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Targeting non-receptor tyrosine kinases using small molecule inhibitors: an overview of recent advances

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Abstract

Protein tyrosine kinases are enzymes that catalyse the transfer of phosphate groups from ATP to tyrosine residues on other proteins as substrate. Phosphorylation at tyrosine residues regulates several functions, including enzyme activity, cellular localization, signal transduction, and interactions between proteins. Non-receptor tyrosine kinases (nRTKs) are one of the main players in intracellular signaling pathways. Dysregulation of nRTKs leads to their constitutive activation, which might contribute to initiation or progression of cancer. Therefore, targeting dysregulated nRTKs may prevent the process of tumorigenesis. Targeted-based cancer therapy (TBCT) methods and agents or personalized medicine have emerged as the main tools for cancer treatment. Currently, several TBCT agents, including monoclonal antibodies (mAbs) and small molecules inhibitors of tyrosine kinases (TKIs) have been developed. TKIs of cytoplasmic kinases inhibit intracellular signaling pathways and interfere with tumor cell functions. In this article, the recent progresses in development of TKIs of nRTKs approved by the US Food and Drug Administration (FDA) and current promising TKIs in preclinical and clinical settings have been reviewed.

Introduction

Recent outstanding progresses in tumor biology have improved and changed the traditional concept of cancer treatment. It is known that most tumors express dysregulated and specific molecules. These molecules may be classified as tumor specific antigens (TSA) and tumor associated antigens (TAA). A few TSAs have been shown to be pan-tumor markers [1]. ROR1 receptor tyrosine kinase-like orphan receptor 1 is an example of a pan-tumor associated marker, which is expressed by several tumors [2-8]. These dysregulated molecules are the major players that are required for tumorigenesis process [2, 4-5].

Protein kinases (PKs) are one of the most investigated classes of tumor targets for different approaches of targeted based-cancer therapy (TBCT). Phosphorylation of protein substrates by PKs results in several functional changes of the target protein by altering the activity of the target molecule. These changes modify the cellular location and association of target proteins with other proteins as well as activation or inactivation of signaling pathways. Currently, 500 kinases (2% of genome) that modify up to 30% of cellular proteins have been known to control the majority of intracellular pathways, especially those which are involved in the process of signal transduction [9].

Protein tyrosine kinases (PTKs) are a major subtype of PKs that catalyse and phosphorylate tyrosine (Tyr) residues and are mainly involved in signal transduction. They usually behave as growth-factor receptors (receptor tyrosine kinases (RTKs)) or downstream signaling molecules [10]. PTKs that are downstream of RTKs are called non-receptor TKs (nRTKs) and constitute different proteins involve in different signaling pathways. The vast majority of these proteins are complicated in several diseases, including cancer and inhibition of these abnormal kinases has gained the attention of several researchers for cancer treatment using TBCT agents [11].

TBCT has seen a rapid development of numerous specific agents and new methods. This field has shown the development of promising drugs that selectively targets tumor antigens that are necessary for cancer cell survival [12]. Currently, monoclonal antibodies (mAbs) and small molecule inhibitors of tyrosine kinases (TKIs) are the most specific agents of TBCT [10, 12-13].

Among several molecules, targeting nRTKs has shown rapid and impressive therapeutic applications. Currently, most TKIs are multi-targeted inhibitors that suppress several proteins.

Due to the lack of cumulative data on selective TKIs of nRTKs, in this article, recent advances in targeting nRTKs using the most selective/specific TKIs have been reviewed.

Tyrosine kinase inhibitors (TKIs)

TKIs have been recently classified into five classes. Type 1 TKIs are the most common type of inhibitors, which are adenosine 3-phosphate (ATP)-competitors. These inhibitors detect the active structure of the substrate and mimic ATP [14].

Functional forms of a kinase might be described by the place of a conserved Asp–Phe–Gly (DFG) amino acid sequence (conserved activation loop in several kinases) in the activation site. Type I usually targets the ATP binding site in the active DFG-in conformation. Type II inhibitors induce a different DFG-out (inactive form) conformation and occupy an additional hydrophobic pocket created by this sequence rearrangement. These inhibitors have several advantages compared to type I, including a better selectivity and slower off-rate for targeted kinase (due to the stabilization of inactive kinase conformation, the off rates are significantly lower than their type I counterparts) [15-16]. ABL proto-oncogene inhibitors such as imatinib (Gleevec or Glivec) and nilotinib (Tasigna), as well as KIT and RAF inhibitor sorafenib (Nexavar), are among this type.

Type III or allosteric inhibitors bind to the allosteric site of the substrate proximal to the ATP binding pocket and have shown to be highly selective and specific for kinases. IκB kinase (IKK) inhibitor (BMS-345541) and the mitogen-activated protein kinase (also known as MAP2K, MEK, MAPK) inhibitor (CI-1040) are among this group [14].

Type IV inhibitors (pure allosteric enzyme inhibitors) form an irreversible bond to the active site of the substrate through binding to a cysteine (Cys) residue. Epidermal growth factor receptor (EGFR) inhibitor HKI-272 and Bruton's tyrosine kinase (BTK) inhibitor ibrutinib belong to this group [16].

Type V inhibitors are classified as a new type, and a few TKIs hve been classified in this group. These inhibitors are bivalent compounds that target two distinct regions of the kinase. This bivalent binding will significantly increase affinities and enhances the selectivity of TKI for the targeted kinase. These new type of inhibitors might be the most useful drugs for personalized medicine and investigating signal transduction (Fig 1).

Currently, several selective/specific nRTK-TKIs have been developed and most of them are in preclinical investigations. Due to the large number of these TKIs, in the next sections, the most important and selective nRTK-TKIs have been briefly described.

Intracytoplasmic tyrosine kinases and TKIs

Proto-oncogene tyrosine-protein kinase SRC family

SRC family kinases belong to nRTKs and contain nine protein members divided into three subfamilies, including SRC, gardner-rasheed feline sarcoma oncogene (FGR), YES, and FYN (subfamily A) as well as B lymphocyte kinase (BLK), HCK, LCK, and LYN (subfamily B) and FYN-related kinase (FRK) subfamily [17].

SRC kinases are controlled by several types of receptors, including RTKs, cytokine receptors, G-protein coupled receptors, steroid hormone receptors, and integrin receptors and are involved in cell migration and motility, cell proliferation, survival, and gene expression regulation through communication with integrins, E-cadherin and focal adhesion kinase (FAK) [18].

SRC interacts with several RTKs such as EGFR, human epidermal receptor (HER) 2, MET, and platelet-derived growth factor (PDGF) that are overexpressed or mutated in several tumors [19].

SRC members have highly similar structure; therefore it is difficult to selectively target SRC members by TKIs. However, it has been demonstrated that the orientation and linker between the SRC homology (SH) domains of some SRC members are different from each other [20]. Based on these differences several selective TKIs have been produced, but none of them have entered preclinical and clinical trials [21].

Currently, several multi-targeted TKIs, including bosutinib (bosulif/SKI-606), dasatinib (Sprycel, BMS-354825), saracatinib (AZD0530), and imatinib have been shown to target SRC kinases and are approved by the US Food and Drug Administration (FDA) for the treatment of different malignancies (Table 1).

Bosutinib

Bosutinib is a dual SRC- and B-cell receptor (BCR)/ABL-TKI with IC_{50} values of 1.2 and 1 nM, respectively in cell-free assays. It received the FDA approval on September 4, 2012, for the treatment of Philadelphia chromosome positive (Ph^+) chronic myeloid leukemia (CML) patients with resistance or intolerance to prior therapy [22].

The combination effects of bosutinib versus imatinib were evaluated in BELA trial in newly diagnosed chronic myeloid leukemia patients. The trial did not meet the primary endpoint results. Moreover, the drugs showed partial and distinct safety profile. Liver and gastrointestinal complications were frequent in bosutinib treated patients, whereas musculoskeletal disorders, neutropenia, and edema were observed in imatinib treated group [22].

Bosutinib is currently in clinical trials for the treatment of breast and pancreatic cancers, acute lymphocytic leukemia (ALL) and glioblastoma [18]. Moreover, the effects in combination with other inhibitors and cytotoxic agents are testing in several clinical trials (www.clinicaltrials.gov).

Dasatinib

Among several multi-targeted TKIs, dasatinib is an appropriate TKI that target most SRC family members, including HCK, FGR, FRK, SRC, LYN, LCK, FYN, YES, and BLK. Moreover, this TKI targets discoidin domain receptor 1 (DDR1), ABL, c-KIT, c-FMS, platelet-derived growth factor receptors (PDGFR) α and β, and ephrin receptors [23]. Dasatinib has been shown to block several cellular functions such as cell duplication and proliferation through G0-G1 arrest, migration, invasion, and induced tumor cells apoptosis [23]. This SMI was approved on June 28, 2006, for the treatment of Ph^+ CML and ALL. On October 28, 2010, the FDA granted accelerated approval to dasatinib for the treatment of newly diagnosed adult patients with Ph^+ CML in chronic phase (CP-CML). This drug is testing in several clinical trials for the treatment of patients with solid tumors and ALL [18]. Hematopoietic toxicity, diarrhea, hypertension, and fatigue have been described the major side effects of dasatinib [24].

Saracatinib

Saracatinib is more selective for SRC members than other non-specific SRC inhibitors and targets BLK, LYN, YES, FYN, LCK and, FGR. It also inhibits ABL and EGFR (with L858R and L861Q mutations) kinases and is testing in several clinical trials for the treatment of ovarian, colorectal, gastric and small cell lung cancers, non-small cell lung carcinoma (NSCLC), and osteosarcoma (www.clinicaltrials.gov).

Preclinical studies have shown promising effects of this TKI. Saracatinib has shown good pharmacokinetic, aqueous solubility and moderate binding to plasma proteins in animal models. It blocked the growth of tumor cells in a 3T3-fibroblast xenografted model transfected with c-SRC *in vivo* and increased the survival of animal transplanted with human pancreatic cancer [25].

Saracatinib reduced CML and Ph^+ ALL cell lines growth but has no effects on the Ph^- ALL cells. It inhibited imatinib-resistant Ba/F3 cells growth, which express the mutations E255K and Y253F [26] and in xenografted mice, it reduced tumor cell growth [26].

Combination of saracatinib and anti-estrogen drug tamoxifen reduced SRC phosphorylation, activity and blocked expression of c-MYC and cyclin D1 in MCF7 and T47D breast cancer cell lines. The combination treatment inhibited tumor cell proliferation, abrogated invasive behavior of cells *in vitro* and prevented the emergence of tumor cell resistance to tamoxifen alone therapy [27].

Saracatinib has been shown to be effective in lung cancer cell lines and decreased migration and invasion of cells, inhibited AKT activation and sensitized tumor cells to irradiation [28] as well as inhibition of pancreatic tumors growth in mice xenografts [29].

This TKI is testing in clinical trials for the treatment of solid tumors, including prostate, head and neck, pancreatic, breast, colorectal, NSCLC cancers, and osteosarcoma, as single agent therapy or in combination with other drugs.

A phase II trial of saracatinib has been done in patients with advanced castration resistant prostate cancer [30]. The primary endpoint was 30% or greater decrease of prostate-specific antigen (PSA). Twenty eight patients were treated with 175 mg orally once daily continuously. Five

patients showed transient PSA reduction. Saracatinib showed to be well-tolerated and the median progression-free survival (PFS) time was determined to be 8 weeks [30].

JAK/STATs pathway

Janus kinase (JAK)/ signal transducers and activators of transcription (STAT) signaling pathway is one of the most important pathways, involved in the immune system for direct communication from transmembrane signals to the nucleus [31]. This pathway is activated by cytokines. Upon stimulation by ligand, cytokine receptor-associated JAK molecules phosphorylate the intracellular tail of their receptors and create docking sites for STAT transcription factors. STATs phosphorylation by JAKs activates STATs and induces their transfer to the nucleus to regulate gene expression [32-33].

JAK family consists of four members, including JAK1, JAK2, JAK3, and TYK2 that bind to different receptors with distinct roles and thereby discrete functional outcomes are sought. Moreover, STAT family has seven members (STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6).

Constitutive phosphorylation and activation of JAKs and STATs was first reported as being associated with malignancy in the 1990s [34]. The JAK/STAT pathway is activated by several mechanisms such as activating mutations of receptors and autocrine or paracrine cytokine secretion which then activates STATs. Hyperactivation of STAT members has been demonstrated in several tumors. Of several members of STATs, constitutive activation of STAT3 has been shown in several types of solid tumors and hematologic malignancies [32-33, 35-38].

Among STAT members, STAT3 is vital for several RTK signaling pathways, including ROR1 [1, 4-5, 39-40] and EGFR [41] and is constitutively phosphorylated in several solid tumors and is involved in cell proliferation and immune tolerance as well as tumor angiogenesis. Inhibition of this molecule induces apoptosis of tumor cells in STAT3-activated cells [42].

OPB-51602

OPB-51602 is a SMI of STAT3 with proper preclinical results. OPB-51602 prevents the phosphorylation and activation of STAT3 molecule, impeding it from moving from the cell cytoplasm to the cell nucleus and therefore inhibiting the regulation of gene expression by STAT3 via binding to the respective gene promoters. This TKI blocks the phosphorylation of STAT3 at Tyr and serine (Ser) residues 705 and 727, respectively. These residues are essential for full activation of STAT3.

OPB-51602 has been evaluated in a phase I trial for the treatment of refractory solid malignancies, including NSCLC [42]. In this study, the maximum tolerated dose showed to be 5 mg per day dosing. Data demonstrated the dephosphorylation of STAT3 (Tyr 705) following OPB-51602 exposure. Of fifty-one patients, two patients showed partial response. First cycle dose-limiting toxicities were observed in two patients (grade 3 hyponatremia and dehydration) and common side effects were diarrhea, fatigue, vomiting, anorexia and early-onset peripheral neuropathy [42].

OPB-31121

OPB-31121 is another STATs inhibitor, which its effects have been investigated in gastric cancer and xenograft models [43]. OPB-31121 reduces JAK2 expression and phosphorylation leading to inhibition of STAT3 activation. Moreover, it induces the apoptosis of tumor cells, reduces the expression of anti-apoptotic proteins, preventes interleukin-6 (IL-6)-induced JAK/STAT signaling pathway, reduces tumor cell proliferation *in vitro* and in a xenograft model, and exhibits synergism with 5-fluorouracil and cisplatin drugs [43].

Furthermore, OPB-31121 inhibited the phosphorylation of both STAT3 and STAT5 and prevented tumor cell growth in multiple myeloma, Burkitt lymphoma and leukemias with expression of fms-like tyrosine kinase (FLT3)/ internal tandem duplication (ITD), JAK2-V617F and BCR/ABL oncoproteins. It also showed anti-tumor effects against primary human leukemia cells in an immunodeficient mouse transplant model [44].

In the first clinical trial, OPB-31121 was investigated for the maximum tolerated dose and biologic activity, safety and pharmacokinetics in thirty patients with advanced tumors [45]. In

this study, patients received twice daily of the OPB-31121 at six doses (50-350 mg). Grade 3 lactic acidosis as well as grade 3 vomiting and diarrhea were observed at 300 and 350 mg, respectively. Grade 1 and 2 side effects were nausea, vomiting, diarrhea, and fatigue. Pharmacokinetics showed high variability between different patients. However, no objective responses were observed after two cycles of treatment [45].

Ruxolitinib

Ruxolitinib (INCB018424 or INC424) is a selective inhibitor of JAK1 and JAK2 [46]. It was approved by the FDA on November 16, 2011, for the treatment of myelofibrosis, polycythemia vera and thrombocythemia as well as December 4, 2014 for the treatment of patients with polycythemia vera who have had an inadequate response to or are intolerant of hydroxyurea (www.fda.gov) [46].

Preclinical studies have shown that it dephosphorylated JAK1 and JAK2 and inhibited IL-6 signaling with an IC_{50} value of 281 nM in cell-free assays [47]. Cell lines carrying JAK2 V617F mutation exhibited growth inhibition and apoptosis when treated with ruxolitinib. In a mouse model of JAK2 V617F myeloproliferative neoplasm, ruxolitinib induced apoptosis of neoplastic cells and decreased splenomegaly and inflammatory cytokines and resulted in prolonged mice survival [47].

Ruxolitinib has been investigated in several preclinical and clinical trials for the treatment of myelofibrosis patients [48-50]. In the COMFORT-II clinical trial ruxolitinib treatment showed 42% reduction in risk of death. After 3.5 years follow-up, the probability of survival was shown to be 71% in the ruxolitinib arm [51].

Tofacitinib

Tofacitinib (Xeljanz or Jakvinus) is a TKI of the JAK1/JAK3 that was approved by the FDA for the treatment of rheumatoid arthritis (RA). It is investigating for the treatment of psoriasis and inflammatory bowel disease. The cytotoxic effects of this TKI have been extensively studied in autoimmune disorders [52-54]. This TKI selectively inhibits proliferating cells. It interferes with

T cell blast stimulation through blocking IL-2/STAT5 and inhibits the expression of inflammatory cytokines through IL-6/STAT3 and STAT1 [55].

The therapeutic effects of tofacitinib have been studied in a few cancers such as B-cell lymphoma [56] and breast cancer [57]. It has been demonstrated that oncostatin M (OSM) induced phosphorylation of STAT3 at Tyr 705 and Ser 727 residues in breast cancer cells as well as overexpression of STAT3-dependent genes, including S100 family members S100A7, S100A8 and S100A9. Activation of STAT3 in MCF-7 breast cancer by OSM induced cellular scattering and tumor vascularization of orthotopic xenografts. Selective inhibition of OSM by tofacitinib inhibited STAT3 signaling and tumor angiogenesis [57].

Xenografted mice, which have been transplanted with bone marrow progenitor cells expressing JAK3 mutants, developed T-ALL-like leukemia and are characterized by growth of immature $CD8^+$ T cells. Treatment of these mice with tofacitinib decreased the number of lymphocytes and induced apoptosis of leukemic cells [58].

Tofacitinib has shown inhibitory effects on nasal-type natural killer (NK)/T-cell lymphoma (NKCL) cells in xenograft mice. NKCL is an aggressive cancer with poor prognosis in which JAK3/STAT3 pathway is constitutively active [59]. Data show that tofacitinib has anti-tumor activity and might be a proper TKI for the treatment of more progressive tumors. Further investigations are necessary to evaluate the potential anti-tumor effects of tofacitinib in cancer.

The effects of several other JAKs-TKIs, including oclacitinib [60], baricitinib [61], momelotinib (CYT387), pacritinib [62], LY2784544 [63], and lestaurtinib [64] are testing in autoimmune and allergic disorders and cancer (multiple myeloma) (Table 1).

BCR/ABL kinase

BCR/ABL is an oncokinase involved in cancer development. BCR/ABL is encoded by the Ph chromosome that forms due to the translocation between chromosomes 9 and 22 (t(9;22) (q34;q11) reciprocal translocation) [65]. Therefore, this translocation produces a chimeric BCR/ABL oncoprotein that is constitutively active and enhances the growth of leukemic cells. This kinase is active in more than 90% of CML patients. The ABL protein normally shuttles between the cytoplasm and nucleus and following fusion with BCR, it loses this property and

retains in the cytoplasm and reacts with other intracellular proteins complicated in the process of tumorigenesis [66].

BCR/ABL is subjected to several point mutations that affect the structure of this kinase, leading either to disruption of major contact points between the TKIs and the BCR/ABL or change the kinase conformation, resulting in a kinase that is resistant to TKIs. These mutations increase during disease progression from chronic disease to the blast phase. T315I is the most frequent mutation that happens at the P-loop [67].

Due to the homology between SRC and ABL kinases in the catalytic domains (47%), TKIs that were produced as SRC inhibitors have shown to inhibit ABL. Currently, several non-specific BCR/ABL-TKIs, including imatinib, dasatinib, nilotinib, ponatinib (Iclusig, Ariad), and bosutinib have been approved by the FDA for cancer treatment, mostly in Ph^+ ALL and acute myeloid leukemia (AML) patients (Table 1).

Ponatinib

Ponatinib (AP24534) is a selective BCR/ABL-TKI, which is used for the treatment of AML patients with BCR/ABL-mutation; however it targets other kinases. This TKI was discovered by O'Hare T et al. at 2009. It has IC_{50} values of 0.37 nM for native BCR/ABL and 2.0 nM for the T315I mutant form (in cell-free assays) [68].

The activity of ponatinib, imatinib, nilotinib and dasatinib has been tested in biochemical assays. All inhibitors reduce the catalytic activity of native ABL; however, only ponatinib is the potent inhibitor of the ABL-T315I mutant form. Ponatinib has displayed similar inhibitory effects on other imatinib-resistant ABL mutants, including ABL-Y253F, ABL-E255K, and ABL-G250E [68].

Ponatinib prevented the kinase activity of native ABL and the T315I mutants and suppressed proliferation of Ba/F3-derived cell lines. Moreover, treatment of xenografted mice with BCR/ABL mutant (T315I) expressing Ba/F3 cells, prolonged the survival of transplanted mice [69]. Based on the positive effects on patients with ALL and CML with BCR/ABL-T315I mutation, it was approved on December 14, 2012 for the treatment of ALL and CML with this mutation [18].

The effects of ponatinib have been tested against other ABL inhibitor-resistant leukemia cells. Treatment with ponatinib has been shown to reduce BCR/ABL and LYN phosphorylation and prevented the proliferation and growth of imatinib or nilotinib resistant K562 cells [70].

GNF-5

GNF-5 is a selective/specific and allosteric BCR/ABL-TKI with IC_{50} value of 220 nM in cell-free assays. This TKI possesses proper selectivity towards BCR/ABL mutants found in CML patients and binds to the myristate pocket of ABL [71].

It has been noted that combination of GNF-5 with imatinib or nilotinib (the ATP-competitive inhibitors), suppressed the development of resistance mutations and this combination is more effective in suppressing tumor cells with ABL-T315I mutation *in vitro* and in mice models. The results demonstrated that inhibition of BCR/ABL using inhibitors that react with myristate residue and in combination with BCR/ABL type I inhibitors may overcome tumor cells resistance compared to either inhibitor alone [72].

Focal adhesion kinase (FAK)

FAK (PTK2) regulates several intracellular activities involved in cell motility, reorganization of actin, cell migration, adhesion, spreading and cell polarization, bone remodeling, and transcriptional events promoting epithelial-to-mesenchymal transition (EMT) [73-75]. This nRTK is necessary for retention of marginal-zone B cells in the spleen, migration of B cells located in the spleen and macrophage polarization and migration towards sites of inflammation. FAK is localized to the sites of integrin adhesion and modulates growth-factor signaling, proliferation, survival, and cell migration.

Several studies have noted that FAK overexpression is associated with cancer poor prognosis and drug resistance [73, 76]. This nRTK is dysregulated and overexpressed in several cancers such as ovarian [76], skin and breast cancers [77]. Therefore, it might be a proper target for TBCT (Fig 1).

Several TKIs have been developed to target FAK, including defactinib, GSK2256098, TAE226, PF-573,228, PF-562,271, VS‑6062, and VS‑4718 (PND‑1186). These TKIs prevented FAK Tyr 397 autophosphorylation and inhibited cell movement as described in cell lines and animal models, but they induced low apoptosis in tumor cells [78].

Defactinib

Defactinib (VS-6063) is a selective FAK inhibitor and has been shown to be a potent cancer stem cell inhibitor [79]. Defactinib induces FAK dephosphorylation at the Tyr 397 in a time- and dose-dependent manner. The combination of defactinib with other cytotoxic agents such as paclitaxel inhibited proliferation and increased apoptosis of tumor cells. Defactinib reduced AKT levels in taxane-resistant cell lines. Moreover, inhibition of FAK improved chemosensitivity in taxane-resistant cells. [79].

Currently, defactinib is evaluating in a few phase I and II clinical trials for the treatment of patients with malignant pleural mesothelioma, advanced ovarian cancer and KRAS mutant NSCLC (ClinicalTrials.gov).

Other FAK inhibitors such as GSK2256098, VS‑4718 and VS‑6062 are in phase I and II trials and NVP-TAC544, PF-573,228, TAE226, 1H‑Pyrrolo (2,3‑b) pyridine, compound 1 and 2, Y15, C4, R2, and Y11 are in preclinical investigations [75].

TEC protein kinase

The TEC family is the second largest family of nRTKs consists of five members, including BTK, IL-2 inducible tyrosine kinase (ITK/TSK/EMT), BMX, resting lymphocyte kinase (RLK/TXK), and TEC, which are expressed by several normal tissues. BTK is the prototype of this family [80]. TEC members are important players in the regulation of the immune system and have important functions in T cell activation, differentiation and cell signaling [81].

TEC kinases are dysregulated in several malignancies, including chronic lymphocytic leukemia (CLL), mantle cell lymphoma (MCL), multiple myeloma, and diffuse large B-cell lymphoma (DLBCL) [81-83].

Based on the crucial role of this family, several inhibitors have been designed and produced to target TEC family members. Ibrutinib (PCI-32765) is the most famous TKI that targets BTK and has been approved by the FDA for the treatment of MCL and CLL (Fig. 1) [84-85].

Ibrutinib

Ibrutinib is the first-in-class covalent inhibitor of BTK reported by Pan et al. [86]. This TKI binds covalently to Cys 481 in the ATP-binding pocket of BTK (type IV TKI) (Table 1). Ibrutinib has IC_{50} values of 0.5, 0.5, 0.8, 5.6, 10.7, and 16.1 nM for BTK, BLK, BMX, EGFR, ITK and JAK3 in cell-free assays, respectively [87-88].

Ibrutinib has been tested in several preclinical and clinical experiments for the treatment of various lymphomas and leukemias. Preclinical studies have demonstrated blocking of BCR signaling by ibrutinib [87] leads to induction of apoptosis in primary CLL, MCL, hairy cell leukemia (HCL), and DLBCL cells [89-93]. The anti-tumor effects of this TKI have been described in several review articles [94-95] and will not be described here.

BMS-509744

BMS-509744 is a selective ATP-competitive inhibitor of ITK with an IC_{50} value of 19 nM (Table 1) [83]. The inhibitory effects of this TKI have not been well studied in malignancies; however, several studies have shown the inhibitory effects in inflammatory and infectious diseases [96-97]. Interestingly, it has been demonstrated that BMS-509744 blocked wild-type HIV-1 infectivity and replication [96]. The HIV-1 NEF virulence factor interacts with several signaling proteins, including the SH3 domain of SRC family, BMX, BTK, and ITK kinases resulting in the activation of essential kinases for viral infectivity and replication [96].

PRN694

PRN694 has been considered as a new inhibitor of ITK and RLK that selectively and covalently binds to Cys 442 and Cys 350 residues of ITK and RLK, respectively (type IV TKI) [98]. Cellular experiments showed that PRN694 inhibited TCR signaling as well as TCR-induced T-cell proliferation and prevented release of proinflammatory cytokines. PRN694 prevented T-cell prolymphocytic leukemia (T-PLL) cells growth with durable pharmacodynamic effects on ITK [98].

CTN06

CTN06 is a potent BMX inhibitor that induces autophagy and apoptosis in prostate cancer cells and blocks xenograft tumor growth *in vivo* [99]. Treatment of PC3 prostate cancer cells with CTN06 induced effective tumor cell killing through inhibition of BTK/ BMX signals. CTN06 induced apoptosis of prostate cancer cells as well as sensitizing tumor cells to cytotoxic agents such as docetaxel. Moreover, CTN06 has been shown to impede the motility of prostate cancer cells [99].

Spleen tyrosine kinase (SYK)

SYK is mainly expressed in hematopoietic lineage cells. It is normally involved in downstream signaling of immune receptors (e.g. BCR). SYK contributes to several biologic functions, including cell development, adhesion, proliferation, and differentiation [100].

SYK is a prosurvival factor in B-cell malignancies and tumors of epithelial origins. It is constitutively active in malignancies such as CLL, non-Hodgkin's lymphoma (NHL), ALL, MCL, and Epstein–Barr virus-associated B-cell lymphomas. It is important for cell proliferation and survival and inhibition of SYK leads to tumor cells apoptosis [101-103].

SYK overexpression has been noted in non-B cell hematologic malignancies such as AML [104] and T-cell lymphomas [105] in which immunoreceptor tyrosine-based activation motif (ITAM)-containing receptors (e.g. TCR complex) are coupled to the activated SYK through SH2 domains [106]. SYK overexpression has been shown in melanoma cell lines and tumors [107].

In contrast, SYK has been shown to act as a tumor suppressor as described that it is absent in invasive breast carcinomas and the expression is associated with inhibition of the metastasis of tumor cells in invasive carcinomas [108]. Lack of SYK mRNA expression has been observed in

patient with ductal carcinoma [109]. Low expression of SYK has been reported in hepatocellular carcinoma, and it has been reported to act as tumor-suppressor in pancreatic ductal adenocarcinoma [110].

Current data shows that SYK has dual roles and acts as a tumor promoter and tumor suppressor in cancer [111] and it is important to consider that it may not be a proper target in all types of tumors with SYK overexpression.

Fostamatinib

Fostamatinib (R788) was produced as the first-in-class SYK-TKI. It inhibits BCR signaling and antagonizes the supportive effects of stromal cells, decreases cells migration to chemokines and induces apoptosis of cells with SYK overexpression [112]. The inhibitory effects of this TKI are testing in several clinical trials of rheumatoid arthritis [113].

Moreover, fostamatinib has been noted to be effective in inhibition of tumor cell growth in several malignancies [114].

Fostamatinib has been evaluated in clinical trials for cancer treatment. In the first trial, the effects have been investigated in relapsed B-cell NHL and CLL patients [114]. Diarrhea, neutropenia and thrombocytopenia were dose limiting toxicity and 200 mg twice daily was defined to be well-tolerated.

In the second phase of this study, sixty-eight patients with B-NHL were recruited in 3 cohorts, including DLBCL, follicular lymphoma (FL), and other NHL, including small lymphocytic leukemia (SLL)/CLL, marginal zone lymphoma (MZL), lymphoplasmacytic lymphomas, MCL, and mucosa-associated lymphoid tissue lymphoma [114]. Diarrhea, cytopenias, nausea, fatigue, and hypertension were the most common side effects. Median PFS was shown to be 4.2 months. Objective response rates were 55% (6 of 11) for SLL/CLL, 22% (5 of 23) for DLBCL, 11% (1 of 9) for MCL, and 10% (2 of 21) for FL patients [114].

PRT318 and P505-15

PRT318 (PRT-060318) and P505-15 (PRT062607) are two selective SYK-TKIs and the effects have been evaluated on CLL cells survival and the interactions with tumor environment [115]. Both TKIs have shown to antagonize CLL leukemic cells survival following the stimulation of BCR and in coculture with nurse-like cells and suppress BCR-dependent secretion of chemokine

(C-C motif) ligand (CCL) 3 and CCL4 by leukemic CLL cells. Moreover, these TKIs inhibited CLL cell migration toward the chemokines chemokine (C-X-C motif) ligand (CXCL) 12, CXCL13 as well as inhibiting SYK phosphorylation after BCR triggering [115].

P505-15 inhibits SYK with IC_{50} value of 1-2 nM and blocks BCR-mediated signaling and activation in B cells with IC_{50} values of 0.27 and 0.28 μM, respectively [116].

It has been shown that P505-15 suppressed signaling and reduced cell viability in NHL and CLL leukemic cells. Treatment of mice with this TKI inhibited the growth of NHL tumor in a xenograft model. Combination of P505-15 plus fludarabine showed the synergistic enhancement of primary CLL cells growth inhibition at nanomolar concentrations [117]. These TKIs have not been tested in human cancer clinical trials.

Entospletinib

Entospletinib (GS-9973) is another selective inhibitor of SYK. The effects have been tested in CLL. Inhibition of SYK decreased the survival of CLL cells and in combination with phosphoinositide 3-kinase (PI3K) δ-TKIs, induced synergistic inhibition of cell growth and disrupted chemokine effects at low concentrations [118].

This TKI is currently testing in a few clinical trials for the treatment of AML and B-cell malignancies and promising results have been observed in CLL. In a recent phase II study, the effects of entospletinib have been evaluated in patients with relapsed or refractory CLL (n=41) and NHL (n=145) patients [119]. Patients were treated with 800 mg of entospletinib twice daily. The most common treatment-associated side effects were dehydration, pyrexia, febrile neutropenia, pneumonia, and dyspnea. Neutropenia (14.5%) and reversible aspartate aminotransferase/alanine aminotransferase (AST/ALT) elevations (13.4%) were the most common grade 3/4 abnormalities. The PFS and objective response rates were 70.1% (95% confidence interval [CI]: 51.3%, 82.7%) and 61.0% (95% CI: 44.5%, 75.8%), respectively. The median PFS was 13.8 months (95% CI: 7.7 months, not reached) [119]. Overall, the data indicated proper efficacy and safety in all tested CLL patients.

Currently two phase I and II trials are recruiting patients for testing the effects of entospletinib in relapsed or refractory hematologic malignancies (NCT01799889) and AML (NCT02343939).

MEK1/MEK2 tyrosine kinases and MAPK/ERK signaling pathway

The mitogen-activated protein kinases or extracellular signal-regulated kinases (MAPK/ERK) pathway consists of several signaling molecules involved in communication of signals from cell membrane receptors to the nucleus. MAPK/ERK pathway is consisting of two classical and non-classical pathways. MAPK pathway is the well-known pathway and is considered the classical pathway. The non-classical pathway involves MEK4, MEK7, c-Jun N-terminal kinases (JNK) 1-3, MEK3, and MEK6/p38 molecules (p38α/β/γ/δ) as well as ERK5/MEK5 pathways [120]. These pathways are involved in several cellular functions, including regulation of gene expression, G1 cell cycle progression before starting S cycle and cell division.

MEK1 and MEK2 proteins are involved in cell proliferation, tumorigenesis and inhibition of apoptosis. The overexpression is associated with aberrant activation of the RAS/RAF/MEK/ERK1/2 pathway in several tumors and, therefore, is the ideal target for TBCT [121].

Several selective/specific non-ATP-competitive inhibitors of MEK1 and MEK2 have been tested in preclinical and clinical settings (Table 1, Fig. 1). Moreover, combination of these inhibitors and cytotoxic agents as well as other TBCT agents has been studied [121].

Trametinib

Trametinib (GSK1120212, JTP 74057) is a selective TKI of MEK1/MEK2. It is a non-ATP-competitive inhibitor. The effects of this TKI have been extensively tested in several preclinical and clinical experiments for the treatment of gastrointestinal cancers [122], NSCLC [123], colorectal carcinoma, and metastatic/BRAF mutant melanomas [124-127].

On May 29, 2013, FDA approved trametinib for the treatment of patients with metastatic melanoma with V600E and V600K BRAF mutations. This approval was based on the improved PFS of 322 melanoma patients with BRAF V600E or V600K mutations in a phase III clinical trial [125]. In this trial, metastatic melanoma patients received either trametinib (2 mg once daily) or chemotherapy (dacarbazine or paclitaxel every 3 weeks). The primary and secondary end points were PFS and overall survival, respectively. Results showed a median PFS of 4.8 and 1.5 months in the trametinib and chemotherapy groups, respectively (hazard ratio for disease progression or death in the trametinib group, 0.45; 95% CI: 0.33 to 0.63; $P<0.001$). The rate of

overall survival was 81% in the trametinib group and 67% in the chemotherapy group at 6 months (hazard ratio for death, 0.54; 95% CI: 0.32 to 0.92; P=0.01). The most common side effects were rash, diarrhea and peripheral edema in the trametinib group [125].

Combination of trametinib with other SMIs or cytotoxic agents has been studied in several trials.

In a phase I dose escalation trial (PACMEL), combination of trametinib with paclitaxel has been evaluated [128]. In this trial a fixed dose of paclitaxel and escalated dose of trametinib (maximum 2 mg daily) was used for the treatment of 15 patients with advanced melanoma or those who have received up to two previous lines of treatment for metastatic disease. The maximal dose of trametinib alone showed to be tolerable with weekly paclitaxel. The most frequent adverse effects were rash and fatigue. Overall survival and median PFS were 14.1 (95% CI: 4.6-not reached) and 5.5 months (95% CI: 1.8-7.8 months), respectively. Partial response was observed in 40% of patients (4 of 8 patients had NRAS mutations). Data showed that trametinib can be safe in combination with weekly paclitaxel in melanoma patients without V600 BRAF mutations [128].

In a phase Ib dose-escalation trial, the combination of the trametinib with buparlisib (PI3K inhibitor) was evaluated in 113 NSCLC patients with RAS- or BRAF-mutants, ovarian and pancreatic cancers [129]. Maximum tolerance dose was determined to be 70 mg for buparlisib plus 1.5 mg daily for trametinib. The major dose limited toxicities were stomatitis, diarrhea, dysphagia, and creatine kinase (CK) increase. Grade 3 and 4 side effects were observed in 73 patients (65%), including CK increase, stomatitis, AST/ALT increase, and rash. For 21 ovarian cancer patients, the overall response and disease control rates were 29% and 76%, respectively with median PFS of 7 months. The least effects of combination therapy was observed in NSCLC (1/17 partial response) and pancreatic cancers (no response) [129].

In another phase I trial, the effects of trametinib in combination with afuresertib (AKT inhibitor) were evaluated in twenty patients with solid tumors and multiple myeloma [130]. Grade 2 esophagitis, grade 3 elevated AST, mucosal inflammation and hypokalemia were dose-limiting toxicities that were observed at once daily doses 1.5 and 50 mg of trametinib and afuresertib, respectively. Diarrhea, acneiform dermatitis, maculopapular rash, fatigue, dry skin, nausea, dyspnea, and vomiting were observed in 10% of patients. One and four patients showed partial

response (BRAF wild-type melanoma) and stable disease, respectively. Overall, the results showed that the combination of trametinib/afuresertib (continuous daily dosing) was poorly tolerated; however, intermittent dose schedule was tolerated better [130].

AZD8330

AZD8330 (ARRY-704) is a selective MEK1/MEK2 [131] and non-ATP-competitive inhibitor, with an IC_{50} value of 7 nM.

In vitro effects of AZD8330 have been investigated in multiple myeloma cell lines IM9 and NCI-H929. Data showed that AZD8330 decreased the cell viability, induced apoptosis and cell cycle arrest at G1 phase with IC_{50} values of 20 and 30 nM for IM9 and NCI-H929 cells, respectively [132]. AZD8330 could also induce apoptosis in Burkitt's lymphoma cells in high concentrations (1 μM) [133].

AZD8330 effects have been evaluated in one clinical trial. In this phase I study, safety and tolerability were tested in eighty-two patients with advanced malignancies [131]. Patients were treated with either once or twice daily of AZD8330 with starting dose of 0.5 mg with dose escalations until a non-tolerated dose was observed. Dose-dependent dephosphorylated ERK was observed in peripheral blood mononuclear cells in doses higher than 3 mg. The most frequent adverse reactions were acneiform dermatitis, fatigue, diarrhea, and vomiting. Maximum tolerated dose was defined to be 40 mg/day. One and thirty-two patients showed partial response and stable disease, respectively [131].

Selumetinib

Selumetinib (AZD6244 or ARRY-142886) is a selective MEK1/MEK2-TKI with an average IC_{50} value of 14 nM [134]. Strong anti-tumor activity has been shown in preclinical studies in both *in vitro* (cell-growth experiments) and *in vivo* (xenograft models) [135-137]. The effects of selumetinib have been investigated in different tumors either alone or in combination with other agents [135-137].

Pharmacokinetic and pharmacodynamic of selumetinib have been checked in patients with advanced cancers [138]. The most frequent adverse effects were diarrhea, rash, fatigue, nausea,

and transient blurred vision. 8.3 hours was the median half-life of the drug in plasma after a single dose of selumetinib. ERK dephosphorylation was shown in 19 tested tumor biopsies; however, no partial responses were observed, and stable disease was only showed in two patients [138].

Several phase II trials have tested the effects of selumetinib as monotherapy in serous carcinoma of the ovary or peritoneum, metastatic biliary cancers, melanoma, AML, hepatocellular carcinoma, and papillary thyroid carcinoma [139-143].

Several trials have checked selumetinib in combination with other inhibitors and cytotoxic agents, including cixutumumab, vandetanib, temsirolimus, pemetrexed, gemcitabine, irinotecan, paclitaxel and carboplatin, and pemetrexed and cisplatin in several types of melanoma and other types of cancer [120].

Several other MEK inhibitors such as cobimetinib (melanoma and pancreatic cancer) [144-145], PD0325901 [146-147], pimasertib (lung, colorectal cancer, pancreatic adenocarcinoma, melanoma, and multiple myeloma) [148], binimetinib (MEK162, ARRY-162, ARRY-438162) (melanoma), and refametinib (hepatocellular carcinoma) [149-150] are ongoing in clinical trials for the treatment of different cancers.

Phosphoinositide 3-kinases (PI3Ks)

PI3K kinases are one of the most important families of nRTKs that are involved in proliferative, differentiation and survival pathways, including those of hematopoietic origin. PI3K kinases are classified into three subtypes. Class I is divided into IA and IB subsets and converts phosphatidylinositol 4,5-bisphosphate (PIP2) to phosphatidylinositol 3,4,5-trisphosphate (PIP3) in response to cell stimulators that act as intracellular second messenger for activating other signaling proteins. Class IA PI3Ks, including PI3Kα, β, δ are activated by RTKs stimulation. PI3Kβ and class IB PI3K (PI3Kγ) are stimulated via G-protein–coupled hormone receptors [151]. The function and structure of class II and III are differentiated from the class I and will not be discussed here.

Among various PI3K members, PI3Kδ has been extensively studied for TBCT. PI3Kδ is widely expressed in blood leukocytes, suggesting that it may be a proper target for different

hematological malignancies, including NHL, CLL and MCL in which PI3K pathways are constitutively active in the cells [152]. Thus, PI3K-TKIs might have therapeutic potential either as single drug or in combination with other therapies for different cancer indications. Several PI3K-TKIs have been developed against different PI3Ks members and have been approved by the FDA (e.g. idelalisib) or are in experimental settings (Table 1, Fig. 1).

Idelalisib

Idelalisib (CAL-101 or GS-1101) is the first-in-class inhibitor of the p110δ (catalytic subunit of PI3Kδ) isoform of PI3K, which has an expression pattern mainly restricted to cells of hematopoietic origin [153-154]. This SMI is a reversible and selective with an IC_{50} value of 2.5 nM for p110δ isoform. It is 40 to 300 fold more selective for p110δ than other p110 isoforms [151].

For the first time, the effects of idelalisib on tumor cells were examined in 2011 [155]. In this study, primary tumor cells from different malignancies were evaluated for sensitivity to idelalisib *in vitro*. Data showed significant sensitivity (efficient concentration, (EC) 50<1μM) in 23% of B-cell ALL (B-ALL) cells, 26% of CLL and 3% of AML, but no effect on myeloproliferative neoplasm samples was observed. Results showed a strong activity of idelalisib in B-ALL and CLL cells suggesting a superior potential effects on malignant B cells [155].

The consequence of PI3K inhibition by idelalisib is to prevent signaling pathways that are involved in the homing of B cells in the bone marrow and lymph nodes [156]. The inhibition starts with blocking PI3K phosphorylation in malignant B cells. This inhibition results in AKT dephosphorylation and ERK phosphorylation. The chemotaxis, migration and secretion of the CCL3, CCL4 and CXCL13 by leukemic cells will be inhibited by idelalisib and finally the viability and adhesion of leukemic cells to endothelial cells and bone marrow stromal cells will be impaired [157].

Idelalisib is a very effective agent that leads to rapid resolution of lymphadenopathy and splenomegaly. Moreover, it is effective in CLL patients with *p53* gene mutations that have poorer prognosis [158].

FDA approved idelalisib on July 23, 2014, for the treatment of patients with relapsed CLL, in combination with rituximab (anti-CD20 antibody). Moreover, FDA granted accelerated approval for the treatment of FL or SLL patients who have received more than two prior therapies as a single agent therapy [159]. The approval for CLL patients was based on the results of an international, placebo-controlled trial of patients in combination with rituximab to placebo in combination with rituximab [160].

In one study, the effects of idelalisib were evaluated in multiple myeloma [161]. SiRNA silencing of p110δ induced significant inhibition of tumor cell growth. Moreover, treatment of multiple myeloma cell lines and primary cells with idelalisib showed cytotoxic effects as well as inactivation of AKT; however, no effects were observed on normal peripheral blood mononuclear cells. Prior treatment of multiple myeloma cells with IL-6, insulin-like growth factor-1 (IGF-1) and coculture with bone marrow stromal cell followed by idelalisib treatment did not rescue cells from apoptosis. On the other hand, it was shown that inhibition of p110δ induced autophagy of malignant cells. Combination of idelalisib with bortezomib showed synergistic cytotoxic effects against multiple myeloma cells [161]. Although the effects have been shown to be promising in multiple myeloma; however, there is no clinical trial testing idelalisib for the treatment of this malignancy.

Idelalisib is investigating in clinical settings for the treatment of several B-cell malignancies, including relapsed/refractory MCL, FL, CLL, indolent NHL, MZL, SLL, and lymphoplasmacytic lymphoma as single agent or in combination with other drugs. It is in phase III clinical trials evaluating drug combination with rituximab and/or bendamustine (NCT01569295).

Pictilisib

Pictilisib or GDC-0941 is a selective/specific PI3Kα/δ-TKI with an IC_{50} value of 3 nM, proper clinical tolerability and anti-neoplastic activity against tumor cells. It inhibited proliferation and survival of medulloblastoma cell lines. Cell treatment with this TKI decreased AKT phosphorylation and activation. Moreover, pictilisib attenuated the migration of medulloblastoma cells and a population of $CD133^{+}$ stem cells [162]. Pictilisib augmented the anti-tumor activity of the standard medulloblastoma chemotherapeutic etoposide (VP16). Furthermore, it impaired tumor cell growth and prolonged the survival of tumor cells in a xenografted mice model [162].

Pictilisib reduced the growth of prostate cancer cell lines such as LNCaP and C4-2B through dephosphorylation of PI3Kα/δ followed by inactivation of AKT [163]. MAbs targeting HER2 and HER3 have shown synergistic anti-tumor effects when combined with pictilisib [163].

In the first phase I study of pictilisib, tolerability, safety, dose-limiting toxicities, maximum-tolerated dose, pharmacodynamics, pharmacokinetics, and clinical activity were investigated in sixty patients with solid tumors [164]. Data showed that pictilisib is well tolerated. Grade 1-2 rash, nausea, and fatigue were the most common toxicities, whereas grade 3 maculopapular rash with dosage of 450 mg was observed in 2 of 3 patients and in 1 of 7 patients with dosage of 330 mg. AKT dephosphorylation was noted at Ser 473 residue with >90% inhibition in platelet-rich plasma. Moreover, a melanoma patient with BRAF mutation (V600E) and one ovarian cancer patient with platinum-refractory epithelial showed PTEN loss and amplification of PIK3CA and exhibited partial response [164].

In a high-throughput screening of more than 500 cancer cell lines, including 46 pancreatic ductal adenocarcinoma lines, the combination of the MEK1/ MEK2 inhibitor, AZD6244 and pictilisib has been tested [165]. Data showed that pancreatic ductal adenocarcinoma cell lines were mostly non-responder to single-agent therapies; however, the combination induced apoptosis of tumor cells [165].

Other investigations have shown the promising effects of pictilisib in different types of tumors, including pediatric glioblastoma [166], T-ALL leukemia [167], breast [168] and colorectal cancers [169], melanoma [170], AML [171], multiple myeloma [172], and refractory/aggressive MCL patients [173].

Buparlisib

Buparlisib (NVP-BKM120) is a selective pan-PI3K inhibitor with IC_{50} values of 52, 166, 116, and 262 nM for PI3Kα, β, δ, and γ, respectively. The effects have been investigated in several tumors and have exhibited strong anti-tumor activity in several preclinical animal models. Several clinical trials are currently undergoing in patients with solid tumors. Moreover, it has anti-tumor activity in multiple myeloma [174] and FL [175].

In a phase I dose-escalation study of buparlisib, thirty-five patients with advanced solid tumors were treated with 12.5 to 150 mg/day buparlisib [176]. The most common dose-limiting toxicities were grade 2-4 mood alteration, epigastralgia, rash, and hyperglycemia at doses of 80-150 mg/day and the maximum-tolerated dose was shown to be 100 mg/d. Rash, hyperglycemia, diarrhea, anorexia, mood alteration (37% each), nausea (31%), fatigue (26%), pruritus (23%), and mucositis (23%) were also the most common treatment-associated side effects. These data were supported by another phase I dose-escalation study on Japanese patients [177].

In a phase Ib trial of buparlisib, the effects in combination with letrozole in estrogen receptor-positive (ER^+)/HER2-negative metastatic breast cancer patients have been tested [178]. The maximum-tolerated dose of buparlisib was 100 mg/day and grade 2 disorders such as nausea, hyperglycemia, transaminitis, fatigue, and mood disorders were the most common drug-related side effects. 31% of patients that were treated with maximum-tolerated dose showed clinical benefit rate e.g. lack of progression $\geq$ 6 months after treatment. Data showed a safe profile of letrozole and buparlisib combination [178].

Several phase I and II clinical trials are investigating the effects of buparlisib alone or in combination in CLL, recurrent glioblastoma, recurrent/refractory primary central nervous system lymphoma, NSCLC, advanced breast cancer, relapsed or refractory NHL, and other tumors (clinicaltrials.gov).

Pilaralisib

Pilaralisib (SAR245408, XL147) is a pan-class I PI3K inhibitor. This TKI is type I ATP-competitor with IC_{50} values of 39, 383, 36 and 23 nM for PI3Kα, β, δ and γ, respectively. It is currently in clinical trials for cancer treatment.

Pilaralisib prevented the formation of PIP3 from PIP2 and inhibited the activation of AKT, p70S6K and S6 phosphorylation in different tumor cell lines such as breast cancer in cellular assays and xenograft mice models. Repeated treatment with pilaralisib has been shown to induce substantial inhibition of tumor cells growth in xenograft mice models. Combination of pilaralisib with cytotoxic drugs showed strong anti-tumor activity in mice xenograft models compared to either single agent treatment [179].

Treatment of breast cancer cells with constitutive activation of PI3K inhibited cell growth, reduced AKT, pS6 and expression of signal transducers in the PI3K/AKT/mTOR pathway. Inhibition of PI3K in HER2-overexpressing cell lines induced the upregulation of expression as well as phosphorylation of several RTKs such as HER3, insulin receptor (INSR), insulin-like growth factor 1 receptor (IGF-1R), and fibroblast growth factor receptor (FGFR) 2. Furthermore, inhibition of forkhead box protein (Fox)O1 and FoxO3a transcription factors prevented the mRNAs expression of these RTKs upon suppression of PI3K. Downregulation of HER3 by specific siRNA and treatment of $HER2^+$ cells with trastuzumab or lapatinib enhanced the effects of pilaralisib on breast cancer cells. In BT474 xenografts, trastuzumab and lapatinib augmented the effects of pilaralisib and inhibited the activation of PI3K/AKT signaling pathway and inhibited the growth of tumor cells [180].

The maximum tolerated dose, safety, pharmacokinetics, and pharmacodynamics have been investigated in phase I trial of pilaralisib alone or in combination with erlotinib [181]. Pharmacodynamic analyses showed inhibition of PI3K, MAPK and EGFR signaling pathways. Of 27 patients, one and 14 patients showed partial response and stable disease, respectively. Rash, diarrhea and fatigue were the most common treatment-related side effects. Overall, data showed limited anti-tumor activity of the combination treatment.

In a phase II clinical trial, the efficacy and safety of the pilaralisib were evaluated in 67 patients with advanced or recurrent endometrial carcinoma [182]. Objective response rate, proportion of patients with PFS>6 months and safety were the primary endpoints. Tumor responses (complete or partial) were observed in two patients. Rate of PFS (>6 months) was 11.9%. Diarrhea, rash and fatigue were the most common treatment-related cytotoxicities and rash, diarrhea and elevated ALT were reported as grade ≥3 treatment-related side effects.

The maximum tolerated dose, pharmacokinetics, efficacy, and safety of pilaralisib in combination with trastuzumab (arm 1) or trastuzumab plus paclitaxel (arm 2) were tested in a phase I/II dose-escalation study in 42 patients with $HER2^+$ metastatic breast cancer [183]. The maximum tolerated dose of pilaralisib was defined at 400 mg once daily in all patients. Three patients in arm 1 and two in arm 2 showed dose-limiting toxicity, including rash and neutropenia. Diarrhea (23.8 % vs. 66.7 % in arm 1 and arm 2, respectively), rash (33.3 vs. 38.1 %) and fatigue (14.3 vs.

42.9 %) were the most common side effects. Erythematous rash (9.5 %) in arm 1 and diarrhea, peripheral neuropathy and neutropenia (14.3 % each) in arm 2 were the most common treatment-associated grade ≥3 side effects. No significant treatment responses were observed in arm 1; however, four of 20 tested patients in arm 2 showed a partial response. Data showed that pilaralisib plus trastuzumab with or without paclitaxel had proper safety in breast cancer patients and proper clinical activity in treated patients in arm 2 [183].

NVP-BYL719

NVP-BYL719 is a potent and selective PI3Kα inhibitor that was identified in 2013 [184]. Treatment of $HER2^+$ cell lines as well as trastuzumab resistant cells with NVP-BYL719 demonstrated strong anti-tumor activity *in vitro* and in xenograft models [185]. Moreover, it exhibited significant anti-tumor effects on erlotinib resistant pancreatic ductal adenocarcinoma cells *in vitro* and in xenograft mice models [186].

The *in vitro* impacts of NVP-BYL719 have been investigated on proliferation, apoptosis and cell cycle arrest of human and murine osteosarcoma cells [187]. This class I TKI reduced proliferation of tumor cells by blocking cell cycle in G0/G1 phase and inhibited osteosarcoma cells migration. NVP-BYL719 significantly decreased tumor progression and tumor bone formation in mice model of osteosarcoma, as noted by the reduction of $Ki67^+$ cells and vascularization of tumor [187].

NVP-BYL719 is currently in phase I/II trial as a potent therapeutic TKI to treat recurrent or metastatic head and neck squamous cell carcinoma (NCT01602315).

Other selective PI3K inhibitors, including AMG 511, GNE-490, CNX-1351, HSW 243, and AS-252424 are testing in preclinical experiments [188].

Zeta-chain-associated protein kinase 70 (ZAP-70) tyrosine kinase

ZAP-70 is a member of SYK family and is normally expressed by T cells and NK cells and is a major part of the TCR proximal signaling protein. The molecular weight is 70 kDa and plays a

crucial role in T-cell signaling [189]. It plays a major role in immune responses by T cells and is involved in inflammatory and autoimmune diseases [190].

TCR is the major activator of T lymphocytes through binding to antigens presented by antigen presenting cells (e.g. dendritic cells and B cells) via the human leukocyte antigens (HLA) [191]. Upon activation, the coreceptor complex, including TCR-CD4 or TCR-CD8 binds to the HLA molecule and activates the LCK as the coreceptor associated tyrosine kinase. Then the intracellular parts of the CD3 complex (ITAM) become phosphorylated by LCK and creates a docking site for ZAP-70. The outcome of these reactions is the activation of T cell and the transcription of different genes that induce T cells proliferation, differentiation and cytokine secretion [191].

Several compounds have been produced that selectively inhibit ZAP-70 [192-193]. Currently, most of these inhibitors are used for studying the function of ZAP-70 in T cells and up to date none of these inhibitors has been tested for inhibition of tumor cells [194-195]. Moreover, targeting ZAP-70 in cancer patients might induce deep tumor suppression [196]; however, these inhibitors may be useful for the treatment of autoimmune and allergic disorders as well as preventing transplants rejection by the immune system.

FES and FER kinases

Feline sarcoma proto-oncogene (FES/FPS) and FES-related (FER) tyrosine kinases are two nRTKs involved in cell signaling. FES is the counterpart of feline sarcoma retrovirus protein. FES is involved in the maintenance of cellular transformation, modulates cellular signaling, differentiation, normal hematopoiesis, the innate immune response, and vasculogenesis [197]. Moreover, it acts downstream of cell surface receptors such as FCεR1 signaling in mast cells and regulates several functions, including actin cytoskeleton, microtubule assembly, cell to cell contacts, mast cell degranulation, cell scattering and migration. FES phosphorylates BCR and acts as a downregulator of BCR through phosphorylation of hematopoietic lineage cell-specific protein (HCLS1/HS1), platelet endothelial cell adhesion molecule (PECAM) 1, STAT3, and tripartite motif-containing (TRIM) 28 molecules [198].

Overexpression, hyperactivation and gain-of-function mutations of *FES* gene have not shown to be associated with outgrowth and uncontrolled proliferation of many cancers. In lymphoid

cancers hyperactivation of FES has shown to be important in supporting constitutively active receptors such as the FLT3 mutants expressed in AML cells. Inhibition of FES expression using siRNAs has been shown to decrease AML cell survival [199].

Targeting FES kinase by TKIs is at the preliminary stages and a few studies have described the production of selective FES-TKIs.

In the first report, the inhibitory effects of 21 FES-TKIs for preventing autophosphorylation and tubulin polymerization have been described in Cos-7 cell line [197]. Of 21 compounds, the TAE684 compound showed the highest effects and prevented autophosphorylation of FES at Tyr 713 residue as well as inhibition of microtubule association. Nine inhibitors reduced FES autophosphorylation and microtubule association in at least a subset of cells. In contrast to these compounds, HG-7-27-01 (type II TKI) decreased FES autophosphorylation in 0–15% of cells at concentrations below the cytotoxicity threshold [197].

Several other nRTKs are involved in cell signaling pathways; however, some of them act as tumor suppressors and are not ideal candidates for targeting by TKIs. C-terminal SRC kinase (CSK) and megakaryocyte-associated tyrosine kinase (MATK) or CSK-homologous kinase (CHK) are examples and have not been described in this article.

Conclusions and future prospective

Tyrosine kinase inhibitors (TKIs) introduction to the list of agents for cancer treatment has greatly improved the outcome of cancer treatments. However, still several undesirable effects such as off-target effects have dampened the benefits. The high sequence homology of more than 500 kinases has blunted the development of selective/specific kinase inhibitors. Most of current TKIs in preclinical or clinical developments as well as those, which have been approved for cancer treatment, belong to the type I TKIs and are non-selective and multi-targeted inhibitors. These inhibitors that target multiple kinases might be acceptable and desirable as a therapeutic agent; however, they may not be proper for personalized medicine. Selective inhibitors are more pharmacologically relevant for the dissection of complicated signaling pathways. Currently, several researches have focused on developing TKIs with increasing selectivity by targeting different regions outside the ATP-binding pocket. Non-type I inhibitors have fewer off-target

effects and may have less side effects for cancer treatment than type I. In addition to cancer, these TKIs might have desirable effects on other types of life-threatening diseases such as autoimmune disorders. These new attitudes may pave the way to potentially new areas for drug development and provide valuable tools for evaluating signal transduction pathways. Moreover, several combinations of TKIs with other inhibitors, mAbs and chemotherapy agents have shown proper clinical outcomes. On the other hand, proper combination regimens might prevent undesirable effects and target cancer stem cells, as the main cells for drug resistance and cancer recurrence. A deeper understanding of the structure of drug targets in cancer as well as developing new methods for discovering and evaluating new TKIs might be necessary to improve the field of TBCT using small molecule inhibitors.

Competing interest

The author has no relevant affiliation or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript.

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References

1. Hojjat-Farsangi M, Moshfegh A, Daneshmanesh AH, Khan AS, Mikaelsson E, et al. The receptor tyrosine kinase ROR1--an oncofetal antigen for targeted cancer therapy. Semin Cancer Biol 2014; 29:21-31.
2. Daneshmanesh AH, Hojjat-Farsangi M, Khan AS, Jeddi-Tehrani M, Akhondi MM, et al. Monoclonal antibodies against ROR1 induce apoptosis of chronic lymphocytic leukemia (CLL) cells. Leukemia 2012; 26:1348-1355.
3. Daneshmanesh AH, Porwit A, Hojjat-Farsangi M, Jeddi-Tehrani M, Tamm KP, et al. Orphan receptor tyrosine kinases ROR1 and ROR2 in hematological malignancies. Leuk Lymphoma 2013; 54:843-850.
4. Hojjat-Farsangi M, Ghaemimanesh F, Daneshmanesh AH, Bayat AA, Mahmoudian J, et al. Inhibition of the receptor tyrosine kinase ROR1 by anti-ROR1 monoclonal antibodies and siRNA induced apoptosis of melanoma cells. PLoS One 2013; 8:e61167.
5. Hojjat-Farsangi M, Khan AS, Daneshmanesh AH, Moshfegh A, Sandin A, et al. The tyrosine kinase receptor ROR1 is constitutively phosphorylated in chronic lymphocytic leukemia (CLL) cells. PLoS One 2013; 8:e78339.
6. Broome HE, Rassenti LZ, Wang HY, Meyer LM, Kipps TJ. ROR1 is expressed on hematogones (non-neoplastic human B-lymphocyte precursors) and a minority of precursor-B acute lymphoblastic leukemia. Leuk Res 2011; 35:1390-1394.
7. Zhang S, Chen L, Cui B, Chuang HY, Yu J, et al. ROR1 is expressed in human breast cancer and associated with enhanced tumor-cell growth. PLoS One 2012; 7:e31127.
8. Zhang S, Chen L, Wang-Rodriguez J, Zhang L, Cui B, et al. The onco-embryonic antigen ROR1 is expressed by a variety of human cancers. Am J Pathol 2012; 181:1903-1910.
9. Manning G, Whyte DB, Martinez R, Hunter T, Sudarsanam S. The protein kinase complement of the human genome. Science 2002; 298:1912-1934.
10. Hojjat-Farsangi M. Small-molecule inhibitors of the receptor tyrosine kinases: promising tools for targeted cancer therapies. Int J Mol Sci 2014; 15:13768-13801.
11. Cohen P. Protein kinases--the major drug targets of the twenty-first century? Nat Rev Drug Discov 2002; 1:309-315.
12. Hojjat-Farsangi M. Novel and emerging targeted-based cancer therapy agents and methods. Tumour Biol 2015; 36:543-556.
13. Zwick E, Bange J, Ullrich A. Receptor tyrosine kinase signalling as a target for cancer intervention strategies. Endocr Relat Cancer 2001; 8:161-173.
14. Lamba V, Ghosh I. New directions in targeting protein kinases: focusing upon true allosteric and bivalent inhibitors. Curr Pharm Des 2012; 18:2936-2945.
15. Kufareva I, Abagyan R. Type-II kinase inhibitor docking, screening, and profiling using modified structures of active kinase states. J Med Chem 2008; 51:7921-7932.
16. Cox KJ, Shomin CD, Ghosh I. Tinkering outside the kinase ATP box: allosteric (type IV) and bivalent (type V) inhibitors of protein kinases. Future Med Chem 2011; 3:29-43.
17. Bergman M, Mustelin T, Oetken C, Partanen J, Flint NA, et al. The human p50csk tyrosine kinase phosphorylates p56lck at Tyr-505 and down regulates its catalytic activity. EMBO J 1992; 11:2919-2924.
18. Roskoski R, Jr. Src protein-tyrosine kinase structure, mechanism, and small molecule inhibitors. Pharmacol Res 2015; 94C:9-25.
19. Roskoski R, Jr. ErbB/HER protein-tyrosine kinases: Structures and small molecule inhibitors. Pharmacol Res 2014; 87:42-59.

20. Williams JC, Wierenga RK, Saraste M. Insights into Src kinase functions: structural comparisons. Trends Biochem Sci 1998; 23:179-184.
21. Hah JM, Sharma V, Li H, Lawrence DS. Acquisition of a "Group A"-selective Src kinase inhibitor via a global targeting strategy. J Am Chem Soc 2006; 128:5996-5997.
22. Cortes JE, Kim DW, Kantarjian HM, Brummendorf TH, Dyagil I, et al. Bosutinib versus imatinib in newly diagnosed chronic-phase chronic myeloid leukemia: results from the BELA trial. J Clin Oncol 2012; 30:3486-3492.
23. Montero JC, Seoane S, Ocana A, Pandiella A. Inhibition of SRC family kinases and receptor tyrosine kinases by dasatinib: possible combinations in solid tumors. Clin Cancer Res 2011; 17:5546-5552.
24. Spreafico A, Chi KN, Sridhar SS, Smith DC, Carducci MA, et al. A randomized phase II study of cediranib alone versus cediranib in combination with dasatinib in docetaxel resistant, castration resistant prostate cancer patients. Invest New Drugs 2014; 32:1005-1016.
25. Hennequin LF, Allen J, Breed J, Curwen J, Fennell M, et al. N-(5-chloro-1,3-benzodioxol-4-yl)-7-[2-(4-methylpiperazin-1-yl)ethoxy]-5- (tetrahydro-2H-pyran-4-yloxy)quinazolin-4-amine, a novel, highly selective, orally available, dual-specific c-Src/Abl kinase inhibitor. J Med Chem 2006; 49:6465-6488.
26. Musumeci F, Schenone S, Brullo C, Botta M. An update on dual Src/Abl inhibitors. Future Med Chem 2012; 4:799-822.
27. Hiscox S, Jordan NJ, Smith C, James M, Morgan L, et al. Dual targeting of Src and ER prevents acquired antihormone resistance in breast cancer cells. Breast Cancer Res Treat 2009; 115:57-67.
28. Purnell PR, Mack PC, Tepper CG, Evans CP, Green TP, et al. The Src inhibitor AZD0530 blocks invasion and may act as a radiosensitizer in lung cancer cells. J Thorac Oncol 2009; 4:448-454.
29. Rajeshkumar NV, Tan AC, De Oliveira E, Womack C, Wombwell H, et al. Antitumor effects and biomarkers of activity of AZD0530, a Src inhibitor, in pancreatic cancer. Clin Cancer Res 2009; 15:4138-4146.
30. Lara PN, Jr., Longmate J, Evans CP, Quinn DI, Twardowski P, et al. A phase II trial of the Src-kinase inhibitor AZD0530 in patients with advanced castration-resistant prostate cancer: a California Cancer Consortium study. Anticancer Drugs 2009; 20:179-184.
31. Murray PJ. The JAK-STAT signaling pathway: input and output integration. J Immunol 2007; 178:2623-2629.
32. Yu H, Pardoll D, Jove R. STATs in cancer inflammation and immunity: a leading role for STAT3. Nat Rev Cancer 2009; 9:798-809.
33. Kryczek I, Lin Y, Nagarsheth N, Peng D, Zhao L, et al. IL-22(+)CD4(+) T cells promote colorectal cancer stemness via STAT3 transcription factor activation and induction of the methyltransferase DOT1L. Immunity 2014; 40:772-784.
34. Leonard WJ, O'Shea JJ. Jaks and STATs: biological implications. Annu Rev Immunol 1998; 16:293-322.
35. Wang BX, Platanias LC, Fish EN. STAT activation in malignancies: roles in tumor progression and in the generation of antineoplastic effects of IFNs. J Interferon Cytokine Res 2013; 33:181-188.
36. Furth PA. STAT signaling in different breast cancer sub-types. Mol Cell Endocrinol 2014; 382:612-615.
37. Lam LT, Wright G, Davis RE, Lenz G, Farinha P, et al. Cooperative signaling through the signal transducer and activator of transcription 3 and nuclear factor-{kappa}B pathways in subtypes of diffuse large B-cell lymphoma. Blood 2008; 111:3701-3713.
38. Song JI, Grandis JR. STAT signaling in head and neck cancer. Oncogene 2000; 19:2489-2495.
39. Li P, Harris D, Liu Z, Liu J, Keating M, et al. Stat3 activates the receptor tyrosine kinase like orphan receptor-1 gene in chronic lymphocytic leukemia cells. PLoS One 2010; 5:e11859.

40. Hogfeldt T, Bahnassy AA, Kwiecinska A, Osterborg A, Tamm KP, et al. Patients with activated B-cell like diffuse large B-cell lymphoma in high and low infectious disease areas have different inflammatory gene signatures. Leuk Lymphoma 2013; 54:996-1003.
41. Pan Y, Zheng M, Zhong L, Yang J, Zhou S, et al. A preclinical evaluation of SKLB261, a multikinase inhibitor of EGFR/Src/VEGFR2, as a therapeutic agent against pancreatic cancer. Mol Cancer Ther 2015; 14:407-418.
42. Wong AL, Soo RA, Tan DS, Lee SC, Lim JS, et al. Phase I and biomarker study of OPB-51602, a novel signal transducer and activator of transcription (STAT) 3 inhibitor, in patients with refractory solid malignancies. Ann Oncol 2015; 26:998-1005.
43. Kim MJ, Nam HJ, Kim HP, Han SW, Im SA, et al. OPB-31121, a novel small molecular inhibitor, disrupts the JAK2/STAT3 pathway and exhibits an antitumor activity in gastric cancer cells. Cancer Lett 2013; 335:145-152.
44. Hayakawa F, Sugimoto K, Harada Y, Hashimoto N, Ohi N, et al. A novel STAT inhibitor, OPB-31121, has a significant antitumor effect on leukemia with STAT-addictive oncokinases. Blood Cancer J 2013; 3:e166.
45. Bendell JC, Hong DS, Burris HA, 3rd, Naing A, Jones SF, et al. Phase 1, open-label, dose-escalation, and pharmacokinetic study of STAT3 inhibitor OPB-31121 in subjects with advanced solid tumors. Cancer Chemother Pharmacol 2014; 74:125-130.
46. Becker H, Engelhardt M, von Bubnoff N, Wasch R. Ruxolitinib. Recent Results Cancer Res 2014; 201:249-257.
47. Quintas-Cardama A, Vaddi K, Liu P, Manshouri T, Li J, et al. Preclinical characterization of the selective JAK1/2 inhibitor INCB018424: therapeutic implications for the treatment of myeloproliferative neoplasms. Blood 2010; 115:3109-3117.
48. Muxi PJ, Oliver AC. Jak-2 positive myeloproliferative neoplasms. Curr Treat Options Oncol 2014; 15:147-156.
49. Swaim SJ. Ruxolitinib for the treatment of primary myelofibrosis. Am J Health Syst Pharm 2014; 71:453-462.
50. Pozdnyakova O, Hasserjian RP, Verstovsek S, Orazi A. Impact of Bone Marrow Pathology on the Clinical Management of Philadelphia Chromosome-Negative Myeloproliferative Neoplasms. Clin Lymphoma Myeloma Leuk 2015; 15:253-261.
51. Geyer HL, Mesa RA. Therapy for myeloproliferative neoplasms: when, which agent, and how? Blood 2014; 124:3529-3537.
52. Yuan K, Chen J, Xu A. Tofacitinib versus methotrexate in rheumatoid arthritis. N Engl J Med 2014; 371:1163-1164.
53. Kremer JM, Cohen S, Wilkinson BE, Connell CA, French JL, et al. A phase IIb dose-ranging study of the oral JAK inhibitor tofacitinib (CP-690,550) versus placebo in combination with background methotrexate in patients with active rheumatoid arthritis and an inadequate response to methotrexate alone. Arthritis Rheum 2012; 64:970-981.
54. Sexton D. Tofacitinib versus methotrexate in rheumatoid arthritis. N Engl J Med 2014; 371:1163.
55. Meyer SC, Levine RL. Molecular pathways: molecular basis for sensitivity and resistance to JAK kinase inhibitors. Clin Cancer Res 2014; 20:2051-2059.
56. Hanna DM, Fellowes A, Vedururu R, Mechinaud F, Hansford JR. A unique case of refractory primary mediastinal B-cell lymphoma with JAK3 mutation and the role for targeted therapy. Haematologica 2014; 99:e156-158.
57. Lapeire L, Hendrix A, Lambein K, Van Bockstal M, Braems G, et al. Cancer-associated adipose tissue promotes breast cancer progression by paracrine oncostatin M and Jak/STAT3 signaling. Cancer Res 2014; 74:6806-6819.

58. Degryse S, de Bock CE, Cox L, Demeyer S, Gielen O, et al. JAK3 mutants transform hematopoietic cells through JAK1 activation, causing T-cell acute lymphoblastic leukemia in a mouse model. Blood 2014; 124:3092-3100.
59. Bouchekioua A, Scourzic L, de Wever O, Zhang Y, Cervera P, et al. JAK3 deregulation by activating mutations confers invasive growth advantage in extranodal nasal-type natural killer cell lymphoma. Leukemia 2014; 28:338-348.
60. Cosgrove SB, Wren JA, Cleaver DM, Martin DD, Walsh KF, et al. Efficacy and safety of oclacitinib for the control of pruritus and associated skin lesions in dogs with canine allergic dermatitis. Vet Dermatol 2013; 24:479-e114.
61. van Vollenhoven RF. Small molecular compounds in development for rheumatoid arthritis. Curr Opin Rheumatol 2013; 25:391-397.
62. Hatzimichael E, Tsolas E, Briasoulis E. Profile of pacritinib and its potential in the treatment of hematologic disorders. J Blood Med 2014; 5:143-152.
63. Rosenthal A, Mesa RA. Janus kinase inhibitors for the treatment of myeloproliferative neoplasms. Expert Opin Pharmacother 2014; 15:1265-1276.
64. Shabbir M, Stuart R. Lestaurtinib, a multitargeted tyrosine kinase inhibitor: from bench to bedside. Expert Opin Investig Drugs 2010; 19:427-436.
65. Dhut S, Chaplin T, Young BD. BCR-ABL and BCR proteins: biochemical characterization and localization. Leukemia 1990; 4:745-750.
66. Cilloni D, Saglio G. Molecular pathways: BCR-ABL. Clin Cancer Res 2012; 18:930-937.
67. An X, Tiwari AK, Sun Y, Ding PR, Ashby CR, Jr., et al. BCR-ABL tyrosine kinase inhibitors in the treatment of Philadelphia chromosome positive chronic myeloid leukemia: a review. Leuk Res 2010; 34:1255-1268.
68. O'Hare T, Shakespeare WC, Zhu X, Eide CA, Rivera VM, et al. AP24534, a pan-BCR-ABL inhibitor for chronic myeloid leukemia, potently inhibits the T315I mutant and overcomes mutation-based resistance. Cancer Cell 2009; 16:401-412.
69. Huang WS, Metcalf CA, Sundaramoorthi R, Wang Y, Zou D, et al. Discovery of 3-[2-(imidazo[1,2-b]pyridazin-3-yl)ethynyl]-4-methyl-N-{4-[(4-methylpiperazin-1-y l)methyl]-3-(trifluoromethyl)phenyl}benzamide (AP24534), a potent, orally active pan-inhibitor of breakpoint cluster region-abelson (BCR-ABL) kinase including the T315I gatekeeper mutant. J Med Chem 2010; 53:4701-4719.
70. Okabe S, Tauchi T, Tanaka Y, Ohyashiki K. Efficacy of ponatinib against ABL tyrosine kinase inhibitor-resistant leukemia cells. Biochem Biophys Res Commun 2013; 435:506-511.
71. Iacob RE, Zhang J, Gray NS, Engen JR. Allosteric interactions between the myristate- and ATP-site of the Abl kinase. PLoS One 2011; 6:e15929.
72. Zhang J, Adrian FJ, Jahnke W, Cowan-Jacob SW, Li AG, et al. Targeting Bcr-Abl by combining allosteric with ATP-binding-site inhibitors. Nature 2010; 463:501-506.
73. Yoon H, Choi YL, Song JY, Do I, Kang SY, et al. Targeted inhibition of FAK, PYK2 and BCL-XL synergistically enhances apoptosis in ovarian clear cell carcinoma cell lines. PLoS One 2014; 9:e88587.
74. McLean GW, Carragher NO, Avizienyte E, Evans J, Brunton VG, et al. The role of focal-adhesion kinase in cancer - a new therapeutic opportunity. Nat Rev Cancer 2005; 5:505-515.
75. Sulzmaier FJ, Jean C, Schlaepfer DD. FAK in cancer: mechanistic findings and clinical applications. Nat Rev Cancer 2014; 14:598-610.
76. Halder J, Landen CN, Jr., Lutgendorf SK, Li Y, Jennings NB, et al. Focal adhesion kinase silencing augments docetaxel-mediated apoptosis in ovarian cancer cells. Clin Cancer Res 2005; 11:8829-8836.

77. Lim ST, Mikolon D, Stupack DG, Schlaepfer DD. FERM control of FAK function: implications for cancer therapy. Cell Cycle 2008; 7:2306-2314.
78. Slack-Davis JK, Martin KH, Tilghman RW, Iwanicki M, Ung EJ, et al. Cellular characterization of a novel focal adhesion kinase inhibitor. J Biol Chem 2007; 282:14845-14852.
79. Kang Y, Hu W, Ivan C, Dalton HJ, Miyake T, et al. Role of focal adhesion kinase in regulating YB-1-mediated paclitaxel resistance in ovarian cancer. J Natl Cancer Inst 2013; 105:1485-1495.
80. Guo W, Liu R, Bhardwaj G, Yang JC, Changou C, et al. Targeting Btk/Etk of prostate cancer cells by a novel dual inhibitor. Cell Death Dis 2014; 5:e1409.
81. Horwood NJ, Urbaniak AM, Danks L. Tec family kinases in inflammation and disease. Int Rev Immunol 2012; 31:87-103.
82. Lo HY. Itk inhibitors: a patent review. Expert Opin Ther Pat 2010; 20:459-469.
83. Charrier JD, Knegtel RM. Advances in the design of ITK inhibitors. Expert Opin Drug Discov 2013; 8:369-381.
84. Davids MS, Brown JR. Ibrutinib: a first in class covalent inhibitor of Bruton's tyrosine kinase. Future Oncol 2014; 10:957-967.
85. Rajabi B, Sweetenham JW. Mantle cell lymphoma: observation to transplantation. Ther Adv Hematol 2015; 6:37-48.
86. Pan Z, Scheerens H, Li SJ, Schultz BE, Sprengeler PA, et al. Discovery of selective irreversible inhibitors for Bruton's tyrosine kinase. ChemMedChem 2007; 2:58-61.
87. Honigberg LA, Smith AM, Sirisawad M, Verner E, Loury D, et al. The Bruton tyrosine kinase inhibitor PCI-32765 blocks B-cell activation and is efficacious in models of autoimmune disease and B-cell malignancy. Proc Natl Acad Sci U S A 2010; 107:13075-13080.
88. Xing L, Huang A. Bruton's TK inhibitors: structural insights and evolution of clinical candidates. Future Med Chem 2014; 6:675-695.
89. Herman SE, Gordon AL, Wagner AJ, Heerema NA, Zhao W, et al. Phosphatidylinositol 3-kinase-delta inhibitor CAL-101 shows promising preclinical activity in chronic lymphocytic leukemia by antagonizing intrinsic and extrinsic cellular survival signals. Blood 2010; 116:2078-2088.
90. Choi MY, Kipps TJ. Inhibitors of B-cell receptor signaling for patients with B-cell malignancies. Cancer J 2012; 18:404-410.
91. Sivina M, Kreitman RJ, Arons E, Ravandi F, Burger JA. The bruton tyrosine kinase inhibitor ibrutinib (PCI-32765) blocks hairy cell leukaemia survival, proliferation and B cell receptor signalling: a new therapeutic approach. Br J Haematol 2014; 166:177-188.
92. Jain N, O'Brien S. Ibrutinib (PCI-32765) in chronic lymphocytic leukemia. Hematol Oncol Clin North Am 2013; 27:851-860.
93. Winer ES, Ingham RR, Castillo JJ. PCI-32765: a novel Bruton's tyrosine kinase inhibitor for the treatment of lymphoid malignancies. Expert Opin Investig Drugs 2012; 21:355-361.
94. Aalipour A, Advani RH. Bruton's tyrosine kinase inhibitors and their clinical potential in the treatment of B-cell malignancies: focus on ibrutinib. Ther Adv Hematol 2014; 5:121-133.
95. Davids MS, Brown JR. Ibrutinib: a first in class covalent inhibitor of Bruton's tyrosine kinase. Future Oncology 2014; 10:957-967.
96. Tarafdar S, Poe JA, Smithgall TE. The accessory factor Nef links HIV-1 to Tec/Btk kinases in an Src homology 3 domain-dependent manner. J Biol Chem 2014; 289:15718-15728.
97. Lin TA, McIntyre KW, Das J, Liu C, O'Day KD, et al. Selective Itk inhibitors block T-cell activation and murine lung inflammation. Biochemistry 2004; 43:11056-11062.
98. Zhong Y, Dong S, Strattan E, Ren L, Butchar JP, et al. Targeting Interleukin-2-inducible T-cell Kinase (ITK) and Resting Lymphocyte Kinase (RLK) Using a Novel Covalent Inhibitor PRN694. J Biol Chem 2015; 290:5960-5978.

99. Guo S, Sun F, Guo Z, Li W, Alfano A, et al. Tyrosine kinase ETK/BMX is up-regulated in bladder cancer and predicts poor prognosis in patients with cystectomy. PLoS One 2011; 6:e17778.
100. Bradshaw JM. The Src, Syk, and Tec family kinases: distinct types of molecular switches. Cell Signal 2010; 22:1175-1184.
101. Mraz M, Kipps TJ. MicroRNAs and B cell receptor signaling in chronic lymphocytic leukemia. Leuk Lymphoma 2013; 54:1836-1839.
102. Lankester AC, Schijndel GM, Pakker NG, Van Oers RH, van Lier RA. Antigen receptor function in chronic lymphocytic leukemia B cells. Leuk Lymphoma 1996; 24:27-33.
103. Ernst A, Hofmann S, Ahmadi R, Becker N, Korshunov A, et al. Genomic and expression profiling of glioblastoma stem cell-like spheroid cultures identifies novel tumor-relevant genes associated with survival. Clin Cancer Res 2009; 15:6541-6550.
104. Hahn CK, Berchuck JE, Ross KN, Kakoza RM, Clauser K, et al. Proteomic and genetic approaches identify Syk as an AML target. Cancer Cell 2009; 16:281-294.
105. Feldman AL, Sun DX, Law ME, Novak AJ, Attygalle AD, et al. Overexpression of Syk tyrosine kinase in peripheral T-cell lymphomas. Leukemia 2008; 22:1139-1143.
106. Geahlen RL. Getting Syk: spleen tyrosine kinase as a therapeutic target. Trends Pharmacol Sci 2014; 35:414-422.
107. Bailet O, Fenouille N, Abbe P, Robert G, Rocchi S, et al. Spleen tyrosine kinase functions as a tumor suppressor in melanoma cells by inducing senescence-like growth arrest. Cancer Res 2009; 69:2748-2756.
108. Coopman PJ, Do MT, Barth M, Bowden ET, Hayes AJ, et al. The Syk tyrosine kinase suppresses malignant growth of human breast cancer cells. Nature 2000; 406:742-747.
109. Moroni M, Soldatenkov V, Zhang L, Zhang Y, Stoica G, et al. Progressive loss of Syk and abnormal proliferation in breast cancer cells. Cancer Res 2004; 64:7346-7354.
110. Layton T, Stalens C, Gunderson F, Goodison S, Silletti S. Syk tyrosine kinase acts as a pancreatic adenocarcinoma tumor suppressor by regulating cellular growth and invasion. Am J Pathol 2009; 175:2625-2636.
111. Krisenko MO, Geahlen RL. Calling in SYK: SYK's dual role as a tumor promoter and tumor suppressor in cancer. Biochim Biophys Acta 2015; 1853:254-263.
112. Suljagic M, Longo PG, Bennardo S, Perlas E, Leone G, et al. The Syk inhibitor fostamatinib disodium (R788) inhibits tumor growth in the Emu- TCL1 transgenic mouse model of CLL by blocking antigen-dependent B-cell receptor signaling. Blood 2010; 116:4894-4905.
113. Weinblatt ME, Genovese MC, Ho M, Hollis S, Rosiak-Jedrychowicz K, et al. Effects of fostamatinib, an oral spleen tyrosine kinase inhibitor, in rheumatoid arthritis patients with an inadequate response to methotrexate: results from a phase III, multicenter, randomized, double-blind, placebo-controlled, parallel-group study. Arthritis Rheumatol 2014; 66:3255-3264.
114. Friedberg JW, Sharman J, Sweetenham J, Johnston PB, Vose JM, et al. Inhibition of Syk with fostamatinib disodium has significant clinical activity in non-Hodgkin lymphoma and chronic lymphocytic leukemia. Blood 2010; 115:2578-2585.
115. Hoellenriegel J, Coffey GP, Sinha U, Pandey A, Sivina M, et al. Selective, novel spleen tyrosine kinase (Syk) inhibitors suppress chronic lymphocytic leukemia B-cell activation and migration. Leukemia 2012; 26:1576-1583.
116. Coffey G, DeGuzman F, Inagaki M, Pak Y, Delaney SM, et al. Specific inhibition of spleen tyrosine kinase suppresses leukocyte immune function and inflammation in animal models of rheumatoid arthritis. J Pharmacol Exp Ther 2012; 340:350-359.
117. Spurgeon SE, Coffey G, Fletcher LB, Burke R, Tyner JW, et al. The selective SYK inhibitor P505-15 (PRT062607) inhibits B cell signaling and function in vitro and in vivo and augments the activity of fludarabine in chronic lymphocytic leukemia. J Pharmacol Exp Ther 2013; 344:378-387.

118. Burke RT, Meadows S, Loriaux MM, Currie KS, Mitchell SA, et al. A potential therapeutic strategy for chronic lymphocytic leukemia by combining Idelalisib and GS-9973, a novel spleen tyrosine kinase (Syk) inhibitor. Oncotarget 2014; 5:908-915.
119. Sharman J, Hawkins M, Kolibaba K, Boxer M, Klein L, et al. An open-label phase 2 trial of entospletinib (GS-9973), a selective spleen tyrosine kinase inhibitor, in chronic lymphocytic leukemia. Blood 2015; 125:2336-2343.
120. Zhao Y, Adjei AA. The clinical development of MEK inhibitors. Nat Rev Clin Oncol 2014; 11:385-400.
121. Fischmann TO, Smith CK, Mayhood TW, Myers JE, Reichert P, et al. Crystal structures of MEK1 binary and ternary complexes with nucleotides and inhibitors. Biochemistry 2009; 48:2661-2674.
122. Dimou AT, Syrigos KN, Saif MW. Novel agents in the management of pancreatic adenocarcinoma: phase I studies. Highlights from the "2011 ASCO Gastrointestinal Cancers Symposium". San Francisco, CA, USA. January 20-22, 2011. JOP 2011; 12:114-116.
123. Joshi M, Rice SJ, Liu X, Miller B, Belani CP. Trametinib with or without Vemurafenib in BRAF Mutated Non-Small Cell Lung Cancer. PLoS One 2015; 10:e0118210.
124. Sullivan RJ, Flaherty KT. Resistance to BRAF-targeted therapy in melanoma. Eur J Cancer 2013; 49:1297-1304.
125. Flaherty KT, Robert C, Hersey P, Nathan P, Garbe C, et al. Improved survival with MEK inhibition in BRAF-mutated melanoma. N Engl J Med 2012; 367:107-114.
126. Falchook GS, Lewis KD, Infante JR, Gordon MS, Vogelzang NJ, et al. Activity of the oral MEK inhibitor trametinib in patients with advanced melanoma: a phase 1 dose-escalation trial. Lancet Oncol 2012; 13:782-789.
127. Andrews MC, Behren A, Chionh F, Mariadason J, Vella LJ, et al. BRAF inhibitor-driven tumor proliferation in a KRAS-mutated colon carcinoma is not overcome by MEK1/2 inhibition. J Clin Oncol 2013; 31:e448-451.
128. Coupe N, Corrie P, Hategan M, Larkin J, Gore M, et al. PACMEL: A phase 1 dose escalation trial of trametinib (GSK1120212) in combination with paclitaxel. Eur J Cancer 2015; 51:359-366.
129. Bedard PL, Tabernero J, Janku F, Wainberg ZA, Paz-Ares L, et al. A Phase Ib Dose-Escalation Study of the Oral Pan-PI3K Inhibitor Buparlisib (BKM120) in Combination with the Oral MEK1/2 Inhibitor Trametinib (GSK1120212) in Patients with Selected Advanced Solid Tumors. Clin Cancer Res 2015; 21:730-738.
130. Tolcher AW, Patnaik A, Papadopoulos KP, Rasco DW, Becerra CR, et al. Phase I study of the MEK inhibitor trametinib in combination with the AKT inhibitor afuresertib in patients with solid tumors and multiple myeloma. Cancer Chemother Pharmacol 2015; 75:183-189.
131. Cohen RB, Aamdal S, Nyakas M, Cavallin M, Green D, et al. A phase I dose-finding, safety and tolerability study of AZD8330 in patients with advanced malignancies. Eur J Cancer 2013; 49:1521-1529.
132. Yao Y, Bian YP, Xia DD, Pan B, Niu MS, et al. [Effect of AZD8330 on proliferation and apoptosis of multiple myeloma cells]. Zhongguo Shi Yan Xue Ye Xue Za Zhi 2014; 22:1311-1315.
133. Feng K, Wang C, Zhou H, Yang J, Dong L, et al. [Effect of ERK1/2 inhibitor AZD8330 on human Burkitt's lymphoma cell line Raji cells and its mechanism]. Zhonghua Xue Ye Xue Za Zhi 2015; 36:148-152.
134. Yeh TC, Marsh V, Bernat BA, Ballard J, Colwell H, et al. Biological characterization of ARRY-142886 (AZD6244), a potent, highly selective mitogen-activated protein kinase kinase 1/2 inhibitor. Clin Cancer Res 2007; 13:1576-1583.
135. Haass NK, Sproesser K, Nguyen TK, Contractor R, Medina CA, et al. The mitogen-activated protein/extracellular signal-regulated kinase kinase inhibitor AZD6244 (ARRY-142886) induces

growth arrest in melanoma cells and tumor regression when combined with docetaxel. Clin Cancer Res 2008; 14:230-239.
136. Davies BR, Logie A, McKay JS, Martin P, Steele S, et al. AZD6244 (ARRY-142886), a potent inhibitor of mitogen-activated protein kinase/extracellular signal-regulated kinase kinase 1/2 kinases: mechanism of action in vivo, pharmacokinetic/pharmacodynamic relationship, and potential for combination in preclinical models. Mol Cancer Ther 2007; 6:2209-2219.
137. Huynh H, Soo KC, Chow PK, Tran E. Targeted inhibition of the extracellular signal-regulated kinase kinase pathway with AZD6244 (ARRY-142886) in the treatment of hepatocellular carcinoma. Mol Cancer Ther 2007; 6:138-146.
138. Adjei AA, Cohen RB, Franklin W, Morris C, Wilson D, et al. Phase I pharmacokinetic and pharmacodynamic study of the oral, small-molecule mitogen-activated protein kinase kinase 1/2 inhibitor AZD6244 (ARRY-142886) in patients with advanced cancers. J Clin Oncol 2008; 26:2139-2146.
139. Farley J, Brady WE, Vathipadiekal V, Lankes HA, Coleman R, et al. Selumetinib in women with recurrent low-grade serous carcinoma of the ovary or peritoneum: an open-label, single-arm, phase 2 study. Lancet Oncol 2013; 14:134-140.
140. Catalanotti F, Solit DB, Pulitzer MP, Berger MF, Scott SN, et al. Phase II trial of MEK inhibitor selumetinib (AZD6244, ARRY-142886) in patients with BRAFV600E/K-mutated melanoma. Clin Cancer Res 2013; 19:2257-2264.
141. Hayes DN, Lucas AS, Tanvetyanon T, Krzyzanowska MK, Chung CH, et al. Phase II efficacy and pharmacogenomic study of Selumetinib (AZD6244; ARRY-142886) in iodine-131 refractory papillary thyroid carcinoma with or without follicular elements. Clin Cancer Res 2012; 18:2056-2065.
142. O'Neil BH, Goff LW, Kauh JS, Strosberg JR, Bekaii-Saab TS, et al. Phase II study of the mitogen-activated protein kinase 1/2 inhibitor selumetinib in patients with advanced hepatocellular carcinoma. J Clin Oncol 2011; 29:2350-2356.
143. Jain N, Curran E, Iyengar NM, Diaz-Flores E, Kunnavakkam R, et al. Phase II study of the oral MEK inhibitor selumetinib in advanced acute myelogenous leukemia: a University of Chicago phase II consortium trial. Clin Cancer Res 2014; 20:490-498.
144. Larkin J, Ascierto PA, Dreno B, Atkinson V, Liszkay G, et al. Combined vemurafenib and cobimetinib in BRAF-mutated melanoma. N Engl J Med 2014; 371:1867-1876.
145. Ribas A, Gonzalez R, Pavlick A, Hamid O, Gajewski TF, et al. Combination of vemurafenib and cobimetinib in patients with advanced BRAF(V600)-mutated melanoma: a phase 1b study. Lancet Oncol 2014; 15:954-965.
146. Paulo JA, McAllister FE, Everley RA, Beausoleil SA, Banks AS, et al. Effects of MEK inhibitors GSK1120212 and PD0325901 in vivo using 10-plex quantitative proteomics and phosphoproteomics. Proteomics 2015; 15:462-473.
147. El-Hoss J, Cheng T, Carpenter EC, Sullivan K, Deo N, et al. A Combination of rhBMP-2 (Recombinant Human Bone Morphogenetic Protein-2) and MEK (MAP Kinase/ERK Kinase) Inhibitor PD0325901 Increases Bone Formation in a Murine Model of Neurofibromatosis Type I Pseudarthrosis. J Bone Joint Surg Am 2014; 96:e117.
148. Martinelli E, Troiani T, D'Aiuto E, Morgillo F, Vitagliano D, et al. Antitumor activity of pimasertib, a selective MEK 1/2 inhibitor, in combination with PI3K/mTOR inhibitors or with multi-targeted kinase inhibitors in pimasertib-resistant human lung and colorectal cancer cells. Int J Cancer 2013; 133:2089-2101.
149. Lim HY, Heo J, Choi HJ, Lin CY, Yoon JH, et al. A phase II study of the efficacy and safety of the combination therapy of the MEK inhibitor refametinib (BAY 86-9766) plus sorafenib for Asian patients with unresectable hepatocellular carcinoma. Clin Cancer Res 2014; 20:5976-5985.

150. Schmieder R, Puehler F, Neuhaus R, Kissel M, Adjei AA, et al. Allosteric MEK1/2 inhibitor refametinib (BAY 86-9766) in combination with sorafenib exhibits antitumor activity in preclinical murine and rat models of hepatocellular carcinoma. Neoplasia 2013; 15:1161-1171.
151. Somoza JR, Koditek D, Villasenor AG, Novikov N, Wong MH, et al. Structural, biochemical, and biophysical characterization of idelalisib binding to phosphoinositide 3-kinase delta. J Biol Chem 2015; 290:8439-8446.
152. Bernal A, Pastore RD, Asgary Z, Keller SA, Cesarman E, et al. Survival of leukemic B cells promoted by engagement of the antigen receptor. Blood 2001; 98:3050-3057.
153. Sharman J, de Vos S, Leonard JP, Furman RR, Coutre SE, et al. A Phase 1 Study of the Selective Phosphatidylinositol 3-Kinase-Delta (PI3K delta) Inhibitor, CAL-101 (GS-1101), in Combination with Rituximab and/or Bendamustine in Patients with Relapsed or Refractory Chronic Lymphocytic Leukemia (CLL). Blood 2011; 118:779-780.
154. Leonard J, Schreeder M, Coutre S, Flinn I, Wagner-Johnston N, et al. A Phase 1 Study of Cal-101, an Isoform-Selective Inhibitor of Phosphatidylinositol 3-Kinase P110d, in Combination with Anti-Cd20 Monoclonal Antibody Therapy and/or Bendamustine in Patients with Previously Treated B-Cell Malignancies. Annals of Oncology 2011; 22:137-137.
155. Lannutti BJ, Meadows SA, Herman SE, Kashishian A, Steiner B, et al. CAL-101, a p110delta selective phosphatidylinositol-3-kinase inhibitor for the treatment of B-cell malignancies, inhibits PI3K signaling and cellular viability. Blood 2011; 117:591-594.
156. Hoellenriegel J, Meadows SA, Sivina M, Wierda WG, Kantarjian H, et al. The phosphoinositide 3'-kinase delta inhibitor, CAL-101, inhibits B-cell receptor signaling and chemokine networks in chronic lymphocytic leukemia. Blood 2011; 118:3603-3612.
157. Fiorcari S, Brown WS, McIntyre BW, Estrov Z, Maffei R, et al. The PI3-kinase delta inhibitor idelalisib (GS-1101) targets integrin-mediated adhesion of chronic lymphocytic leukemia (CLL) cell to endothelial and marrow stromal cells. PLoS One 2013; 8:e83830.
158. Chung C, Lee R. Ibrutinib, obinutuzumab, idelalisib, and beyond: review of novel and evolving therapies for chronic lymphocytic leukemia. Pharmacotherapy 2014; 34:1298-1316.
159. Miller BW, Przepiorka D, de Claro RA, Lee K, Nie L, et al. FDA approval: idelalisib monotherapy for the treatment of patients with follicular lymphoma and small lymphocytic lymphoma. Clin Cancer Res 2015; 21:1525-1529.
160. Furman RR, Sharman JP, Coutre SE, Cheson BD, Pagel JM, et al. Idelalisib and rituximab in relapsed chronic lymphocytic leukemia. N Engl J Med 2014; 370:997-1007.
161. Ikeda H, Hideshima T, Fulciniti M, Perrone G, Miura N, et al. PI3K/p110{delta} is a novel therapeutic target in multiple myeloma. Blood 2010; 116:1460-1468.
162. Ehrhardt M, Craveiro RB, Holst MI, Pietsch T, Dilloo D. The PI3K inhibitor GDC-0941 displays promising in vitro and in vivo efficacy for targeted medulloblastoma therapy. Oncotarget 2015; 6:802-813.
163. Poovassery JS, Kang JC, Kim D, Ober RJ, Ward ES. Antibody targeting of HER2/HER3 signaling overcomes heregulin-induced resistance to PI3K inhibition in prostate cancer. Int J Cancer 2014.
164. Sarker D, Ang JE, Baird R, Kristeleit R, Shah K, et al. First-in-human phase I study of pictilisib (GDC-0941), a potent pan-class I phosphatidylinositol-3-kinase (PI3K) inhibitor, in patients with advanced solid tumors. Clin Cancer Res 2015; 21:77-86.
165. Alagesan B, Contino G, Guimaraes AR, Corcoran RB, Deshpande V, et al. Combined MEK and PI3K inhibition in a mouse model of pancreatic cancer. Clin Cancer Res 2015; 21:396-404.
166. Al-Saffar NM, Marshall LV, Jackson LE, Balarajah G, Eykyn TR, et al. Lactate and choline metabolites detected in vitro by nuclear magnetic resonance spectroscopy are potential metabolic biomarkers for PI3K inhibition in pediatric glioblastoma. PLoS One 2014; 9:e103835.

167. Dail M, Wong J, Lawrence J, O'Connor D, Nakitandwe J, et al. Loss of oncogenic Notch1 with resistance to a PI3K inhibitor in T-cell leukaemia. Nature 2014; 513:512-516.
168. Reikvam H, Tamburini J, Skrede S, Holdhus R, Poulain L, et al. Antileukaemic effect of PI3K-mTOR inhibitors in acute myeloid leukaemia-gene expression profiles reveal CDC25B expression as determinate of pharmacological effect. Br J Haematol 2014; 164:200-211.
169. Haagensen EJ, Thomas HD, Wilson I, Harnor SJ, Payne SL, et al. The enhanced in vivo activity of the combination of a MEK and a PI3K inhibitor correlates with [18F]-FLT PET in human colorectal cancer xenograft tumour-bearing mice. PLoS One 2013; 8:e81763.
170. Kirkpatrick DS, Bustos DJ, Dogan T, Chan J, Phu L, et al. Phosphoproteomic characterization of DNA damage response in melanoma cells following MEK/PI3K dual inhibition. Proc Natl Acad Sci U S A 2013; 110:19426-19431.
171. Reikvam H, Nepstad I, Bruserud O, Hatfield KJ. Pharmacological targeting of the PI3K/mTOR pathway alters the release of angioregulatory mediators both from primary human acute myeloid leukemia cells and their neighboring stromal cells. Oncotarget 2013; 4:830-843.
172. Li C, Takahashi C, Zhang L, Huseni M, Stankovich B, et al. Development of a robust flow cytometry-based pharmacodynamic assay to detect phospho-protein signals for phosphatidylinositol 3-kinase inhibitors in multiple myeloma. J Transl Med 2013; 11:76.
173. Tabe Y, Jin L, Konopleva M, Shikami M, Kimura S, et al. Class IA PI3K inhibition inhibits cell growth and proliferation in mantle cell lymphoma. Acta Haematol 2014; 131:59-69.
174. Martin SK, Gan ZY, Fitter S, To LB, Zannettino AC. The effect of the PI3K inhibitor BKM120 on tumour growth and osteolytic bone disease in multiple myeloma. Leuk Res 2015; 39:380-387.
175. Matas-Cespedes A, Rodriguez V, Kalko SG, Vidal-Crespo A, Rosich L, et al. Disruption of follicular dendritic cells-follicular lymphoma cross-talk by the pan-PI3K inhibitor BKM120 (Buparlisib). Clin Cancer Res 2014; 20:3458-3471.
176. Bendell JC, Rodon J, Burris HA, de Jonge M, Verweij J, et al. Phase I, dose-escalation study of BKM120, an oral pan-Class I PI3K inhibitor, in patients with advanced solid tumors. J Clin Oncol 2012; 30:282-290.
177. Ando Y, Inada-Inoue M, Mitsuma A, Yoshino T, Ohtsu A, et al. Phase I dose-escalation study of buparlisib (BKM120), an oral pan-class I PI3K inhibitor, in Japanese patients with advanced solid tumors. Cancer Sci 2014; 105:347-353.
178. Mayer IA, Abramson VG, Isakoff SJ, Forero A, Balko JM, et al. Stand up to cancer phase Ib study of pan-phosphoinositide-3-kinase inhibitor buparlisib with letrozole in estrogen receptor-positive/human epidermal growth factor receptor 2-negative metastatic breast cancer. J Clin Oncol 2014; 32:1202-1209.
179. Foster P, Yamaguchi K, Hsu PP, Qian F, Du X, et al. The Selective PI3K Inhibitor XL147 (SAR245408) Inhibits Tumor Growth and Survival and Potentiates the Activity of Chemotherapeutic Agents in Preclinical Tumor Models. Mol Cancer Ther 2015; 14:931-940.
180. Chakrabarty A, Sanchez V, Kuba MG, Rinehart C, Arteaga CL. Feedback upregulation of HER3 (ErbB3) expression and activity attenuates antitumor effect of PI3K inhibitors. Proc Natl Acad Sci U S A 2012; 109:2718-2723.
181. Soria JC, LoRusso P, Bahleda R, Lager J, Liu L, et al. Phase I Dose-Escalation Study of Pilaralisib (SAR245408, XL147), a Pan-Class I PI3K Inhibitor, in Combination With Erlotinib in Patients With Solid Tumors. Oncologist 2015; 20:245-246.
182. Matulonis U, Vergote I, Backes F, Martin LP, McMeekin S, et al. Phase II study of the PI3K inhibitor pilaralisib (SAR245408; XL147) in patients with advanced or recurrent endometrial carcinoma. Gynecol Oncol 2015; 136:246-253.

183. Tolaney S, Burris H, Gartner E, Mayer IA, Saura C, et al. Phase I/II study of pilaralisib (SAR245408) in combination with trastuzumab or trastuzumab plus paclitaxel in trastuzumab-refractory HER2-positive metastatic breast cancer. Breast Cancer Res Treat 2015; 149:151-161.
184. Furet P, Guagnano V, Fairhurst RA, Imbach-Weese P, Bruce I, et al. Discovery of NVP-BYL719 a potent and selective phosphatidylinositol-3 kinase alpha inhibitor selected for clinical evaluation. Bioorg Med Chem Lett 2013; 23:3741-3748.
185. O'Brien NA, McDonald K, Tong L, von Euw E, Kalous O, et al. Targeting PI3K/mTOR overcomes resistance to HER2-targeted therapy independent of feedback activation of AKT. Clin Cancer Res 2014; 20:3507-3520.
186. Wong MH, Xue A, Julovi SM, Pavlakis N, Samra JS, et al. Cotargeting of epidermal growth factor receptor and PI3K overcomes PI3K-Akt oncogenic dependence in pancreatic ductal adenocarcinoma. Clin Cancer Res 2014; 20:4047-4058.
187. Gobin B, Huin MB, Lamoureux F, Ory B, Charrier C, et al. BYL719, a new alpha-specific PI3K inhibitor: single administration and in combination with conventional chemotherapy for the treatment of osteosarcoma. Int J Cancer 2015; 136:784-796.
188. Jeong Y, Kwon D, Hong S. Selective and potent small-molecule inhibitors of PI3Ks. Future Medicinal Chemistry 2014; 6:737-756.
189. Chan AC, Iwashima M, Turck CW, Weiss A. ZAP-70: a 70 kd protein-tyrosine kinase that associates with the TCR zeta chain. Cell 1992; 71:649-662.
190. Wang H, Kadlecek TA, Au-Yeung BB, Goodfellow HE, Hsu LY, et al. ZAP-70: an essential kinase in T-cell signaling. Cold Spring Harb Perspect Biol 2010; 2:a002279.
191. Williams WV, Weiner DB, Wadsworth S, Greene MI. The antigen-major histocompatibility complex-T cell receptor interaction. A structural analysis. Immunol Res 1988; 7:339-350.
192. Sanam R, Vadivelan S, Tajne S, Narasu L, Rambabu G, et al. Discovery of potential ZAP-70 kinase inhibitors: pharmacophore design, database screening and docking studies. Eur J Med Chem 2009; 44:4793-4800.
193. Kohler K, Lellouch AC, Vollmer S, Stoevesandt O, Hoff A, et al. Chemical inhibitors when timing is critical: a pharmacological concept for the maturation of T cell contacts. Chembiochem 2005; 6:152-161.
194. Au-Yeung BB, Zikherman J, Mueller JL, Ashouri JF, Matloubian M, et al. A sharp T-cell antigen receptor signaling threshold for T-cell proliferation. Proc Natl Acad Sci U S A 2014; 111:E3679-3688.
195. Levin SE, Zhang C, Kadlecek TA, Shokat KM, Weiss A. Inhibition of ZAP-70 kinase activity via an analog-sensitive allele blocks T cell receptor and CD28 superagonist signaling. J Biol Chem 2008; 283:15419-15430.
196. Hussain SF, Kong LY, Jordan J, Conrad C, Madden T, et al. A novel small molecule inhibitor of signal transducers and activators of transcription 3 reverses immune tolerance in malignant glioma patients. Cancer Res 2007; 67:9630-9636.
197. Hellwig S, Miduturu CV, Kanda S, Zhang J, Filippakopoulos P, et al. Small-molecule inhibitors of the c-Fes protein-tyrosine kinase. Chem Biol 2012; 19:529-540.
198. Laurent CE, Delfino FJ, Cheng HY, Smithgall TE. The human c-Fes tyrosine kinase binds tubulin and microtubules through separate domains and promotes microtubule assembly. Mol Cell Biol 2004; 24:9351-9358.
199. Voisset E, Lopez S, Chaix A, Georges C, Hanssens K, et al. FES kinases are required for oncogenic FLT3 signaling. Leukemia 2010; 24:721-728.

Figures legend:

Figure 1. Downstream signaling events and interruption by small molecule inhibitors (SMIs). SMIs of intracellular kinases in cell signaling pathways with significant activity are indicated. Different small molecule inhibitors target and inhibit specific molecules that are essential for tumor survival, proliferation, differentiation, and invasion.