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ACK1 Tyrosine Kinase: Targeted Inhibition to Block Cancer Cell Proliferation

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Abstract

ACK1 tyrosine kinase, located on chromosome 3q29, is aberrantly activated, amplified or mutated in a wide variety of human cancers. While the deregulated kinase is oncogenic and its activation correlates with progression to metastatic stage, its inhibition causes cell cycle arrest, sensitizes cells to ionizing radiation and induces apoptosis. Oncogenicity of *ACK1* is not only due to its ability to promote activation of critical pro-survival kinases and receptors by phosphorylating at distinct tyrosine residues, but also by employing a similar mechanism to eliminate a tumor suppressor from cancer cells. Despite the substantial data supporting the oncogenic role of *ACK1*, and the potential clinical benefit of blocking *ACK1* in metastatic disease, to date *ACK1*-specific small molecule inhibitors have not been exploited for cancer therapy. This review highlights recent advances that elucidate how cancer cells employ *ACK1* kinase to their advantage and discusses some of the novel *ACK1* inhibitors that have shown promise in pre-clinical studies.

Keywords

ACK1; TNK2; AKT; tyrosine kinase; small molecule inhibitor; drug resistance

1. Introduction

Tyrosine kinases are being pursued as effective targets in cancer therapy due to the strident dependence of cancer cells on one or more these entities for proliferation and survival. At least 90 unique tyrosine kinases are encoded by the human genome, of which 58 comprise the receptor-tyrosine kinases (RTKs)-grouped into 20 subfamilies, and 32 non-receptor tyrosine kinases (NRTKs) categorized into 10 subfamilies based on structure of the kinase domain [1; 2]. In addition, there are some unusual members such as the WSTF (Williams–Beuren Syndrome Transcription Factor), which tyrosine phosphorylates the variant histone, H2AX in the chromatin [3]. Since the first clinical success of tyrosine kinase inhibitor (TKI), Imatinib or Gleevec, against BCR-ABL in chronic myeloid leukemia (CML), at least

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Conflict of Interest Statement

K.M. and N.P.M. are named as inventors on US patent application (Serial # 13/205,171) and international patent application (Serial # PCT/US2010/023487) titled “AKT Tyrosine 176 Phosphorylation as Cancer Biomarker”

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fifteen other TKIs have been evaluated in clinical setting [4; 5]. Notable are the TKIs targeting HER2/Neu, EGFR, VEGF and B-Raf [6; 7; 8; 9; 10]. Consequently, selective targeting of the deregulated oncogenic kinases- HER2 with Trastuzumab in 'HER2-positive' breast cancers and the mutant B-RafV600E with Vemurafenib (PLX-4032) in melanomas causes significant tumor regression. However, this personalized therapy approach is advantageous only when patients with a specific addictive oncogene have been identified and specific inhibitors are available. With the emergence of large scale sequencing platforms and various gene-profiling methodologies, identification of kinases that promote cancer cell survival, proliferation and metastasis is principally streamlined. In this review, we summarize the latest developments in elucidating the biochemical and pathological role of an understudied tyrosine kinase, ACK1, in cancer cell survival and discuss novel small molecular inhibitors in terms of their suitability for targeting ACK1 in different cancer types (see Tables 1 and 2).

1.1. Activated ACK1- an oncotarget

ACK1/TNK2, a non-receptor tyrosine kinase (NRTK), represents a paradigm of tyrosine kinase signaling that appears to be addictive in cancer cells [11]. Although it is ubiquitously expressed, its multi-domain structure and its ability to interact with a number of transmembrane RTKs suggests that cell type specific activation of ACK1 is dependent on RTKs that are predominantly operational in a particular cell type [11; 12; 13; 14; 15; 16; 17] (Figure 1). For example, while ACK1 is activated by EGF stimulation in some prostate (LAPC4), ovarian (A2780-CP), lung (H292) and pancreatic (Panc-1) cancer cell lines, other cancer derived lines such as the breast cancer cell line (MCF7) and the pancreatic cancer cell line (CD18) are reliant on insulin stimulation for ACK1 activation [12; 13; 14; 18; 19; 20]. ACK1 signaling acquires further importance due to its ability to be activated in the same cell by multiple ligands. For example, it is activated by both insulin and heregulin ligands in the breast cancer cell line, MCF7 [14]. This observation becomes particularly significant in cancers that develop resistance to inhibition of a singular RTK pathway and have activated alternate RTK regulated pathways for their survival.

1.2. ACK1 activation

ACK1 senses extracellular growth signals rapidly by interacting with membrane bound activated RTKs such as MERTK, AXL, HER2, insulin receptor (IR), leukocyte tyrosine kinase (LTK), anaplastic lymphoma kinase (ALK) and EGFR [12; 13; 14; 16]. As a result, it not only activates itself but also transduces the growth promoting signals by activating effector proteins, each with its own distinct functions. ACK1 interacts with Tyr-phosphorylated EGFR via a region at the carboxy terminus termed as MIG6 homology region (MHR), due to its similarity with Gene-33/RALT/Mig-6 [15; 21]. A crystal structure of ACK1 spanning kinase and Src homology 3 or SH3 domains (residues 115–453) indicates that the activated ACK1 kinase domain is a symmetric dimer and is likely to be auto inhibited in its monomeric state [22]. The activation of the kinase is facilitated by dimerization of the amino-terminal sterile alpha motif (SAM) domain which promotes trans-phosphorylation [21]. Further, structural studies indicated that the Met409, located in SH3 domain might recognize the proline-rich region of ACK1 to bring MHR in contact with the kinase domain to negatively regulate the ACK1 kinase activity [22]. In addition, SH3 and CRIB domains have also been suggested to interact with the kinase domain to exert an auto inhibitory effect by restraining the enzyme in an inactive conformation [15]. Another potential inhibitory mechanism may be the interaction of the SH3 domain with the EBD (epidermal growth factor receptor binding domain) which might suppress ACK1 kinase activity; however, the autoinhibitory interaction is relieved and when ACK1 interacts with EGFR via the EBD [17]. Overall, the inter-domain interaction of ACK1 kinase domain with downstream SH3, CRIB, proline-rich and MHR domains appear to keep kinase activity in

check. Two distinct mechanisms of ACK1 activation are likely to be operational in cells; ACK1 overexpression or gene amplification facilitating ACK1 dimerization leading to its activation could be the first mechanism, predominantly seen in cancer cells. The second mode of activation involves binding of ACK1 to activated RTKs which could relieve autoinhibitory inter-domain interaction.

Not surprisingly, cancer cells seem to have exploited the obligatory reliance on domain-interaction of ACK1 by mutating key residues that relieve autoinhibition to transduce survival signals. Somatic point mutations in ACK1 have been identified in ovarian (R99Q in the intra-domain region and E346K in the kinase domain), lung (R34L in the SAM domain), and stomach (M409I in the SH3 domain) cancers, which prevent auto inhibition, consequently leading to deregulated enzyme activation [11; 14; 23]. Additionally, 10 novel somatic and two germ line mutations in the *ACK1* gene were reported by sequencing 261 cancer cell lines of diverse origins [24; 25]. Of these, proline to leucine substitution at 725 in the proline rich region of ACK1 was the most common event observed in 89 of 261 different cancer cell lines. Although highly prevalent in cancer cell lines, the precise role of P725L mutation in ACK1 activation and cancer cell pathology is not fully understood.

ACK1 activation occurs in multiple cancers such as primary endocrine and in hormone-driven tumors [11; 26]. These cancers display increased ACK1 activation by modulating *ACK1* gene expression at the transcriptional level by increased mRNA expression [13; 27; 28; 29; 30; 31]. In addition to increased kinase activation in breast and prostate cancers, *ACK1* gene amplification is a frequent event in lung cancers [32]. Consistent with this finding, reverse-phase protein microarrays reveal ACK1 activation in 47 non-small cell lung cancer (NSCLC) tumors [33]. Recently, micro RNA (miRNA) miR-7 was identified to be a negative regulator of *ACK1* gene at the post-transcriptional level [34]. miR-7 was found to be one of the most down regulated miRNA in these tumors and the expression levels of miR-7 and *ACK1* mRNA was shown to be inversely correlated in human schwannoma, a nerve sheath tumor. Further, overexpression of miR-7 inhibited schwannoma cell growth both in culture and in the xenograft tumor models *in vivo*, suggesting that ACK1 inhibitors could be potential therapeutic molecules for targeting malignant schwannoma.

1.3. ACK1 as a survival kinase

ACK1 promotes cell survival by positively regulating survival pathways to prevent cell death. Most mammalian cells undergo cell cycle arrest followed by apoptosis when ACK1 protein is depleted by silencing RNA, or when treated with ACK1-specific small molecule inhibitors [19; 20; 35]. The pro-proliferative function of ACK1 like proteins is conserved in the fruit fly, *Drosophila* [36]. *Drosophila* Ack resembles human TNK1 in domain organization, but retains significant sequence identity/similarity with ACK1/TNK2 in all conserved domains including the activation loop [36]. While homozygous female flies with *Ack⁸⁶* null alleles are normal, the males are sterile. *Ack⁸⁶* null seminal vesicles were empty suggesting that fly Ack has a role in mature sperm production. Other functions of mammalian ACK1 that overlap in the fly Ack is its ability to promote anti-apoptotic signaling. Expression of Ack in the eye disc reduced the number of TUNEL positive cells while expression of kinase inactive Ack^{K156A} seem to increase the number of TUNEL positive cells indicating that Ack suppresses apoptosis [36]. One of the substrates identified was a transcriptional co-activator, Yorkie, that promoted transcription of proliferative and anti-apoptotic genes and interacted synergistically with fly Ack to promote tissue overgrowth [36].

Based on the conservation of the Cdc42 interacting CRIB domain between human and worm ACK1 (56% identity), SID-3 was identified to be an ortholog of *ACK1* in *Caenorhabditis elegans* [37]. SID-3 is suggested to promote the endocytic uptake of

silencing double stranded RNAs into cells. Over-expression of SID-3 resulted in efficient import of dsRNA that is dependent on an intact kinase domain [37]. The lysine residue K139 in SID-3 corresponds to ACK1(K158) that binds to ATP. Whether other *ACK1* orthologs retain this dsRNA import function, its role in normal physiology and implications in survival or metastasis of cancer cells remains to be determined.

2. ACK1 signaling partners

ACK1 interacts with and tyrosine phosphorylates many cellular proteins regulating critical cellular processes [11]. While ACK1 shares common intracellular effectors such as AKT with other signaling pathways, it imparts specificity to signaling by phosphorylating effectors at distinct sites [11; 14]. Majority of the sites that ACK1 phosphorylates are strikingly unique, which includes AKT at Tyr176, androgen receptor or AR at Tyr267 & Tyr363 and the tumor suppressor Wwox at Tyr287 [11]. This feature is attributed to the unusual peptide substrate binding ability of ACK1 [38]. The significance of ACK1 interactome in cancer cell survival acquired significance due to our understanding of many of its preferred substrates and their activation. For a comprehensive description of all ACK1 interacting proteins, detailed information is available from other reviews [11; 15; 26].

2.1. ACK1-Androgen receptor-ATM

A significant fraction of primary human prostate tumors exhibited *ACK1* mRNA up-regulation [30], and a significant increase in ACK1 tyrosine 284 phosphorylation- a marker of ACK1 activation [13; 14; 19; 20]. Moreover, this increased ACK1 activation negatively correlates with poor prognosis- expression levels of Tyr284-phosphorylated-ACK1 and Tyr267-phosphorylated-AR positively correlate with the severity of disease progression, and inversely correlate with the survival of prostate cancer patients [13; 14; 18]. Consistent with the human data, expression of activated ACK1 is sufficient to promote growth of prostate xenograft tumors in castrated mice, indicating that ACK1 plays a key role in androgen-independent prostate tumor growth [13; 20]. Moreover, transgenic mice expressing activated ACK1 under the control of prostate specific probasin promoter, Prob-ACK1 or PB-ACK1 mice, developed prostatic intraepithelial neoplasia (PINs) by ~30 weeks [14]. A few mice develop prostatic adenocarcinoma by ~50 weeks.

Recent studies have uncovered key mechanistic details of ACK1-AR signaling; ACK1 phosphorylated AR at tyrosine 267 in the transcription activation domain to promote its transcriptional co-activator function at target promoters [13]. Significantly, in contrast to AR bound to androgen, the activated ACK1/pTyr267-AR complex is recruited to a distinct set of genes such as ATM (Ataxia telangiectasia mutated kinase) in the androgen-depleted environment [20]. ATM, a regulator of DNA damage and cell cycle checkpoint signaling pathways, ensures genetic integrity within cells in response to DNA double-strand breaks [39; 40; 41; 42]. Tyr267-phosphorylated-AR was shown to promote ATM transcription under androgen depleted conditions; further, human prostate tumor microarray analysis revealed an increased expression of ATM protein that correlated with human prostate cancer progression to castration resistance [20]. These data suggested that upregulation of genes associated with maintaining genetic integrity may allow CRPC cells to evade cell death. Thus, suppressing ACK1-AR signaling and therefore ATM levels by ACK1 inhibitors could be a new therapeutic strategy for CRPC tumors, which often exhibit radioresistance.

2.2. ACK1-AKT

AKT (also known as PKB) is recently reported as a novel ACK1 interacting protein [11; 14; 19]. AKT plays a central role in various aspects of cellular physiology in a large number of human malignancies. ACK1 strongly associates with all the three isoforms of AKT and

phosphorylates them at tyrosine 176, a site that is invariant from yeast to mammals [14]. Interestingly, ACK1 mediated AKT Tyr176-phosphorylation (pTyr176-AKT) promotes PI3K-independent AKT recruitment to the plasma membrane and subsequent AKT activation [11; 14]. PI3K-independent AKT membrane localization was facilitated by pTyr176-AKT binding to phosphatidic acid (PA), a prominent membrane phospholipid [14]. Consistent with the biochemical data, PB-ACK1 transgenic mice displayed elevated levels of Tyr176-phosphorylated and activated AKT [14]. In addition to prostate cancer, ACK1-AKT signaling has also shown to be critical in breast cancer, pancreatic cancer [14; 19] and myeloma [43]. A high-throughput systematic RNA interference (RNAi) screen identified ACK1 and AKT as the critical targets to inhibit myeloma cell proliferation or survival [43]. ACK1 and pTyr176-AKT expression correlated with disease progression and inversely with patient survival in both breast and pancreatic cancers [14; 19]. With the five-year survival rate at a low 6%, targeted inhibition of ACK1 based on its significant activation in high-grade pancreatic tumors, has potential therapeutic benefits for patients afflicted with this fatal disease.

Several promising AKT inhibitors are currently under clinical trials [44]. Loss of AKT activation by assessing AKT Ser473-phosphorylation (or Thr308-phosphorylation) is often assessed as positive outcome of inhibitor treatment. Since, ACK1 mediated AKT Tyr176-phosphorylation is insensitive to PI3K-inhibition [14; 26], a subset of cancers that exhibit AKT activation may rely on ACK1 mediated AKT Tyr176-phosphorylation for their survival. Therefore, these PI3K-inhibitor insensitive tumors are likely to respond to ACK1 inhibitors. Consistent with that, pancreatic, breast, prostate and lung cancer derived cells lines exhibit significant sensitivity to ACK1 inhibitor [19]. Overall, detection of pTyr176-AKT levels in tumor biopsies could be a 'companion diagnostic' tool for the personalized therapy with ACK1 inhibitors. Indeed, combinatorial treatment with PI3K and ACK1 inhibitors could be highly beneficial in pancreatic, lung, breast and prostate cancers that exhibit robust AKT Tyr176-and Ser473-phosphorylations.

3. ACK1 stabilization in cancers

While in normal cells, such as mouse embryo fibroblast cell lines, ACK1 activation is rapid and transient in response to EGFR stimulation, several cancer cells show constitutive activation of ACK1. One mechanism by which normal cells switch off ACK1 activation is by ubiquitin mediated degradation of the protein, likely facilitated by the UBA domains at the carboxy-terminus of the protein [45; 46]. In support of this finding, an ACK1 S985N mutant, located in the ubiquitin association domain (UBA) and identified in a renal carcinoma cell line, is defective in ubiquitin binding and appears to stabilize ACK1 protein levels [24]. Further, ACK1 S985N mutation promotes cell proliferation, migration and anchorage-independent growth as well as the epithelial-mesenchymal transition.

Consistent with the above observations, over-expression of ACK1 in human cervical cancer derived cell line, HeLa, confined EGFR to early endosomes and prevented sorting to other vesicles for degradation [47]. Indeed, the resistance to EGFR inhibitor, gefitinib, has been suggested to work via preventing EGF-EGFR trafficking out of early endosomes toward the late endosomes/lysosomes [48]. Not surprisingly, loss of ACK1 sensitized the EGFR inhibitor gefitinib resistant renal carcinoma cells to cell death. Thus, combined inhibition of both EGFR and ACK1 could be a novel chemotherapeutic strategy to overcome gefitinib resistance [24; 25].

Notably, ACK1 co-localizes with the E3 ubiquitin ligase NEDD4-2 in clathrin-rich vesicles [45]. The proline-rich sequences in ACK1 interact with WW domain of NEDD4-2. Interestingly, ACK1 uses the same region to interact with the tumor suppressor Wwox [12].

This process is tightly regulated as it requires EGFR-mediated ACK1 activation to drive kinase degradation by NEDD4-2 [45]. Comparing the binding of NEDD4-1 and NEDD4-2 to ACK1, Lin *et. al.*, observed that although the two ubiquitin ligases bound similarly to ACK1, the difference in ubiquitylation of ACK1 mediated by NEDD4-1 was more than 10-fold, suggesting some degree of specificity [46]. Indeed, the Tyr650 in ACK1 seems to be essential for interaction with NEDD4-1. Future cancer genome sequencing efforts may uncover mutations in ubiquitin ligases themselves that block ACK1 degradation and thus stabilize ACK1 to promote oncogenesis.

In contrast to EGF stimulated ACK1 degradation, ACK1 is found to be degraded in a kinase independent fashion by the Seven in absentia homolog (SIAH) ubiquitin ligases, SIAH1 and 2 [49]. These ligases interact with ACK1 via a highly conserved SIAH-binding motif located at the C-terminal proline rich region of ACK1. A point mutation in the C terminus abolished the interaction of ACK1 with SIAH ligases. SIAH2 mediates the ubiquitylation of ACK1 for proteolysis by the proteasome machinery in estrogen (E2) stimulated breast cancer cells. Furthermore, SIAH2 may be a target gene of E2 in breast cancer cells. This work has significant implications particularly for triple negative breast cancers (TNBCs). Since degradation of ACK1 depends on intact E2/ER (estrogen receptor) signaling, TNBCs that lack ER could potentially increase stability of ACK1 and thus likely to be important in promoting breast cancer survival and metastasis.

4. ACK1 inhibitors

Since ACK1 activation is correlated with poor prognosis in various cancers, strong efforts are being directed by multiple groups towards developing highly potent and specific small molecule inhibitors targeting the ACK1 kinase. At least eight small molecule kinase inhibitors have so far been reported in literature (Table 1). The effectiveness of these small molecule inhibitors *in vitro* and their ability to block cancer cell growth is discussed below.

4.1. AIM-100 (4-Amino-5,6-biaryl-furo[2,3-d]pyrimidine)

AIM-100 is the first and the best-studied ACK1 inhibitor, identified in a high throughput screening [18; 20; 50]. A kinase assay performed in the presence of increasing concentrations of AIM-100 revealed that it specifically inhibits ACK1 with an IC₅₀ of 21 nM but not 30 other kinases, including members of the AKT, AXL, HER, JAK, ERK and PI3K subfamily *in vitro* [20]. Further, AIM-100 also inhibited AKT Tyr176-phosphorylation and activation in multiple cancers cell lines, arresting cells in G1 phase of cell cycle promoting eventual cell death [18; 19; 20]. Additionally, treatment with AIM-100 not only suppressed ACK1 and AR Tyr267-phosphorylation and subsequent chromatin recruitment but also inhibited castration and radio resistant prostate xenograft tumor growth [18; 19; 20]. Thus, AIM-100 is a prospective therapeutic agent for breast, prostate, lung and pancreatic cancers.

4.2. Compound 2 (N3,N6-diaryl-1H-pyrazolo[3,4-d]pyrimidine-3,6-diamines)

N3, N6-diaryl-1H-pyrazolo[3, 4-d]pyrimidine-3, 6-diamines were designed and synthesized as potent ACK1 inhibitors [51]. X-ray crystallography studies revealed that the compound 2 anchors to the ATP binding pocket via two hydrogen bonds to the hinge region and one hydrogen bond to the carboxyl oxygen of Thr205 [51]. Although the compound 2 has emerged to be one of the most potent ACK1 inhibitor (IC₅₀ of 20 nM), it exhibited high metabolic degradation and was rapidly cleared from plasma. The analogs of compound 2 exhibited significant improvement in oral bioavailability; however, none of the compounds of this series are currently in clinical trials.

4.3. B19 (Pyrimido benzodiazepines)

High throughput library screening of 118 compounds against 353 kinases identified B19 as a selective ACK1 inhibitor with K_d of 15 nM *in vitro* [52]. However, a relatively strong dose of B19 (10 μ M) was required to suppress EGF-induced ACK1 autophosphorylation in HEK293 cells [52]. Co-transfection of a GTPase defective mutant of Cdc42, Cdc42Q61L, which activates ACK1 kinase, recovered part of the inhibition indicating that Cdc42 binding to ACK1 could cause conformational change that might reduce the B19 binding [52]. Although selective, lung cancer cells treated with 10 μ M of B19 for 7 days exhibited only a four fold decrease in cell number, indicating that this inhibitor possesses limited cancer cell growth inhibitory potential.

4.4. Compound 6 (4,5,6-trisubstituted furo[2,3-d]pyrimidin-4-amine) and 37 (1,3-Dithiolane-substituted pyrrolopyrimidine)

Recently, high throughput screen of small molecule compounds unveiled two new classes of ACK1 inhibitors, 4,5,6-trisubstituted furo[2,3-d]pyrimidin-4-amine and 4,5,6-trisubstituted 7H-pyrrolo[2,3-d]pyrimidin-4-amine [53]. Although Compound 6, a furanopyrimidine potently inhibited ACK1 activity *in vitro* (K_i =70 nM), its ability to inhibit ACK1 *in vivo* was observed to be less impressive (cell IC_{50} of 5.6 μ M). In contrast, the second class of inhibitors, compounds 35 and 37, which were pyrrolopyrimidine dithiolanes, turned out to be excellent ACK1 inhibitors, exhibiting robust ACK1 inhibitory activity both *in vitro* (K_i =0.02 nM) and *in vivo* (cell IC_{50} of 5–10 nM). Compound 35 binds to the ATP-binding site of ACK1 kinase domain *via* hydrogen bond interactions to two amino acids, L207 and A208. Compound 35 also made two additional van der Waals interaction with amino acids D134 and G269 [53]. These multiple interactions were suggested to be the primary reason for high degree of biochemical and cellular inhibition exhibited by compound 35. In spite of the highly desirable properties such as cellular ACK1 inhibition at nanomolar concentrations and selectivity, both the compounds exhibited poor oral bioavailability and were cleared rapidly from plasma, mitigating their effectiveness for *in vivo* use [53].

4.5. Compound 42 (Imidazo[1,5-a]pyrazine derivative)

A series of imidazo[1,5-a]pyrazine derived ACK1 inhibitors were recently identified through a combination of structure-based drug design and empirical medicinal chemistry efforts [54]. Virtual screening led to the discovery of an initial hit, derivatives of which were examined for structure-activity relationships and drug metabolism and pharmacokinetic (PK) properties. It led to the identification of compound 42 that demonstrated good *in vivo* PK properties and selectivity when profiled against 216 purified protein kinases representing the tyrosine and serine/threonine kinase families [54]. While it is a potent ACK1 inhibitor, compound 42 displayed >80% inhibition of 12 other kinases, predominantly non-receptor kinases (YES, FRK, FYN, HCK, LCK, SRC, LYNB, BLK, SRMS, TEC, TXK and FGR). Significantly, physicochemical and ADMET studies revealed that the compound 42 possesses favorable drug-like properties suggesting that it is a potent, selective and orally bio-available ACK1 inhibitor [54].

4.6. PLX-4032 (Vemurafenib)

Researchers from Plexxicon developed a small molecule, PLX-4032 (Vemurafenib), which is marketed primarily as BRAf-V600E inhibitor. Interestingly it is also a potent inhibitor of ACK1 kinase *in vitro* with an IC_{50} of 19 nM [55]. Since Vemurafenib is FDA approved small molecule inhibitor; treatment of cancers that exhibit high ACK1 expression could be a potential therapeutic strategy with significant benefits. However, its ability to inhibit ACK1 kinase activity *in vivo* has not been evaluated in pre-clinical studies, curtailing its use against cancers with activated ACK1. Future studies exploring the use of PLX4032 or its

derivatives as potent ACK1 inhibitors will be valuable to take this ACK1 inhibitor rapidly from benchside to bedside.

4.7. Dasatinib (BMS-354825 or Sprycel)

Dasatinib was originally identified as Src and Abl kinase inhibitor [56]. It is renamed as Sprycel and is commercially available as an oral multi-family tyrosine kinase inhibitor for use in patients with chronic myelogenous leukemia (CML). High affinity binding of Dasatinib to ACK1 ($K_d=6$ nM) was observed when screening existing inhibitors against drug resistant mutant kinases [57]. Later, a drug-affinity chromatography approach using Dasatinib as a bait revealed ACK1 as potential drug target in lung cancer cells [58]. Dasatinib treatment resulted in significant loss of ACK1 tyr-phosphorylation at four major autophosphorylation sites, Tyr284, Tyr518, Tyr857 and Tyr858. Interestingly, while other dasatinib-interacting kinases such as Src, Fyn, Lyn and Lck could rescue lung cancer cells HCC827 from the drug induced cell death, ACK1 failed to do so [58]. Significantly, Dasatinib not only inhibited ACK1 phosphorylation but also its substrate AR Tyr-phosphorylation [59]. In another study involving screening of over 1,400 marketed drugs, four drugs were identified as potent ACK1 inhibitors. Of these four, Dasatinib was as noted to be the most potent inhibitor with IC_{50} of 1nM [60].

5. Future perspective: ACK1 allosteric inhibitors

The ACK1 inhibitors that have so far been reported in literature are all ATP analogs; indeed, large majority of small molecule kinase inhibitors reported in literature target the ATP-binding site of kinases [61]. ATP competitive inhibitors (type I inhibitors) often do not discriminate effectively between ATP binding sites of multiple kinases [62]. This potentially limits their clinical use and increases the likelihood of off-target toxicity. Specificity is particularly important not only to interrogate the cellular biochemistry but also its effectiveness in suppressing tumor growth—ultimate utility as a personal therapeutic strategy. Approaches that seek non-competitive or purely allosteric inhibitors (type II-III inhibitors), which target kinase-specific regulatory sites, may offer better opportunities for selective inhibitor design [61; 63; 64; 65; 66]. An allosteric inhibitor of ACK1 would represent a novel type of ACK1 inhibitor and a preferred therapeutic agent. Because ACK1 is large multi-domain protein and inter-domain interactions are critical for its kinase activation, it is possible to achieve noncompetitive inhibition of ACK1 from a change in the shape of the active site when a small molecule or a peptide binds to an allosteric site located within the kinase domain or even neighboring SAM, SH3 or CRIB domains. Affinity Selection-Mass Spectrometry (AS-MS) has emerged to be a screening technology of choice for identification of potential allosteric inhibitors [67; 68; 69; 70; 71]. However, no ACK1-specific allosteric inhibitor has been identified so far.

6. Conclusion

Collectively, a better understanding of the activated ACK1 signaling paradigm will not only uncover the specific cell signaling networks that the oncogene engages to promote growth and suppress apoptosis, but, will also provide insights into how cellular environment is altered to solely depend on ACK1 for survival. Development of novel and potent ATP competitive and allosteric ACK1 inhibitors can be used to minimize off target effects and reduce toxicities. Moreover, a subset of cancer patients who exhibit high levels of ACK1 activation, identified by immunohistochemical staining of primary tumor biopsies with the phospho-ACK1, -AKT or -AR antibodies, or by DNA sequencing or by real time RT-PCR are likely to benefit from therapy with ACK1 inhibitors, a ‘personalized medicine’ approach.

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Figure 1.

ACK1 inhibitors

Table 1

Inhibitor	Chemical name	Chemical structure	IC ₅₀	Cancer	References
AIM-100	4-Amino-5,6-biaryl-furo[2,3-d]pyrimidines		24 nM	Prostate, Breast, Lung, Pancreatic, Melanoma	[18; 19; 20; 50]
Compound 2	N3,N6-diaryl-1H-pyrazolo[3,4-d]pyrimidine-3,6-diamines		20 nM	Not tested	[51]
B19	Pyrimido benzodiazepines		-	Lung	[52]
Compound 6	2,3-diphenyl-N-((tetrahydrofuran-2-yl)methyl)furo[2,3-b]pyridin-4-amine		70 nM	Not tested	[53]
Compound 37	1,3-Dithiolane-substituted pyrrolopyrimidine		0.3 nM	Not tested	[53]

Inhibitor	Chemical name	Chemical structure	IC ₅₀	Cancer	References
Compound 42	Imidazo[1,5-a]pyrazine derivative		110 nM	Not tested	[54]
Dasatinib	N-(2-chloro-6-methylphenyl)-2-(6-(4-(2-hydroxyethyl)piperazin-1-yl)-2-methylpyrimidin-4-ylamino)thiazole-5-carboxamide		1 nM	Lung	[58]
PLX-4032	N-(3-(5-(4-chlorophenyl)-1H-pyrrolo[2,3-b]pyridine-3-carbonyl)-2,4-difluorophenyl)propane-1-sulfonamide		19 nM	Not tested	[55]

Table 2

Mechanisms of ACK1 activation in cancers

Cancer	Nature of Anomaly	Known Effector/s	Reference
Prostate	Hyperactivation of kinase, gene amplification, inhibition of tumor suppressor	AR, AKT, ATM, Wwox	[12; 13; 14; 20; 32]
Breast	Hyperactivation of kinase	AKT	[14]
Pancreatic	Hyperactivation of kinase	AKT	[19]
Ovarian	Mutation	AKT	[14]
Gastric	Mutation	Not identified	[14]
Lung	Mutation, gene amplification	AKT	[14; 32]
Schwannoma	miRNA-7 suppression	Not identified	[34]
Renal carcinoma	Mutation	Not identified	[24]