Target Cancer Therapy

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Additional information is available at the end of the chapter

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1. Introduction

Over the past decade, cancer therapy has changed drastically by the introduction of the target therapies, which focus on unique molecules present in tumors or protein whose expression or function is enriched within the neoplastic tissue; the so called molecular targets. In this context, target therapies may include monoclonal antibodies, drugs or small inhibitors capable of inhibiting specific molecules, such as kinases. The major targets in cancer therapy are pathways directing cell growth, proliferation and survival, as well as, those interfering on tumors microenvironmental aspects, such as angiogenesis. On the other hand, an emerging field on target therapy is the use of epigenetic drugs, which aim the restoration of the normal epigenetic landscape in cancer cells by targeting the epigenetic machinery of cells. Despite some major side effects associated to some target drugs, these therapies are well tolerated by patients. Moreover, it bears the possibility of developing personal therapies to each individual patient, which is considered the optimum choice in oncology.

2. Kinases and their hole on tumor progression

Kinases are by definition proteins capable of catalyzing the transfer of the terminal phosphate of ATP to substrates that contain, in most cases, a serine, threonine or tyrosine residue. The importance of targeting kinases to fight cancer relies on the central role that these molecules play on tumorigenesis, as uncontrolled tissue growth, and the capacity of cells to invade and metastasize [1]. Considering that, kinases involved in cell growth, division, mi-

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gration and differentiation, as well as, angiogenesis and metastasis have been exploited and targeted in therapeutic oncology [2].

RAF/MEK/ERK and PI3K/AKT/mTOR are particularly important, as aberrant activation of these pathways is frequently observed in many types of cancers. Of interest, they are involved in chemoresistance to conventional chemotherapy, hormonal therapies and radiaotherapy. In addition, upstream elements of these signaling pathways, such as growth factors and growth factor receptors, as well as kinases exclusively found on cancer cells, as the kimeric kinases derived from Philadelphia chromosome (Ph), can also be target for cancer therapy. Thus, inhibitors targeting any of these molecules can potentially suppress tumorigenesis and bypass resistance to conventional treatments to cancer [3, 4, 5].

3. Small molecule kinase inhibitors in cancer therapy

Kinase inhibitors (KI) are divided into several classes based on the site they bind to at the enzyme. Types 1 and 2 bind to the ATP site of kinases, acting as ATP competitors. The difference between these two types is that whereas type I inhibitors target the active conformation of the kinase, type 2 bind to the inactive one. On the other hand, type 3 KI bind outside the ATP site, inhibiting kinases on an allosteric manner. This class of KI is usually more selective, since they bind to unique sequences from specific kinases. The forth class of KI are the covalent inhibitors, which irreversibly bind to the kinase active site, usually by reacting with a nucleophilic cystein residue [6]. KI already approved by the US Food and Drug Administration (FDA) will be discussed next, followed by KI currently under trial.

3.1. Kinase inhibitors approved by FDA for cancer treatment

3.1.1. EGFR and HER2 kinase activity inhibitor

Gefitinib was approved under FDA's accelerated approval regulation in 2003. It acts by inhibiting the phosphorylation of a series of intracellular kinases associated with epidermal growth factor receptor (EGFR), among others. At its initial approval it was recommended for the treatment of patients with locally advanced or metastatic non-small cell lung carcinoma (NSCLC) after the failure of both platinum-based and docetaxel chemotherapies. However, in 2005, FDA published a labeling revision due to the failure demonstrated by gefitinib in increasing NSCLC patients' survival. Since this revision, gefitinib has only been prescribed for patients who are benefiting or have benefited from gefitinib.

In 2004, FDA approved **erlotinib**, which also inhibits the phosphorylation of EGFR-associated TKs, for the treatment of locally advanced or metastatic NSCLC after failure of at least one prior chemotherapy regimen. On the next year, the indication for the use of erlotinib in combination with gemcitabine for the first-line treatment of patients with locally advanced, unresectable or metastatic pancreatic cancer (PC).

Different from the above mentioned KIs, **lapatinib**, approved by FDA in 2007, is a direct inhibitor of the intracellular kinase domain of both EGFR and human growth epidermal growth factor 2 (HER2/neu), inhibiting the tumor driven cell growth. Its initial indication was for the treatment of patients with advanced or metastatic breast cancer (BC) whose tumors overexpress HER2 and who have received prior therapy including an anthracycline, a taxane, and trastuzumab. In 2012, the indications for lapatinib include the combined use of this drug with: capecitabine, for the treatment of patients with advanced or metastatic BC in the same conditions as mentioned above; letrozole for the treatment of postmenopausal women with hormone receptor positive metastatic BC that overexpress the HER2 receptor for whom hormonal therapy is indicated.

3.1.2. Raf inhibitors

A variety of agents have been discovered to interfere with RAF kinases, each of which acting on different ways in order to block Raf protein expression, c-Ras/Raf interaction, Raf kinase activity, Raf's ATP-binding site, or the kinase activity of the Raf target protein MAPKK.

Among these, **sorafenib tyosilate**, a c-Raf inhibitors, has been approved by FDA in 2005. This bi-aryl urea was initially identified as an adenosine triphosphate competitive inhibitor of the c-Raf kinase. Sorafenib targets two kinase classes known to be involved in both tumor proliferation and angiogenesis [7]. The drug blocks the enzyme c-Raf kinase it self, a critical component of the Ras/Raf/MEK/ERK signaling pathway, which is responsible for controlling cell division and proliferation. In addition, sorafenib inhibits the vascular endothelial growth factor receptor (VEGFR)-2/platelet-derived growth factor receptor (PDGFR)-beta signaling cascade, thereby blocking tumor growth and angiogenesis. Sorafenib has been evaluated as a single therapy agent and in combination with various chemotherapy drugs in a number of clinical trials [8-10]. At its first approval sorafenib tosylate was indicated for the treatment of adenois cyst carcinoma (ACC) patients; latter on, at the latest review, in 2012, the indication for the treatment of unresectable hepatocellular carcinoma (HCC) patients was included.

Later on, in 2011, vemurafenib, which targets the mutated form of BRAF protein BRAFv600, was approved by FDA [11]. It has been approved for the treatment of patients with metastatic melanoma (MM) whose tumors presented the mutation, as detected by a FDA approved test. However, it is not recommended for the treatment of MM patients who harbors the wild-type BRAF gene. It has been no review on vermurafenib label so far.

Raf inhibitors that are currently under clinical evaluation have shown promising signs of anti-cancer efficacy with a very tolerable safety profile [12], and will be further discussed on this chapter.

3.1.3. MEK inhibitors

Although MEK mutations are rare in human cancer, MEK inhibitors have been developed as a therapeutic strategy to combat B-RAF inhibitor resistance by targeting downstream effectors. To date, these MEK inhibitors have shown poor efficacy and activity in the clinic. However, with the emergence of resistance to B-RAF therapy, and a higher than previously thought frequency of somatic MEK mutations, these inhibitors are finding renewed clinical use [13].

Several MEK inhibitors have been identified: PD184352 (CI-1040), Selumetinib (AZD6244, ARRY-142886), PD0325901, XL518, GSK1120212 (JTP-74057), ARRY-438162. Worth noticing, most of the known MEK inhibitors are noncompetitive (ie, they do not bind to the ATP–binding site of the kinase) [14]. Despite ATP-biding pockets are highly conserved among human kinases [15], structural analysis of demonstrates tha it harbors a unique site adjacent to the ATP binding site [16]. Thus, biding of inhibitos to this unique MEK site explain the high degree of specificity of the MEK inhibitors compared to other kinase inhibitors with competitive activity.

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3.1.4. mTOR inhibitors

Rapamycin, the canonical mTOR inhibitor, was identified in 1975 as a potent antifungal isolated from *Streptomyces hygroscopicus*, nowadays it is recognized for its immunosuppressive and antitumor activities. However, rapamycin has limited bioavailability duo to its poor aqueous solubility. In an effort to improve its pharmacokinetic characteristics, several rapamycin analogues, named **rapalogs**, have been developed, such the first generation mTOR inhibitors **temsirolimus**, **everolimus**, and **ridaforolimus** [19-21].

In mammalian cells, members of this pharmacological class associate with the intracellular receptor FK506 binding protein 12 (FKBP12). Then, this complex interacts with FKBP12-rapamycin binding (FRB) domain, performing an allosteric mechanism of inhibition of mammalian target of rapamycin (mTOR) kinase activity. Traditionally the rapalogs inhibit only mTOR complex (mTORC) 1, probably because FRB domain is occluded in mTORC2. However, some studies have shown that these compounds are able to disrupt mTORC2 in a dose-, time- and cell type-dependent manner [20, 22]. A possible mechanism by which rapamycin and rapalogs could inhibit mTORC2 would be that rapamycin- or rapalogs-FKBP12 complexes would interact with newly synthesized mTOR molecules. In turn, this interaction would prevent mTOR interaction with RICTOR, inhibiting mTORC2. Indeed, it has been shown that prolonged treatment of cancer cells with rapamycin can promote its binding to mTOR before mTORC2 assembly, and subsequently inhibit Akt signaling [23]. In addition, treatment with temsirolimus or everolimus in acute myeloid leukemia (AML) cell lines blocked mTORC1 as well mTORC2 assembly [24]. In this way, rapalogs inhibit the signal transduction through the mTORCs downstream effectors, as 4E-BP1 and S6K1, resulting in reduction of protein synthesis and cell proliferation, also inhibit cell-cycle progression and angiogenesis, and promote apoptosis. Despite this positive action against cancer cells, these compounds, when inhibiting only mTORC1, lead to relieve negative feedback loop from S6K1 to IRS-1 resulting in PI3K/AKT pathway activation, and, consequently, could promote cell survival and chemoresistance [21, 25].

Temsirolimus and everolimus have been approved by FDA for the treatment of renal cell carcinoma (RCC). Everolimus has been approved for pancreatic neuroendocrine tumors, and recently for HER2-negative BC in combination with exemestane, after letrozole or anastrozole treatment fails. These antineoplastic agents have been investigated in clinical trials for malignancies from many tissues, including breast, gynecologic, gastrointestinal, lung and melanoma, alone or in association with hormonal therapies, EGFR inhibitors, and cytotoxic drugs [3, 19, 26, 27].

The efficacy of rapalogs is partially limited by compensatory mechanism of mTOR activation driven by the loss of negative feedback and because mTOR can be regulated by other signaling pathways such as Ras/Raf/MEK/ERK. Thereby, the inhibition of this pathway alone provides a transient benefit that may result in treatment resistance. It has already been shown the benefits of using mTOR inhibitors in combination with anti-insulin-like growth factor 1 receptor (IGF-1R) monoclonal antibodies. Thus, in order to overcome possible mechanisms of resistance, it would be interesting to establish therapeutic schemes that use combinations of different drugs [4, 27]. Inhibitors of mTOR (TORKinhibs) are still under development/trial, as well as the dual mTOR/PI3K inhibitors and will be discussed further on this chapter.

3.1.5. Ph chromosome-related kinase inhibitors.

In 2003, FDA approved **imatinib mesylate**, a Bcr-Abl fusion tyrosine kinase, leading to impaired proliferation and apoptosis induction of cancer cells. Its indications were for newly diagnosed adult patients with Ph chromosome positive chronic myelogeneous leukemia (CML) in chronic phase, as well as for patients with CML in blast crisis, accelerated phase, or in chronic phase after failure of interferon (IFN)-alfa therapy. Apart from that, imatinib mesylate was also indicated for the treatment of patients with c-Kit positive unresected and/or metastatic malignant gastrointestinal stroma tumors (GIST). The most recent FDA revision on this drug label, from 2012, indicates imatinib mesylate for the treatment of all diseases mentioned above, as well as for: adult patients with relapsed or refractory Ph chromosome positive acute lymphoblastic leukemia (ALL); adult patients with myelodysplastic/myeloproliferative diseases (MDS/MP) associated with PDGFR gene re-arrangements; adult patients with aggressive systemic mastocytosis (ASM) without the D816V c-Kit mutation or with c-Kit mutational status unknown; adult patients with hypereosinophilic syndrome (HES) and/or chronic eosinophilic leukemia (CEL) who have the FIP1L1PDGFRα fusion kinase (mutational analysis or FISH demonstration of CHIC2 allele deletion) and for patients with HES and/or CEL who are FIP1L1-PDGFRα fusion kinase negative or unknown status; adult patients with unresectable, recurrent and/or metastatic dermatofibrosarcoma protuberans (DFSP); and, adjuvant treatment of adult patients following complete gross resection of c-Kit positive GIST.

Dasatinib is a KI of Bcr-Abl, SRC family, c-Kit, ephrin type-A receptor 2 (EPHA2) and PDGFRβ developed to overcome the imatinib-resistance observed in relapsed patients with accelerated phase or blast crisis phase CML [28]. It was approved by FDA in 2006 and is predicted, based on modeling studies, to bind to multiple conformations of ABL kinase. It was initially approved for the treatment of patients with chronic, accelerated, myeloid or lymphoid blast phase CML with resistance or intolerance to prior therapy including imatinib mesylate, as well as for the treatment of adults with Ph chromosome-positive ALL resistant or intolerant to prior therapy. The 2012 label review for this drug also includes its indication for the treatment of newly diagnosed adults with Ph chromosome-positive CML in chronic phase.

Nilotinib hydrochloride monohydrate was approved by FDA in 2007 for the treatment of chronic and accelerated phase Ph chromosome-positive CML adult patients which have developed resistance or intolerance to prior therapy that included imatinib. In 2012, apart from these previous indications, nilotinib hydrochloride monohydrate has also been indicated for the treatment of newly diagnosed adult patients with Ph chromosome-positive CML in chronic phase.

3.1.6. Multi-kinase inhibitors

In 2006 FDA approved **sunitinib malate**, a multi-kinase inhibitor targeting several receptor tyrosine kinases (RTK), such as PDGFR-α and -β, VEGFR-1,-2 and -3, c-Kit, Fms-like tyrosine kinase-3 (FLT3), colony stimulating factor receptor Type 1 (CSF-1R) and the glial cell-line derived neurotrophic factor receptor (RET). At its first approval, sunitinib malate was indicated for the treatment of GIST after disease progression or intolerance to imatinib mesylate; and, for the treatment of advanced RCC, this based on the partial response rates and duration of responses observed for this drug. On the latest FDA review on sunitinib malate indications, in 2012, it was included the indication for the treatment of progressive, well-differentiated pancreatic neuroendocrine tumors (NET) in patients with unresectable locally advanced or metastatic disease.

In 2009, FDA approved the multi-kinase inhibitor **pazopanib hydrochloride** targeting VEGFR-1,-2 and -3, PDGFR-α and –β, fibroblast growth factor receptor (FGFR)-1 and -3, c-Kit, interleukin -2 receptor inducible T-cell kinase (Itk), leukocyte-specific protein kinase (Lck), and transmembrane glycoprotein receptor tyrosine kinase (c-Fms). At its first approval, pazopanib hydrochloride was indicated for the treatment of RCC patients, and at its latest label review, in 2012, it was also indicated for the treatment of patients with advanced soft tissue sarcoma (STS) who have received prior chemotherapy, except for patients with adipocytic STS or GIST, for which pazopanib hydrochloride’s efficacy has not been proved.

During the year of 2011, two KIs with different targets and indications have been approved by FDA. Firstly, **vandetanib** a KI with multiple targets, including VEGFR and EGFR, has been approved for the treatment of symptomatic or progressive medullary thyroid cancer (MTC) in patients with unresectable locally advanced or metastatic disease. Also, **crizotinib** was approved for the treatment of patients with anaplasic lymphoma kinase (ALK) -positive locally advanced or metastatic NSCLC. There was no indication review for this drug so far.

In early 2012, the VEGFR-1, -2 and -3 inhibitor **axitinib** was approved by FDA. This KI was capable of decreasing VEGF-mediated endothelial cell proliferation and growth both in vitro and in animal models. It is indicated for the treatment of advanced RCC patients after failure of previous systemic therapy.

3.2. Kinase inhibitor currently under development or in clinical trial

3.2.1. The PKC inhibitor PKC412/Midostaurin

One of the most promising KI under trial is PKC412/midostaurin. It is a N-Benzoil derivative capable of inhibiting classical protein kinase C (PKC) α, β, γ and the calcium-dependent PKCs δ, ε, η, as well as TK pathways [29]. In 2001, Propper and colleagues published a phase I and pharmacokinetics study on this compound [30]. This study engaged 32 subjects with different types of tumor, which were either refractory to conventional therapy or unresponsive to standard treatment, exposed to seven doses of PKC412/midostaurin (12.5 mg/day – 300 mg/day). From this study it was concluded the PKC412/midostaurin has had as main toxicity nausea/vomiting and fatigue, with significant side effects as diarrhea, anorexia, and headache. A dose-related suppression on circulating lymphocyte and monocyte number was observed after 28 days of treatment. The overall conclusion of this work was that PKC412/midostaurin at 150mg/day would be well tolerated chronically. Currently, 7 active clinical trials using PKC412/midostaurin as single drug or in combination with others can be assessed at the Clinical Trial Search engine from National Cancer Institute [31], as well as, at the U.S. National Institute of Health clinical trial database [32].

Among studies using PKC412/midostaurin in combination with other drugs, two trials from Novartis (CPKC412A2114 and CPCK412AUS06T) evaluate the combined therapy of PKC412/midostaurin with the epigenetic drugs 5-azacytidine and decitabine, respectively. The first trial has been carried out with refractory or relapsed ALL and MDS patients' under 18 years old; the second has been carried out with newly diagnosed or relapsed AML patients over 60 years old. Also from Novartis, a trial (NCT01477606) evaluates PKC412/midostaurin in several combinations with the epigenetic drug cytarabine and the anthracycline daunorubicin, or as single agent, for the treatment of AML patients which express the RTK FLT3-ITD. From the Washington University of Medicine, a study (NCT01161550) evaluated the combination of PKC412/midostaurin with either epigenetic drug cladribine or cytarabine in AML patients. Furthermore, PKC412/midostaurin has been evaluated on a collaborative trial (NCT01174888) from Novartis and Millennium Pharmaceuticals, Inc. in combination with bortezomib (a proteasome inhibitor), mitoxantrone hydrochloride (an anthracenedione), etoposide (a topoisomerase inhibitor) or cytarabine (an epigenetic drug) for the treatment of patients with relapsed or refractory AML. Finally, PKC412/midostaurin has been tested in combination with radiation therapy and 5-fluorouracil for the treatment of patients with advanced rectal cancer in a study (NCT01282502) sponsored by the Massachusetts General Hospital. As a single agent, PKC412/midostaurin has been tested by Novartis (NCT00866281) for the treatment of relapsed or refractory pediatric patients with AML and

ALL. Of interest, by the time this chapter was written, all the above mentioned trials were recruiting participants.

3.2.2. The EGFR inhibitor icotinib

Icotinib has been approved by the State Food and Drug Administration from China, in 2011, under the trade name of Conmana (Beta Pharma Inc.), but it was not yet approved by FDA. It is a reversible EGFR KI capable of inhibiting growth of tumor cell overexpressing EGFR, which underwent two phase I studies reported in 2011 [33, 34]. Both studies demonstrated that icotinib is safe and well tolerated by NSCLC patients and shows positive clinical anti-tumor activities.

3.2.3. PI3 kinase inhibitors

PI3K inhibitors target the p110 catalytic subunit of PI3K, and may be divided into two groups, isoform-specific inhibitors or pan-PI3K inhibitors; the latter can inhibit all class IA PI3Ks. In this way, they block the signal transduction through the PI3K/AKT/mTOR pathway exerting antiproliferative effects. The first-generation of PI3K inhibitors included wortmannin, an irreversible PI3K inhibitor isolated from *Penicillium wortimannin*, and LY294002, a synthetic and reversible PI3K inhibitor. However there are limiting features for their clinical use, which involve low selectivity for PI3K isoforms, poor solubility and toxicity in animals [35, 36].

Several other PI3K inhibitors have been developed in an attempt to overcome these initial limitations. Firstly, CAL-101 has 14 trials on phase I, II or III registered [37]. From these 11 are active, and evaluate either safety or efficacy of the drug alone or in combination with others, for the treatment of indolent non-Hodgkin lymphoma (NHL), chronic lymphocytic leukemia (CLL), mantle cell lymphoma (MCL), follicular lymphoma, small lymphocytic lymphoma (SLL), Hodgkin lymphoma, AML, among others. On the other hand, AMG 319 has only one phase I trial registered on the same data base. This trial is currently recruiting patients with hematologic malignancies. Moreover, XL147 has been on nine clinical trials which evaluate its safety and efficacy, alone or in combination, for the treatment of lymphoma, as well as, several solid tumors such as BC, NSCLC and ovarian cancer (OVCA), among others. Furthermore, GDC-0941 has been on 12 clinical trial, nine of which active. From these, a phase I trials evaluate its effect on solid tumors such as BC and NSCLC, as well as in NHL. Two phase II trial are also active, one with NSCLC patients and the other with BC patients. Other PI3K inhibitor, BYL719, has been on five active phase I and II trials with patients with several solid tumors. Additionally, PX-866 has been on seven pahse I and II trials, five of which active, also for the treatment of solid tumors. Lastly, BKM-120 has been on 43 trials, 42 active, evaluating its effects mostly on solid tumors, such as BC, NSCLC, endometrial carcinoma and glioblastoma, among others [32, 38, 39]. Compounds specifics for a given isoform can be used at lower doses avoiding side effects. Moreover, these isoform-specific compounds have achieved good results in certain cancers. For instance, a specific p110β inhibitor was shown to be more effective in PTEN-deficient cancer [40], whilst it was suggested that PI3K inhibitor specific for p110α might block angiogenesis [41]. However, it is believed that inhibition of one single isoform can lead to activation of another as a compensatory mechanism [42].

3.2.4. Raf inhibitors

As previously mentioned, Raf inhibitors have shown good results on clinical trials. Among these, dabrafenib has been tested as sigle agent or in combination for the treatment of cancer patients, mainly with melanoma. Also, a phase III randomized trial has recently been completed, however the early results have not been relieased by the time this chapter was concluded [43]. Moreover, RAF-265 is currently under phase I and II clinical trials with malignant melanoma patients. This compound is a potent inhibitor of Raf with a highly selective profile and inhibits all 3 isoforms of RAF, as well as mutant BRAF, with high potency [44]. Additionaly, XL281, a specific inhibitor of RAF kinases, including the mutant form of BRAF, and has finished Phase I testing [45].

3.2.5. MEK inhibitors

Although MEK mutations are rare in human cancer, MEK inhibitors have been developed as a therapeutic strategy to combat B-RAF inhibitor resistance by targeting downstream effectors. To date, these MEK inhibitors have shown poor efficacy and activity in the clinic. However, with the emergence of resistance to B-RAF therapy, and a higher than previously thought frequency of somatic MEK mutations, these inhibitors are finding renewed clinical use [13].

Several MEK inhibitors have been identified and are ubdergoing clinical trials: i.e. PD184352 (CI-1040), selumetinib (AZD6244, ARRY-142886), PD0325901, GDC-0973/XL518, trametinib (GSK1120212), MEK162 (ARRY-438162). Worth noticing, most of the known MEK inhibitors are noncompetitive (ie, they do not bind to the ATP–binding site of the kinase) [14]. Despite ATP-biding pockets are highly conserved among human kinases [15], structural analysis of demonstrates tha it harbors a unique site adjacent to the ATP binding site [16]. Thus, biding of inhibitos to this unique MEK site explain the high degree of specificity of the MEK inhibitors compared to other kinase inhibitors with competitive activity.

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3.2.6. AKT inhibitors

Akt inhibition promotes decreasing cancer cell survival and proliferation by preventing signal transduction through its downstream effectors as mTOR. Target Akt is an interesting pharmacological approach due to the Akt activation in consequence of the feedback loop release when mTOR is inhibited. Within this group, we can mention allosteric Akt inhibitors (mK2206), Akt catalytic sites inhibitors (PX316, GSK690693, AT-13148, A-443654) and lipid-based phosphatidylinositol (perifosine, triciribine) [32, 36, 39].

The allosteric Akt inhibitors act preventing the translocation of Akt to the plasma membrane, a crucial step for the activation of this molecule, and are more specific for one Akt isoform than the Akt catalytic sites inhibitors which can target all AKT isoforms [42]. Perifosine, the best-characterized Akt inhibitor, is a lipid-based antitumor agent that inhibits Akt Pleckstrin homology (PH) domain preventing the Akt recruitment to the cell membrane and its activation. Perifosine has shown great efficacy in vitro and in vivo against several human cancers such as breast, ovarian, multiple myeloma and glioma and has been tested in clinical trials [30, 32].

3.2.7. mTOR inhibitors

The ATP-competitive inhibitors of mTOR (TORKinhibs) directly inhibit the mTOR kinase activity affecting both mTORC1 and mTORC2. Thus, resulting in antiproliferative effects by decreasing protein synthesis, inducing cell cycle arrest, and inhibiting angiogenesis in several cancer cell lines [22]. Many TORKinhibs have been developed, including Torin1, Torin2, PP242, PP30, KU0063794, WAY-600, WYE-687, WYE-354, XL-388, INK-128, AZD-2014, AZD8055 and OSI-027. Some of them are currently being tested in human subjects with hematological malignancies, glioma and advanced solid tumors in phase I trials [3, 21, 32].

TORKinhibs have achieved better results than rapamycin and rapalogs. This is due to the additional inhibition of mTORC2, which prevents Akt phosphorylation at S473, and also can inhibit mTORC1 with a higher potency. It has been postulated that complete inhibition of mTORC1 is responsible for this enhanced response to treatment, overcoming the limitations of rapamycin. However, it has been found that loss of feedback on PI3K results in activation of downstream effectors other than Akt. Furthermore, these drugs induce phosphorylation of Akt at residue T308, mediated by PDK-1, configuring a resistance mechanism that requires a different therapeutic approach [18, 22, 46].

3.2.8. PI3K and mTOR dual inhibitors

A strategy to overcome the limitations of rapalogs and TORKinhibs is to target two molecules in the PI3K/Akt/mTOR pathway, PI3K and mTOR. The dual PI3K/mTOR inhibitors include NVP-BEZ235, BGT226, XL765, SF1126, GDC-0980, PI-103, PF-04691502, PKI-587, and SK2126458. These drugs inhibit the catalytic activity of mTOR, targeting both mTORC1 and mTORC2 like the TORKinhibs, beyond that they also inhibit PI3K catalytic subunit. Thus, they act on two fronts in the PI3K/AKT/mTOR signaling pathway decreasing cell proliferation, angiogenesis, apoptosis, and inducing cell cycle arrest [21, 47].

The dual PI3K/mTOR inhibitors have demonstrated a greater antitumor efficacy than rapamycin but also have increased toxicity. Nevertheless, some of them are in phase I/II clinical trials for the treatment of lymphoma, glioma, advanced and refractory solid tumors and presented overall good tolerability [8]. Their potent antitumor effect can be explained by the inhibition of AKT phosphorylation at two sites, S473 and T308, blocking downstream signaling more efficiently than rapamycin/rapalogs and TORinhibs alone, as demonstrated in preclinical studies of NVPBEZ-235 and PI-103 [3, 22, 47].

4. Epigenetic drugs

4.1. Histona deacetylace inhibitors

Due to its ability to regulate gene transcription, histone acetylation has been increasely studied. Histone deacetylases (HDACs) are a group of enzymes that, in conjunction with histone acetyltransferases (HATs), regulate the acetylation status of histone tails. HATs acetylate lysine residues on histone tails resulting in neutralization of their charge and decreased affinity for DNA [48].

There are 18 HDACs, which are classified according to functional and phylogenetic criteria [49]. They are divided into Zn2+-dependent (class I, II and IV), Zn2+-independent and NAD-dependent (classIII) enzymes. Most inhibitors currently under development as anti-cancer agents target class I, II and IV enzymes [50].

There are numerous studies demonstrating that HDACs and HATs also regulate acetylation of nonhistone proteins, including transcription factors, chaperone proteins, and signaling molecules involved in cancer development and progression, such as the tumor suppressor p53 [51]. Furthermore, these enzymes are often overexpressed in various types of cancers, compared with the corresponding normal tissues, and their overexpression is correlated with a poor prognosis [52], because they can drive the silencing of tumor suppressor genes or activation of oncogenes [53].

Over recent years, it has been found that the epigenetic silencing of tumor suppressor genes induced by overexpression of HDACs plays an important role in carcinogenesis, above all in hematological cancers [54]. Thus, HDAC inhibitors (HDACi) have emerged as promising accessory therapeutic agents for multiple human malignancies, as, through their action, tumor suppressor gene expression can be restore, cell differentiation can be induce, and both intrinsic and extrinsic apoptotic pathways can be activated [55]. Also, by targeting HDAC6, for example, these inhibitors can stimulate cell cycle arrest, autophagy, and anti-angiogenic effects, can induce oxidative injury, and interfere with tubulin assembly, and cause disruption of the aggregosome pathways [50].

Several HDACi derived both from natural or synthetic sources have been identified. These compounds share a common pharmacophore containing a cap, a connecting unit, a linker and a zinc binding group that chelates the cation in the catalytic domain of the target HDAC [56]. Thus, this class of inhibitors can be separated into several structurally distinct classes according to their chemical structure [53, 57], and each agent varies in its ability to inhibit individual HDACs.

Regarding short chain fatty acids class, **valproate** (valproic acid, VPA) has been used as an anticonvulsant for three decades, and has only recently been recognized as an HDAC inhibitor. It specifically targets 2 of the 4 classes of HDACs: class I, subclasses Ia and Ib, and class II, subclass IIa. Within subclass IIa, HDAC9 is an exception to this modulation, being activated by VPA, which is also true for HDAC11 [58]. **Butyrate**, also a short chain fatty acid, naturally produced by bacterial fermentation in the colon, has been designated as the most potent fatty acid in arresting cell proliferation [59].

Another class of these inhibitors includes hydroxamic acids. In this group, **vorinostat** (SAHA) and **panobinostat** (LBH 589) are the most extensively studied drugs. The latter is currently under phase II/III clinical trials, and the former has been approved by FDA for the treatment of relapsed and refractory cutaneous T-cell lymphoma [60]. Vorinostat represents the second generation of the polar-planar compounds and is a relatively selective inhibitor for class I HDACs; that is, by inhibiting HDAC-1, −2, −3 and −8, but also with mild activity against class II HDAC-6, −10 and −11 [61]. However, vorinostat lacks activity against class II HDAC-4, −5, −7 and −8. **Belinostat**, other compound of this group, has shown efficacy as monotherapy, and has been the basis for the first pivotal phase I trial of this agent to treat relapsed or refractory peripheral T-cell lymphoma [62]. Belinostat's anticancer effect is thought to be mediated through multiple mechanisms of action, including the inhibition of cell proliferation, induction of apoptosis, inhibition of angiogenesis, and induction of differentiation [63]. Moreover, it has been demonstrated that **resminostat** inhibits proliferation and induces apoptosis in multiple myeloma cells [64]. HDACi **PCI-24781** has been shown to enhance chemotherapy-induced apoptosis in multidrug- resistant sarcoma cell lines [65]. **Givinostat** is currently being tested on three trials, but none of these on neoplasias [32], and **JNJ-26481585** shows results in blood malignancies in phase I trial as monotherapy and in combination with proteasome inhibitor (bortezomib).

On benzamides, **entinostat** (MS-275) is an isotype-selective synthetic benzamide derivative HDACi with predominant class I inhibition. Entinostat has been investigated in patients with advanced refractory acute leukemias, mainly acute myeloid leukemia [66]. Whereas, **mocetinostat** is well-tolerated and exhibits favorable pharmacokinetic and pharmacodynamic profiles indicating target inhibition and clinical responses. It induces cell death and autophagy, synergizes with proteasomal inhibitors and affects non-histone targets, such as microtubules [67]. Yet, mocetinostat shows selectivity for HDAC I/II. It has been used in clinical trials mostly for hematological malignancies, such as AML, CML, NHL and refractory Hodgkin disease, where it has shown very encouraging results [68].

Regarding cyclic tetrapeptides, **romidepsin** (ISTODAX®) shows potential as a new agent, having revealed remarkable activity in the treatment of T-cell lymphomas in preclinical studies and early-phase clinical trials. In 2006, it was approved by FDA for the treatment of CTCL in patients who have received at least one prior systemic therapy [69].

Preclinical studies in cell lines and animal models, HDACi have been proven to be very successful as single-modality agents for the treatment of a variety of cancers. Thus, several structurally different HDACi have been used in numerous clinical trials to test their toxicity and effectiveness [32]. The most common adverse effects associated with HDAC inhibitors include thrombocytopenia, neutropenia, diarrhea, nausea, vomiting and fatigue. Extensive studies have been performed to determine whether HDAC inhibitors are associated with cardiac toxicities. Until now, there is little conclusive evidence to determine whether some or all HDAC inhibitors cause electrocardiac changes [70].

Mechanisms of resistance to HDACi are not well elucidated; however it's believed that it may reflect drug efflux, epigenetic alterations, stress response mechanisms and anti-apoptotic, and pro-survival mechanisms [71]. In this context, it is known that DNA hyper-methylation may cause resistance to HDACi, inducing compact nucleosomes, blocking the access to acetylases, which leads to tumor suppression genes silencing [49].

4.2. Rational combination of HDAC inhibitors with current cancer therapy

HDACi have revealed promise in the clinic but there is clearly space for improvement of therapeutic index. One way to achieve greater clinical efficacy is to use HDACi in combination with other chemotherapeutic agents [53, 72]. There have been numerous preclinical and clinical studies examining rational combinations of HDACi with many current therapies for the treatment of hematological and solid malignancies [60]. Indeed, it has been described that HDACi have synergistic or additive effect with different anticancer agents, including radiation therapy, chemotherapy, hormonal therapies and new targeted agents.

Regarding HDACi in combination with radiotherapy, these inhibitors, including vorinostat, TSA, valproic acid and PCI-24781, enhance the radiosensitivity of cancer cells [73]. Chemotherapeutic agents with additive or synergistic effects with HDACi therapy includes: antitubulin agent (docetaxel) [74]; topoisomerase II inhibitors (doxorubicin, etoposide, and ellipticine) [75, 76]; and DNA cross-linking reagent (i.e. cisplatin) [77].

HDACi combinations with hormonal therapy are also possible. In this context, clinical trials are in progress for BC and prostate cancer (PC). As a monotherapy, the HDACi vorinostat has not shown effectiveness in metastatic BC and PC [78]. On the other hand, preclinical studies have demonstrated that HDACi potentiates the antitumor activity of tamoxifen in a variety of ER-positive BC cell lines [79]; whereas in PC the addition of an HDACi to the anti-androgen bicalutamide have resulted in a synergistic increase in cytotoxicity on hormone-sensitive and resistant preclinical models [80].

Recent studies showed that the combination of some of the specific RTK-targeted therapies with HDCAi can represent a novel way for suppressing tumor growth. Combined therapies with transtuzumab [81], erlotinib and gefitinib [82], sorafenib [83], everolimus [84], imatinib [85], heat shock protein-90 inhibitor 17-N-allylamino-17-demethoxygeldanamycin [86] and bortezomide, a proteasome inhibitor [87], have been studied. The obtained data indicate that, although preclinical studies demonstrated a benefit, it is too early to know whether this combination will prove more beneficial than treatment with RTK pathway inhibitors alone.

Hematological malignancies appear to be particularly sensitive to HDACi therapy. There are well over 100 clinical trials ongoing with HDACi as monotherapy or in combination therapy for several carcinomas. The available results for these clinical trials have recently been reviewed [50]. As mentioned, vorinostat and romidepsin have been approved by FDA for the treatment advanced and refractory cutaneous T-cell lymphoma (CTCL). The clinical value of HDACi in other malignancies remains to be determined.

5. PARPs inhibitors

Poly ADP ribose polymerases (PARPs) are a family of 17 proteins pooled together based on their structural similarity, specifically, they are composed by two ribose moieties and two phosphates per polymer unit [88]. Known since 1963, these enzymes function is to catalyses the polymerization and formation of highly negatively charged poly ADP ribose chains on target proteins, therefore modifying their action [89]. Furthermore, PARPs contain three zinc finger motifs which bind with high affinity to DNA breaks and triggers the enzyme's catalytic module and synthesis of negatively-charged, branched polymers of poly(ADP-ribose) (PAR) from NAD+ [90]. Currently, PARP 1 and PARP 2 are the best understood of these proteins and their key role is to maintain genomic integrity, in particular the repair of single strand DNA lesions and breaks, using the base excision repair (BER) pathway [91]. Moreover, PARPs are also involved in activating apoptosis on both caspase dependent or independent fashion; however this PARP hole is not yet fully understood and will not be discussed in this chapter [92].

5.1. PARPs inhibitors in cancer therapy

Durkacz and colleagues proposed, in 1980, that modulating PARP-1 might augment the effect of alkylating chemotherapy [93]. So far the modulation of its activity by stimulation or inhibition can be applied in therapy or prevention of several pathologies including cardiac infarct [94], septic shock [95], diabetes [96], inflammation [97], neurodegenerative disorders [98], and acute necrotizing pancreatitis [99]. Lately a new potential strategy for therapy has emerged, the PARP inhibitors, using the synthetic lethality and exploiting tumor-specific genetic alterations. Synthetic lethality is defined as the premise, whereby, deletion of one of two genes independently has no effect on cellular viability, whereas, simultaneous loss of both genes is lethal [100]. It has become clear that the genomic instability of some tumor cells allows PARP inhibitors to have selectivity for the tumor cells over normal cells, what explains why this class of drugs shows fewer side effects as a single agent. Taken together, inhibition of these enzymes and, therefore, the BER pathway causes persistence of single strand breaks (SSBs) leading to cell death. Also, PARP inhibitors, when in combination with cytotoxic agents, prevent repair of SSBs caused by these agents in cells with underlying homologous recombination (HR) defects [101].

It has been shown that cancer cells with mutations in the breast and ovarian susceptibility genes BRCA1 and BRCA2 are extremely sensitive to small molecule inhibitors of PARP-1 [102, 103]. Thus, PARP inhibitors have raised as a promise in phase I and phase II clinical trials for the treatment of BRCA1/2-deficient breast, ovarian and prostate tumors [104-106]. However, a recently completed phase III study combining PARP inhibition with chemotherapy did not generate the anticipated survival gains; suggesting that additional, as yet unidentified, molecular factors may influence the *in vivo* anti-tumor effectiveness of this class of drugs [107, 108].

5.2. PARPs inhibitors under clinical development

Some PARP inhibitors, targeting both PARP-1 and PARP-2, were recently under clinical development, which include Pfizer's PF 01367338 (AG014699), AstraZeneca's olaparib (AZD2281, KU-0059436), Sanofi-Aventis' iniparib (BSI 201) and Abbott Laboratories' veliparib (ABT 888) [109].

The first agent analyzed clinically was AG014699 (the phosphate salt of AG14361), in 2003. Publications described preclinical data for 39 OVCA cell lines (without reporting BRCA status of these cell lines) with AG014699 as single-agent or in combination (with carboplatin, doxorubicin, gemcitabine, paclitaxel, or topotecan) using combination index/isobologram analysis for multiple drug effect analysis. The investigators noted a concentration-dependent efficacy across the different cell lines to different degrees. The greatest impact appears to be in combination with carboplatin, topotecan, and doxorubicin. Therefore, an initial phase I was conducted with temozolide (TMZ), both given for 5 days in 28-day cycles, with patients with solid tumors. A subsequent phase II study with melanoma patients has been reported. Overall, there was modest activity with significant myelosuppression. The study started using one-half standard dose (100 mg/m^2) of TMZ and AG014699 was escalated to PARP inhibitory dose (PID) as evaluated from peripheral blood mononuclear cell (PBMCs). The PID, defined as at least 50% of decrease in PARP activity 24 hours after dosing, was determined to be 12 mg/m^2 and at this dose there was 74-97% inhibition of peripheral blood mononuclear cells (PBMCs) PARP. The mean terminal half-life was 7.4-11.7 hours. The PARP in the PBMCs recovered at least 50% function by 72 hours after dosing. The dose limiting toxicity (DLT) for the highest dose level tested of 18 mg/m^2 in combination with standard dose TMZ and lead to myelosuppression [110]. The phase II study evaluated the efficacy of AG014699 at 12 mg/m^2 with TMZ at 200 mg/m^2 in 40 chemotherapy-naive patients with advanced multiple melanoma. Myelosuppression was more significant in the phase II trial than seen in the phase I trial. It was reported several signs of toxicity besides fatigue and nausea: 12% grade four thrombocytopenia, 15% neutropenia, and one death from febrile neutropenia. There were four partial responses (PRs), four prolonged stable diseases, and 10 patients were too early to evaluate at the time of the report [111].

Olaparib is an oral PARP inhibitor (IC_{50} = 4.9 nM for PARP 1) extensively studied for BRCA tumors treatment in combination or as single agent. In a phase I trial, olaparib was given at days 1-4, cisplatin at day 3, and gemcitabine at days 3 and 10, every 21 days. As toxicities effects, five of six patients experienced grade three or four thrombocytopenia. Two PRs were reported in 1 pancreatic cancer and 1 NSCLC patient [112]. Another phase I, this time focusing olaparib as single-agent, enrolled 60 patients with solid tumors, including 22 BRCA mutation patients. This study supported the synthetic lethality concept. Patients were treated at escalating doses and duration. Doses of 10 mg QD 2 out of 3 weeks to 600 mg BID continuously were evaluated. The initial cohort was not restricted to BRCA-deficient patients but was enriched for this population. In the expansion cohort, patients had to have BRCA mutation to enroll and were treated at 200 mg BID continuously. Eight PRs, by response evaluation criteria in solid tumors (RECIST), were observed out of the 15 patients with BRCA mutation-related advanced OVCA group. All the responses in OVCA were seen in BRCA mutated tumors [105].

Iniparib (BSI 201 or 4-iodo-3-nitrobenzamide) is a prodrug which irreversibly inhibits PARP-1 and it is the first PARP inhibitor to show survival advantage in triple-negative breast cancer (TNBC) patients. It has entered in phase III study despite the fact its active metabolite is still unknown. Iniparib is given intravenously twice a week. The phase I study included 23 patients with solid tumors. The concentration that brought about efficacy in preclinical models was 20-30 ng/mL, so achievable levels were well over the preclinical efficacious levels. The 2.8 mg/kg dose caused PARP inhibition in PBMCs by more than 50% with the first dose. Subsequent dosing increased the amount of PARP inhibition to more than 80%. Six of the 23 heavily pretreated patients had stable disease for at least 2 months (up to over 9 months in 1 patient) [113].

Veliparib has been shown to be a potent inhibitor of PARP, as well as, to have a good bioavailability. In pre-clinical studies veliparib potentiated TMZ, platinum agents, cyclophosphamide, and radiation in syngeneic and xenograft tumor models [114]. Combined with topotecan, veliparib has showed significant myelosuppression. The original schedule was topotecan at days 8 and 2-5 at 1.2 mg/m^2, and veliparib 10 mg BID at days 1-7. The schedule was changed to topotecan at days 1-5 when 0.9 mg/m2 of it was not tolerated [115]. Furthermore, PARP inhibitors also augmented the effect of irradiation *in vivo,* as shown in mouse colon cancer xenograft model, where combined therapy increased survival from 23 days with radiation alone to 36 days. One subject also presented complete remission (CR) [108].

Unfortunately, as well as for other therapies, resistance to PARP inhibitors has already been reported. A possible explanation for that would be that a second mutation, a compensatory mutation or a crossover could reestablish the wild-type BCRA protein, reversing the BCRA deficiency [109]. Additionally, upregulation of p-glycoprotein efflux pump, 53BP1 silencing 53BP1 and increased expression of PARP by the tumor have also been shown as a resistance mechanism for PARP inhibitors [116]. Nevertheless, overcoming this resistance could be achieved by: a third mutation on BCRA, which converts the cell back to the mutated form; a mutation that inhibits HR; downregulation of the P-glycoprotein pump; or, upregulation of 53BP1. Recently 6-thioguanine (6-TG) has been shown to be active in cells resistant to PARP inhibitors in BRCA2 deficient tumors [117].

6. Conclusions

Despite the difficulties encountered by physicians and patients in the fight against cancer, we are currently witnessing an ever growing spectrum of new targets and strategies to combat this disease. Considering that an optimal therapy for cancer would be developed based on specific aspect of each patient, target therapies appear as important alternatives to overcome the hurdles presented by currently available strategies. Moreover, as different molecules can be targeted at once, in combination or not with conventional therapies, issues associated to resistance are thought to be milder than with chemotherapy alone. Altogether, we consider that target therapy brings the possibility of increasing patients' overall survival, quality of life, and, maybe, could point to the possibility of vanquishing this disease.

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