

Fig. 6 Chemical structures of MEK inhibitors

8 Next Clinical Developments

The human genome encodes for over 500 kinases which gives ample scope for novel target finding and drug development in cancer therapy [141]. In addition to the 48 FDA-approved kinase inhibitors, a huge range of potential inhibitors are currently in clinical or preclinical trials. For example, clinical phase-I trials of compounds targeting nerve growth factor receptors [142], polo-like kinase 1 [143], phosphatidylinositol 4,5-bisphosphate 3-kinase delta and gamma [144], protein kinase B [145], focal adhesion kinase [146], casein kinase II [147], and Aurora kinases [148] were performed within the last years (Fig. 7).

Notably, the Aurora A kinase inhibitor alisertib could finish two clinical phase-II trials with promising responses in patients with neuroendocrine prostate cancer [NCT01799278] as well as advanced breast cancer and small cell carcinoma of the lung [NCT01045421] (Fig. 7). However, a phase-III trial in patients with relapsed/refractory peripheral T-cell lymphoma [NCT01482962] was announced to be discontinued based on a pre-specified interim analysis by Takeda.

In parallel, the research on PI3K and mTOR inhibiting compounds was heavily impelled in recent years [13]. In addition to idelalisib which was the first FDA-approved compound to inhibit a lipid kinase (PI3Kδ isoform) [149, 150], in total seven dual PI3K/mTOR small molecule inhibitors are tested in advanced clinical trials [13] (Fig. 7). These comprise PKI587 (advanced solid malignancies) [151], quinacrine (various leukemias) [152, 153], GSK2126458 (colorectal, breast, NSCLC, and pancreatic cancer) [154], PF04691502 (breast cancer) [155], GDC0980 (mRC) [156], XL765 (breast cancer) [157], and NVP-BEZ235 (glioblastomas) [158].

The oral pan-PI3K inhibitor buparlisib which targets all four isoforms of class I PI3K was registered for three phase-III clinical trials against breast cancer. Buparlisib was tested in combination with fulvestrant in an advanced breast cancer study (Fig. 7). Due to the safety profile, the results do not endorse an expansion in this clinical setting [159, 160].

Interestingly, patients with a *PIK3CA* mutation have shown a median progression-free survival of 4.2 months (95% CI 2.8–6.7) after buparlisib treatment compared to 1.6 months (95% CI 1.4–2.8) in the placebo group. These results

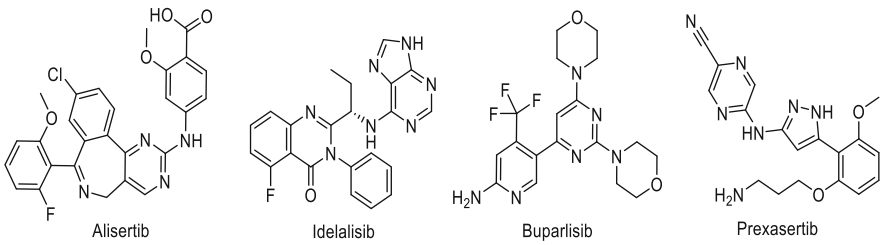

Fig. 7 Chemical structures of next clinical development

support the application of PI3K inhibitors combined with endocrine therapy in this genetic background [160].

Nearly all hallmarks of cancer can be targeted by approved protein kinase inhibitors. However, there are currently no FDA-accepted kinase inhibitors influencing genome instability and DNA damage response. The success story of olaparib, a Poly [ADP-ribose] polymerase 1 (PARP-1) inhibitor, underlines the high potential of small molecules in this field. In general, the incidence of DNA single- or double-strand breaks is mainly controlled by the network of ATR/ATM and CHK1/CHK2 signaling pathways leading to active DNA repair mechanisms and cell cycle checkpoint regulation [161]. At the moment, two different ATR inhibitors, AZD6738 and M6620, are tested in clinical phase-II trials with four and six studies. They are administered in prostate cancer, CLL, recurrent ovarian cancer, progressive metastatic gastric or gastroesophageal junction cancer, small cell lung carcinoma, as well as metastatic tumors including RCC, urothelial carcinoma, ovarian cancer, and PDAC [NCT03787680, NCT03328273, NCT03641313, NCT03517969, NCT03682289, NCT02595892, NCT02567409, NCT03462342, NCT02627443, NCT02487095].

Furthermore, checkpoint kinases are valid targets in DNA damage response. The CHK1/CHK2 inhibitor prexasertib is tested in small cell lung cancer, ovarian cancer, breast cancer, and prostate cancer in phase-II clinical trials [NCT02735980, NCT03414047, NCT02873975, NCT02203513] (Fig. 7).

In *BRCA* wild-type recurrent high-grade serous ovarian cancer, prexasertib could exhibit clinical activity and was in general well tolerated by treated patients [162]. Particularly patients with platinum-resistant or platinum-refractory cancer could profit here from further drug development [162].

9 Challenges

Beside numerous advances of kinase inhibitors, profound understanding of mechanisms in vivo is needed to overcome actual limitations in clinical oncology [13].

Secondary therapy resistance based on kinase mutations is an abundant phenomenon arising after kinase inhibition [163]. The diversity of such mutations among different kinases hampers the overall treatment success in cancer patients [164]. Acquired resistance is the most common resistance type caused by kinase inhibitors and relates to tumors that respond to therapy initially but show posterior resistance to permanent delivered therapy [13]. Secondary resistance can be induced by changes in the kinase gatekeeper residue since hydrophobic interactions in the sub-pocket are decisive for the inhibitor binding affinity [165, 166]. The gatekeeper residue interacts with Type I and Type II kinase inhibitors and sterically influences inhibitor binding to the hydrophobic region in the binding pocket [167]. In addition to gatekeeper mutations in BCR-ABL kinases inducing imatinib resistance, numerous other targets are affected by gatekeeper mutations [168–173]. A prominent example is the T790M mutation in EGFR kinase leading to boosted affinity toward

ATP that triggers resistance to quinazoline inhibitors as well as gefitinib and erlotinib [174–176]. To circumvent drug resistance in the clinic, structural optimization of small molecule inhibitors is required [13]. In case of mutated EGFR-induced resistance to gefitinib and erlotinib, newly developed EGFR inhibitors can covalently bind to the ATP-binding site of EGFR [177, 178]. That represents an example for highly selective inhibitors against mutated targets [13].

To further counter kinase inhibitor resistance, scientists break fresh ground with innovative strategies. In the context of gatekeeper mutations, currently developed inhibitors are going to accept varying amino acids at the gatekeeper mutation site [179, 180]. In a second approach, kinases will be targeted at alternative binding sites to avoid the ubiquitous ATP-binding pocket by a presumable unique cavity [181, 182]. Apart from that, also indirect kinase targeting via inhibition of kinase transformers would be a valid option to overcome resistance [183].

An additional clinical challenge represents the reduction or elimination of critical toxicities associated with kinase inhibitors, such as proteinuria, skin reactions, hypertension, or cardiotoxicity [184, 185].

Well-known examples are associated side effects of BCR-ABL inhibitors, including cytopenia, cardiotoxicity, and cardiac sequela. HER2 and ALK inhibition cause gastric problems and dermatological irregularities. EGFR inhibition is linked to dermatological issues, and VEGFR inhibition can trigger cardiotoxicity [186, 187].

To exclude toxicities triggered by off-target binding of the inhibitor, more specific therapeutic strategies are required. RNA interference is not only a powerful tool for specific gene knockdown in basic research, but it also raises expectations as a therapeutic approach to inhibit crucial players in cancer such as kinases [13]. However, since important drug targets cannot be efficiently eradicated by RNA interference so far, clinical resistance to kinase inhibitors will continue to be an important challenge to kinase-associated therapies [13, 188].

Altogether, the development of clinical relevant kinase inhibition has just started, but the rapid progress in the development of molecular technologies and engineering raises confidence for further success stories.

Compliance with Ethical Standards

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