

## Review Article

# Non-kinase targeting of oncogenic c-Jun N-terminal kinase (JNK) signaling: the future of clinically viable cancer treatments

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c-Jun N-terminal Kinases (JNKs) have been identified as key disease drivers in a number of pathophysiological settings and central oncogenic signaling nodes in various cancers. Their roles in driving primary tumor growth, positively regulating cancer stem cell populations, promoting invasion and facilitating metastatic outgrowth have led JNKs to be considered attractive targets for anti-cancer therapies. However, the homeostatic, apoptotic and tumor-suppressive activities of JNK proteins limit the use of direct JNK inhibitors in a clinical setting. In this review, we will provide an overview of the different JNK targeting strategies developed to date, which include various ATP-competitive, non-kinase and substrate-competitive inhibitors. We aim to summarize their distinct mechanisms of action, review some of the insights they have provided regarding JNK-targeting in cancer, and outline the limitations as well as challenges of all strategies that target JNKs directly. Furthermore, we will highlight alternate drug targets within JNK signaling complexes, including recently identified scaffold proteins, and discuss how these findings may open up novel therapeutic options for targeting discrete oncogenic JNK signaling complexes in specific cancer settings.

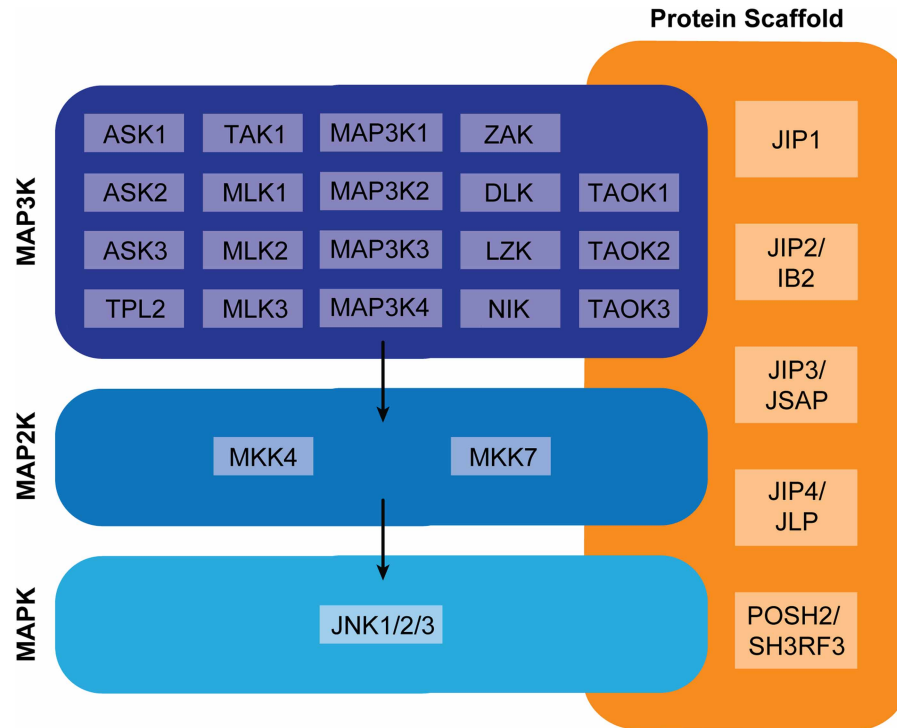
## Introduction

The c-Jun N-terminal Kinases (JNKs) are members of the mitogen-activated protein kinase (MAPK) family that critically regulate a diverse and somewhat opposing range of physiological processes, including cell death, proliferation, differentiation and invasion [1]. This functional diversity is achieved through the assembly of spatially and compositionally discrete multi-protein complexes, which integrate and transmit signals in response to various stimuli. Although originally named stress-activated protein kinases (SAPKs) for their profound response to extracellular stress stimuli such as UV-irradiation, heat shock, osmotic stress, reactive oxygen species and inflammatory cytokines, it is now apparent that JNKs are also activated by intracellular stimuli and biomechanical cues, and that persistent JNK activation underlies pathogenesis in a number of disease contexts, including cancer [2,3].

JNK signaling conforms to the hierarchical MAPK network structure, whereby one of several MAPK-kinase-kinases (MAP3Ks) phosphorylates and activates one of two MAPK-kinases (MAP2K), MKK4 or MKK7, which in turn activate the JNK isoforms, JNK1, JNK2 and/or JNK3 (Figure 1). Each of these JNK isoforms are subject to alternative splicing, with JNK1 and JNK2 variants expressed ubiquitously and JNK3 variants restricted to the brain, heart and testis. Whilst this implies that the composition of JNK signaling complexes is inherently tissue and cell type-dependent, a significant body of research now demonstrates that scaffold proteins also play a key role in dictating MAP3K–MAP2K–MAPK combinations and complex localization [4,5]. By restricting JNK activation to discrete

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**Figure 1. Complexity of JNK signaling.**

JNK proteins are activated downstream of a diverse range of upstream MAP2Ks and MAP3Ks. The precise MAP3K-MAP2K-MAPK composition of a given signaling complex is influenced by tissue and cell type, physiological/pathological context, and scaffold proteins, which bind more than two signaling components and direct them to discrete subcellular locations.

subcellular compartments, scaffold proteins limit JNKs access to substrates such as transcription factors, translation machinery, hormone receptors, apoptotic effectors and cytoskeletal proteins. Once active, JNKs phosphorylate serine/threonine-proline motifs within these spatially segregated substrates in order to drive context specific biological responses [6].

Given the diversity of these JNK-regulated processes, JNK signaling has been implicated in a number of pathophysiological conditions, including neuro-degenerative diseases, diabetes and cancer. In the context of cancer, JNK hyper-activation has been reported in multiple solid tumor types and hematological malignancies, with countless studies demonstrating the therapeutic potential of JNK-targeting strategies [3]. However, a significant body of evidence has also elucidated key tumor-suppressive roles for JNK [7]. This dichotomy of JNK functions is particularly evident in the context of breast tissue, where *in vivo* JNK1/JNK2 knockout models have demonstrated that JNK plays essential roles in maintaining the architecture of normal breast tissue [8], driving the genetic programs required for mammary gland involution post lactation [9] and preventing early tumor initiation events [10,11]. These tumor-suppressive roles starkly contrast JNKs tumor-promoting functions in breast cancer tissue, which includes driving primary tumor growth, promoting an immunosuppressive tumor microenvironment, positively regulating cancer-stem cell populations, promoting tumor cell migration and invasion, and modulating both the structure and immunological landscape of the metastatic niche to support metastatic disease progression [12–15]. Furthermore, an extra layer of complexity is added by the critical requirement for JNK activity in the apoptotic response to chemotherapeutic intervention in breast cancer treatment [16].

Whilst there is significant interest in therapeutically targeting the pro-tumorigenic functions of JNK in various cancers, the pleiotropic nature of JNK signaling means that direct JNK inhibition may have adverse consequences and is unlikely to yield clinically viable cancer treatments. This is reflected in the existing clinical trials that have already been performed with JNK inhibitors (Table 1), which were predominantly performed in

**Table 1 JNK inhibitors assessed in clinical studies**

Inhibitor type	Inhibitor name	Preclinical cancer models		Clinical trials					
		Effects	Refs	Condition/disease	Identifier	Phase	Treatment details	Outcome	Refs
ATP- competitive inhibitor	CC-401	- Inhibits TNBC primary tumor growth and metastasis - Sensitizes colon cancer to chemotherapy <i>in vivo</i>	[12,13,24]	High-risk myeloid leukemia	NCT00126893	I	No information available	Terminated; reason not cited; N/P	
				Healthy adults	Unknown	I	Three-way crossover study, daily oral administration for 6 days with 7 days washout	Completed; well tolerated with no serious adverse events reported	[40]
	CC-930 Tanzisertib	- Not tested in cancer models		Healthy males	Unknown	I	Single-dose of [ <sup>14</sup> C]-Tanzisertib, oral	Completed; eliminated via urinary and fecal excretion with no unique metabolites	[96]
				Idiopathic pulmonary fibrosis	NCT01203943	II	Repeated oral administration for up to 56 weeks	Terminated; benefit/risk profile cited; adverse events in 46.4% of subjects	[40]
	CC-90001 BMS-986360	- Not tested in cancer models		Discoid lupus erythematosus	NCT01466725	II	Daily treatment for 4–8 weeks	Terminated; benefit/risk profile cited; N/P	
				Healthy adults	NCT02110420	I	Single and multiple ascending doses, oral	Completed; safe and well-tolerated	[97]
					NCT02321644	I	Multiple doses and single dose fed/fasting conditions, oral		
					NCT03958864	I	Multiple doses, oral		
					NCT03363815	I	Multiple doses, tested with the following combinations and fed/fasting conditions: + Omeprazole, Midazolam, Warfarin, Vitamin K + Rosuvastatin + Metformin, Digoxin + Nintedanib	Completed; N/P	
	Healthy males	NCT04655898	I	Single-dose of [ <sup>14</sup> C]-CC-90001, oral	Completed; N/P				
Hepatic impairment	NCT03742882	I	Single-dose, oral	Completed; N/P					

Continued

Table 1 JNK inhibitors assessed in clinical studies

Inhibitor type	Inhibitor name	Preclinical cancer models		Clinical trials					
		Effects	Refs	Condition/disease	Identifier	Phase	Treatment details	Outcome	Refs
				Pulmonary fibrosis	NCT02510937	Ib	Daily oral administration for 12 continuous weeks	Completed; N/P	
				Idiopathic pulmonary fibrosis	NCT03142191	II	Daily oral administration for 24-104 weeks	Completed; trial setup published; data N/P	[42]
				Non-alcoholic steatohepatitis and liver fibrosis	NCT04048876	II	Daily oral administration, time not specified	Terminated; changed business objectives cited; N/P	
	AS602801 PGL-5001 Bentamapimod	- Cytotoxic in human pancreatic, non-small cell lung, ovarian and glioblastoma cells - Sensitizes ovarian cancer stem cells to chemotherapy - Perturbs prostate cancer cell invasion <i>in vitro</i> , and tumor growth <i>in vivo</i>	[25-29]	Inflammatory endometriosis	NCT01630252	Ila	Daily oral administration for up to 5 months accompanied by 1-2 Depot Medoxyprogesterone Acetate (DMPA) injections	Completed; N/P	
Substrate-competitive inhibitor	D-JNK11 XG-102 AM-111 Brimapitide	- Reduces HCC proliferation <i>in vitro</i> and tumor growth <i>in vivo</i> - Reduces cancer pain and tumor growth in murine skin cancer model	[55,56]	Healthy males	NCT01570205	I	Single-dose, intravenous	Completed; N/P	[58,59]
				Acute sensorineural hearing loss	Unknown	I/II	Single-dose, intratympanic	Completed; safe and well-tolerated	
					NCT00802425	II	Single-dose, intratympanic	Completed; N/P	
					NCT02561091	III	Frequency and timing not specified; intratympanic	Completed; N/P	
					NCT02809118	III	Single-dose, intratympanic	Terminated; data available from another study cited; N/P	
					EudraCT 2013-002077-21	III	Single-dose, intratympanic	Completed; Otoprotective	
	Intraocular inflammation			EudraCT 2011-000171-14 (cited identifier invalid)		Ib	Single-dose, subconjunctival	Completed; Safe and well tolerated	[98]
				Post-cataract surgery intraocular inflammation	NCT02235272 NCT02508337	III III	Single-dose, subconjunctival Single-dose, subconjunctival	Completed; N/P Completed; N/P	

N/P, Data not published; NCT identifiers from ClinicalTrials.gov; EudraCT identifiers from eudract.ema.europa.eu.



the context of fibrotic or inflammatory diseases. A number of these studies are testing the long-term tolerability of JNK inhibitors, but not within an oncology setting and with no long term monitoring of potential neoplastic activity. With this in mind, we will provide an overview of the different JNK targeting strategies developed to date including various ATP-competitive and non-competitive inhibitors, and outline the insights that these compounds and peptides have provided regarding JNK-targeting in cancer along with their potential limitations. Furthermore, we will highlight recent studies describing novel JNK scaffolds that regulate cancer stemness, and discuss the implications that discoveries such as these may have on the design and development of JNK-targeting therapies in the future.

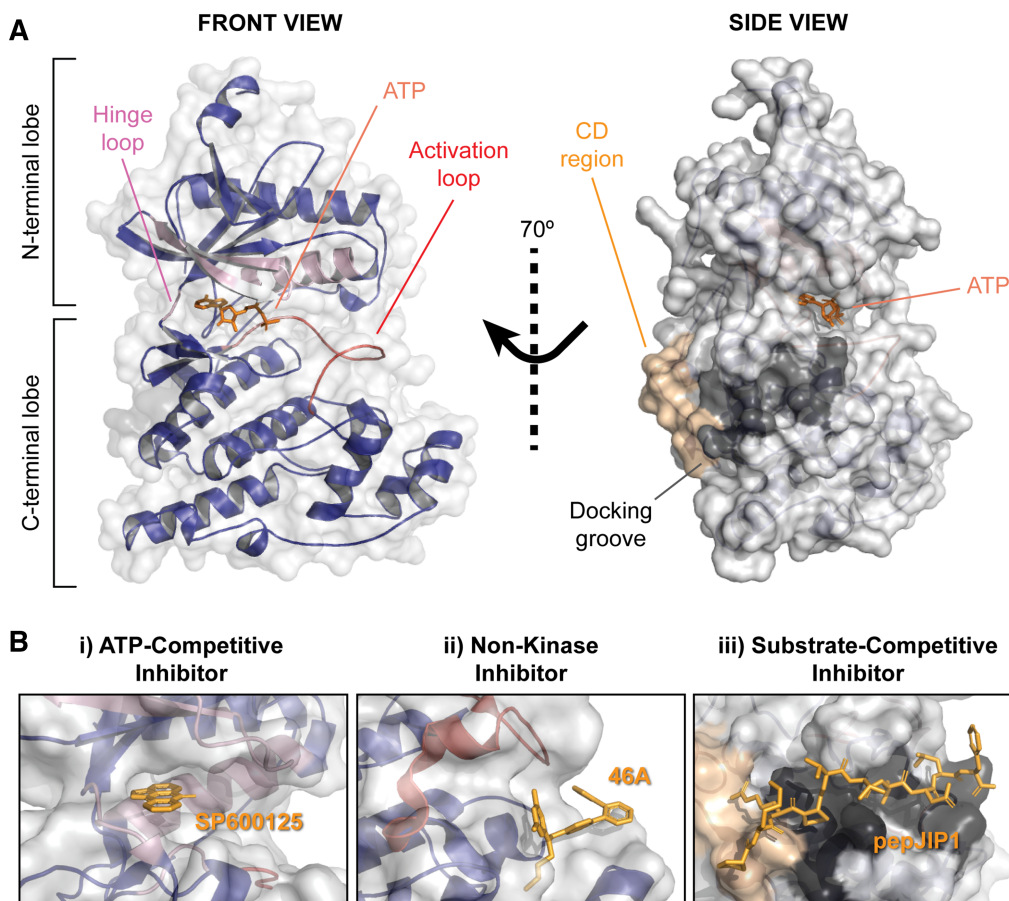
## ATP-competitive JNK inhibitors

In terms of protein structure, JNKs comprise distinct N- and C-terminal lobes that are linked by a flexible hinge loop (Figure 2A). This hinge loop and the structural elements surrounding the central cavity between the two lobes form the ATP-binding cleft. ATP-competitive JNK inhibitors effectively block the functions of JNK proteins by competing with and replacing ATP at this site. Given that JNK dysregulation has been implicated in multiple disease contexts, there has been significant research into the development of these type I inhibitors. The structural characteristics of this extensive list of inhibitors and their precise binding mechanisms have been reviewed elsewhere [17,18].

ATP-competitive JNK inhibitors have been crucial for delineating and defining our understanding of JNK signaling in tumorigenesis, with the anthrapyrazole inhibitor SP600125 amongst the most commonly used in experimental cancer models [19] (Figure 2B-i). Whilst SP600125 has demonstrated significant anti-tumor potential in different cancer types [20–22], its lack of specificity for JNK has generated controversy [23], limited its use and driven the development of second-generation inhibitors, such as CC-401. Although there is limited peer-reviewed biochemical data available for CC-401, recent *in vivo* studies have demonstrated that this well-tolerated compound can sensitize colon cancers to various treatments [24], and inhibit metastatic triple negative breast cancers (TNBCs) by blocking JNK-dependent primary tumor growth, cancer stemness, invasion and metastatic niche development [12,13]. Despite these promising *in vivo* results, a Phase 1 clinical safety, pharmacokinetics and pharmacodynamics study of CC-401 conducted in patients with high-risk myeloid leukemia (NCT00126893; clinicaltrials.gov) was terminated for unknown reasons and the compound not pursued further in clinical settings. Another ATP-competitive JNK inhibitor, AS602801, has also shown promising results within pre-clinical cancer models [25–29] and proceeded to clinical trial for the treatment of inflammatory endometriosis (Table 1). However, the limited biochemical and clinical data available for this compound prevents any discussion of its potential uses and limitations.

Improved JNK specificity has also been achieved through the development of irreversible JNK inhibitors, such as JNK-IN-8, which elicits sustained effects through the formation of a covalent bond with a cysteine in the ATP-binding cleft [30]. Although primarily used as an *in vitro* tool, JNK-IN-8 has been shown to sensitize both pancreatic ductal adenocarcinomas and TNBCs to various therapeutic agents *in vivo* [31,32], and inhibit TNBC primary tumor growth and lung metastasis by modulating the immunological landscape of the tumor microenvironment [15]. Whilst many articles refer to the harmful consequences of type I JNK inhibitors, there is limited literature detailing their side effects. However, it is within reason that these pan-JNK inhibitors may recapitulate the phenotypes observed in compound genetic knockout models, where the homeostatic, apoptotic and tumor-suppressive activities of JNK are adversely perturbed [8,11,33].

These limitations of pan-JNK inhibitors, along with the paradoxical roles of JNK isoforms in various physiological processes and disease settings, including cancer (reviewed in [7,34]), have prompted significant interest in the development of isoform selective inhibitors. Using rational drug design, a number of ATP-competitive inhibitors with enhanced selectivity for JNK3 have been described [35–37]. In terms of JNK1 and JNK2 selectivity, compounds such as the Celgene Corporation inhibitors CC-930 and CC-90001 have been shown to preference one isoform over another, with biases towards JNK2 and JNK1, respectively [38,39]. Whilst Phase 2 studies assessing CC-930 (also Tanzisertib) in patients with Discoid Lupus Erythematosus and Idiopathic Pulmonary Fibrosis were terminated due to the benefit/risk profile [40] (Table 1), CC-90001 passed Phase 1/1b trials with an acceptable safety profile and results are anticipated from a recently completed long-term Phase 2 study in patients with Idiopathic Pulmonary Fibrosis [41,42]. Pending safety results, it would be of great interest to evaluate CC-90001 in the context of hepatocellular carcinoma (HCC) and lung cancer [43–45], where JNK1, but not JNK2, has been implicated in tumor progression.



**Figure 2. Structural features and inhibitor binding sites of JNKs.**

(A) JNK proteins are comprised of distinct N-terminal and C-terminal lobes that surround an inner ATP-binding cleft, where ATP (orange) docks. This ATP binding cleft consists of the hinge loop, G-Rich loop, C-helix (all in light pink) and the activation loop (red), which encompasses the TPY motif for dual MAP2K phosphorylation and activation. The docking site (D-site), the interface through which JNKs interact with most of their binding partners, is located next to the hinge loop and comprises both the CD region (beige) and hydrophobic docking groove (dark grey). This figure was assembled using the AlphaFold structure prediction for JNK1 (AF-P45983-F1) [94,95]. The ATP shown in the nucleotide binding cleft was obtained by overlaying the AlphaFold prediction with a structure of human JNK1 (PDB 2XRW; RMSD = 0.633 Å). (B) Three main types of JNK inhibitors have been described, including (i) ATP-competitive, (ii) non-kinase and (iii) substrate competitive JNK inhibitors. (i) ATP-competitive JNK inhibitors such as SP600125 work by displacing ATP from the nucleotide binding pocket (IPDB 1UKI). (ii) The two biaryl tetrazole non-kinase JNK inhibitors that have been identified, including 46A, bind to inactive JNKs and prevent their phosphorylation by upstream MAP2Ks (PDB 302M). (iii) Alternatively, substrate-competitive JNK inhibitors such as pepJIP1 interact with the D-site and effectively block JNKs interactions with its binding partners, including scaffold proteins, upstream MAP2Ks, downstream substrates and phosphatases (PDB 1UKI).

## Non-kinase JNK inhibitors

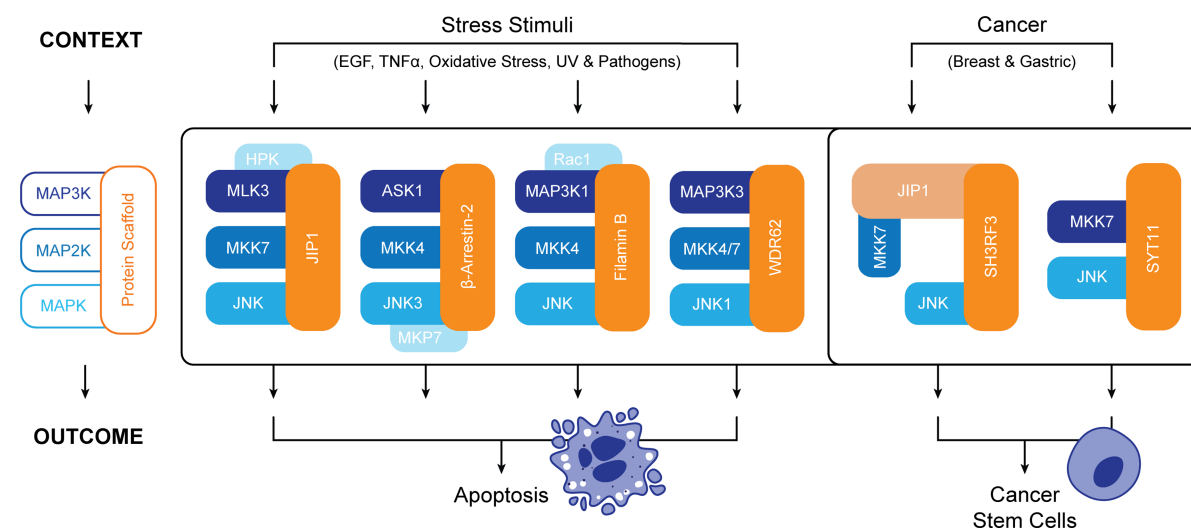
Outside of the ATP-binding pocket, two non-ATP binding sites have been identified for small-molecule and/or peptide-based JNK inhibitors. The first of these, reported by Abbott Laboratories, was identified through an affinity-based screening platform that sought to isolate JNK-targeting candidates from a library of 500 000 small molecules [46]. Whilst NMR revealed that the majority of candidate small molecules interacted with the ATP-binding site, two biaryl tetrazole compounds (including 46A, pubchem ID 15658026) elicited distinct resonance patterns and were found to bind a unique surface pocket bordered by the A-loop; the structural component that encompasses the TPY motif for MAP2K dual phosphorylation and activation (Figure 2B-ii).

Structural analyses revealed that this binding pocket is accessible when JNK is inactive, due to mutations within the TPY motif (Thr183Glu and Tyr183Glu), causing distinct conformational changes in the A-loop. Accordingly, these compounds and their cell-active derivatives block JNK activity at low micro-molar ranges by inhibiting the phosphorylation of JNK by upstream MAP2Ks. To our knowledge, these compounds have not been investigated further in the context of JNK inhibition.

## Substrate-competitive JNK inhibitors

The best-described non-kinase strategy targets the JNK docking site (D-site), which lies next to the hinge loop and comprises the CD region and adjacent hydrophobic docking groove (Figure 2A). Much like the other MAPKs, P38 kinases and extracellular signal-regulated kinases (ERKs), JNKs use this single interface as a means of interacting with many of their binding partners, including scaffold proteins, upstream MAP2Ks, downstream substrates and phosphatases (reviewed in detail in [6]). These interaction partners harbor complementary docking motifs (D-motifs or D-domains), which are typically found in disordered regions of the protein and contain basic residues ( $\theta$ ), followed by short spacer sequences and hydrophobic residues ( $\varphi$ ). Two predominant types of JNK-interacting D-motifs have been identified [6], which resemble the sequences of the transcription factor N-FAT4 ( $\theta$ -X-X- $\varphi$ -X- $\varphi$ -X- $\varphi$ ) or the JNK scaffold protein JNK-interacting protein 1 (JIP1) ( $\theta$ - $\varphi$ -X-X- $\varphi$ -X- $\varphi$ ).

JIP1 is a prototypical scaffold protein in that it facilitates JNK signal transduction by binding all three components of the MAPK cascade, including MAP3Ks of the mixed lineage kinase (MLK) family, MKK7 and JNK (Figure 3) [47]. Significant interest in JIP1 stems from early reports that the overexpression of either JIP1 or the JIP1 JNK binding domain (JBD) is sufficient to block JNK activity [48,49]. These findings led to the development of the D-site targeting peptides pepJIP1 (also known as TI-JIP1; Figure 2B-iii), an 11 amino acid peptide corresponding to the minimal D-motif of JIP1 (residues 153–166) [50,51], and D-JNKI1, a cell-permeable and protease resistant retro-inverso peptide comprising the 20 amino acid JIP1 D-motif and a HIV-TAT sequence [52]. Whilst biochemical analyses reveal that pepJIP1 displays high specificity for JNKs, although with limited isoform selectivity, D-JNKI1 reportedly exhibits higher potency toward P38 kinases than JNKs [53,54]. In spite of this, the JNK inhibiting effects of D-JNKI1 have been assessed in various disease contexts including cancer, where it has been shown to suppress the growth of xenografted HCC cells and



**Figure 3. Scaffold proteins assemble compositionally and functionally discrete JNK signaling complexes in a highly context specific manner.**

JIP1,  $\beta$ -Arrestin-2, Filamin B and WDR62 are amongst the best described JNK scaffold proteins and are known to assemble JNK network components in response to extracellular stress stimuli to induce apoptosis. In the context of gastric and breast cancers, two scaffold proteins, Synaptotagmin 11 (SYT11) and SH3RF3, have recently been shown to promote disease progression through the positive regulation of cancer stem cell populations.

chemically induced liver cancers [55], and attenuate tumor growth and cancer pain development in murine melanoma models [56]. Although D-JNK11 has not progressed as a clinical cancer treatment, its JNK-dependent otoprotective effects (reviewed in [57]) have prompted several preclinical studies and Phase II/III clinical trials evaluating its efficacy as a treatment for acute hearing loss [58,59]. It is likely that local intratympanic administration of D-JNK11 (also known as AM-111 or brimapitide) in this clinical context may mitigate the toxic side effects of systemic JNK inhibition.

Whilst significant focus has been placed on JIP1-like peptides due to their high affinity interaction with JNK, peptide inhibitors have also been derived from the JNK docking protein and substrate, SH3 domain-binding protein 5 (SH3BP-5, or Sab). Expressed on the outer mitochondrial membrane, Sab interacts with and docks activated JNK through its cytoplasmic C-terminal kinase interaction domain (KIM), facilitating its translocation into mitochondria [60,61]. Mitochondrial JNK activity then drives Bcl-xl phosphorylation, cytochrome C release and respiration suppression, which ultimately activate processes such as apoptosis and autophagy [62–66]. Despite containing the essential D-motif components required for JNK docking, Sab has a significantly lower affinity for JNK compared with JIP1 [67,68]. Whilst it was originally thought that this lower affinity interaction would limit the use of Sab-derived peptides, such as TAT-Sab<sub>KIM1</sub>, it has instead been shown to increase their specificity for mitochondrial JNK [69]. Specifically, TAT-Sab<sub>KIM1</sub> is able to inhibit JNK localization to mitochondria and mitochondrial JNK signaling pathways without perturbing cytosolic and nuclear JNK activation [66]. Whilst these peptides represent promising therapeutic targets in diseases where mitochondrial dysfunction is a key driver [64,66], the essential role of mitochondrial JNK in therapy-induced apoptotic response likely limits their use as cancer treatments. For instance, blocking the Sab-JNK interaction with TAT-Sab<sub>KIM1</sub> has already been shown to prevent apoptosis induced by oxidative stress, including that driven by the kinase inhibitor sorafenib in HCC cells [70].

Several small molecule inhibitors of this JIP1-JNK interface have also been identified through large-scale compound screens. For instance, Stebbins et al. [71] utilized a dissociation enhanced lanthanide fluorescent assay to screen 30 000 compounds and identify BI-78D3, a highly specific and potent substrate-competitive JNK inhibitor that can sensitize osteosarcoma cells to doxorubicin treatment *in vitro* [72]. An alternate high-throughput screen of >2 million compounds performed by Pfizer identified an undisclosed number of highly specific JNK inhibitors in this same compound class, although their precise binding site could not be resolved [53]. This study additionally revealed a subset of dual-inhibitors that perturb both the JIP1-JNK and ATP-binding sites through allosteric mechanisms [53]. Based on the behavior of these dual-inhibitors in the presence of excess ATP, and their anticipated binding modes, it was predicted that they elicited their effects through interactions with the ATP-binding site rather than the D-site. Structural and biochemical analyses have since revealed the effects of both substrate-competitive JNK peptides and structurally diverse ATP-competitive compounds on this allosteric communication pathway between the JNK D-site, ATP-binding site and A-loop [68,73]. To our knowledge, no small molecule substrate-competitive JNK inhibitors or dual-inhibitors have progressed through to preclinical testing.

A significant limitation of strategies targeting the JNK D-site appears to be the fact that high-affinity inhibitors end up behaving much like ATP-competitive inhibitors, in that they effectively block all JNK-protein interactions and as such indiscriminately inhibit all JNK functions. Although studies assessing TAT-Sab<sub>KIM1</sub> peptides on mitochondrial JNK indicate that specificity for discrete subcellular JNK pools can be achieved with low affinity inhibitors, it remains to be seen how low-affinity D-site targeting peptides would translate into a clinical setting given that they rely on subtle differences in interaction partner binding affinities to effectively target one JNK pool over another. Regardless, these peptides provide a crucial proof-of-principle that specific JNK functions can be perturbed by targeting protein-protein interaction interfaces within discrete JNK signaling complexes.

## Identifying alternate drug targets within JNK signaling complexes

Given the challenges faced with directly targeting JNK, a number of strategies have been described that perturb JNK signaling through alternate mechanisms. Amongst these is the recent development of specific MAP2K and MAP3K inhibitors, such as those targeting MKK7 [74], ASK1 [75], and TAK1 [76]. However, as each of these kinases is likely to regulate multiple downstream effectors and biological responses, it remains to be seen how they fare in terms of cellular toxicity. For instance, whilst MKK7 has been shown to promote the stemness of



gastric cancers [77] and drive the metastasis of colon cancer cells [78], it also functions as a critical tumor suppressor in lung and mammary cancers through the stabilization of p53 [79]. Much like JNKs, the roles of MAP2Ks and MAP3Ks in tumorigenesis are highly context specific and a better understanding of their involvement in JNK signaling is required for the development of effective treatment strategies.

Although the JIP1/Sab-derived peptides demonstrate that protein-protein interactions represent attractive and effective therapeutic targets, there has been little progress targeting alternate interfaces within JNK signaling complexes. In one case, a 22 amino acid peptide identified through a fragment library screen, known as PYC71, was shown to bind to c-Jun and potently inhibit its interaction with JNKs [80]. This idea of targeting the D-motif interface rather than the D-site itself can also be seen in nature, with Notch1 able to block UV-irradiation induced JNK3 activation through its direct interaction with the JNK binding domain of JIP1 [81]. Whilst these studies support the idea of pursuing alternate protein targets, the identification of complex components (scaffolds and substrates) and interaction interfaces implicated in specific pro-tumorigenic JNK signaling pathways is not a trivial task.

Through their interactions with at least two components of the MAPK tier, scaffold proteins fine-tune the composition and localization of JNK signaling complexes to enhance the specificity and efficacy of signal transduction. Whilst a number of JNK scaffolds have been identified, including JIP family members [82], arrestins [83,84], filamins [85,86] and WDR62 [87,88] (Figure 3), their JNK-related roles have almost exclusively been linked to stress-induced apoptosis and limited literature is available covering their relevance as scaffolds in cancer progression. To this end, SH3 domain containing ring finger 3 (SH3RF3, also POSH2) and Synaptotagmin 11 (SYT11) have both recently been identified as JNK scaffolds that positively regulate cancer stem cells (CSCs) in breast and gastric cancers [14,77]. CSCs are a tumor cell subpopulation that are capable of self-renewal, display tumor-initiating capabilities and are associated with metastasis, therapy resistance and disease recurrence. Recent studies have now demonstrated that JNK signaling is critical for driving the transcriptional programs that maintain CSC phenotypes in multiple cancer contexts [89–93], and also shown that JNK promotes CSC chemoresistance and metastasis in TNBCs by supporting the formation of a CSC niche [12].

With protein-protein interactions partly described for both the SH3RF3 and SYT11 JNK signaling complexes, it is clear that whilst these two scaffolds drive similar biological outcomes, they each regulate JNK through distinct mechanisms [14,77] (Figure 3). In the context of gastric cancer, Kim *et al.* demonstrated that both MKK7 and JNK interact with the SYT11 in the cytoplasm via its N-terminal transmembrane domain. SYT11-dependent JNK phosphorylation in turn activates c-Jun and the subsequent transcription of EMT-related genes that drive tumor formation and liver metastasis [77]. Alternately, Zhang *et al.* reported that whilst MKK7 directly binds the fourth SH3 domain of SH3RF3, JIP1 is required to mediate the interaction between SH3RF3 and JNK. JNK phosphorylation in this context promotes breast CSC phenotypes through the downstream activation of c-Jun and increased expression of pentraxin 3 (PTX3) [14]. In both cases, JNK inhibition achieved through scaffold depletion or small-molecule inhibitors significantly perturbed oncogenesis. Whilst further work is required to fully resolve these binding interfaces, the protein-protein interactions uncovered in these studies open up novel therapeutic options for targeting the discrete oncogenic JNK signaling complexes that regulate cancer stem cell populations in specific tumor types.

## Concluding remarks

Whilst JNK proteins play critical tumor-promoting roles in a number of cancers, their tumor-suppressive, homeostatic and apoptotic functions limit the use of direct JNK inhibitors as anti-cancer treatments. Despite the significant efforts that have been made concerning the development and optimization of ATP-competitive and substrate-competitive JNK inhibitors, it is increasingly clear that strategies targeting JNK directly are unlikely to yield clinical success as they indiscriminately block all JNK functions and lack the subtlety required to selectively suppress oncogenic JNK signaling. For the future of JNK targeting in cancer, we believe that this specificity can be achieved by inhibiting unique protein-protein interactions within oncogenic JNK signaling complexes, including the interactions between scaffold proteins and MAP3K/MAP2K/JNK network components. Whilst recent studies have made progress in identifying oncogenic JNK complexes that regulate cancer stem cell populations in gastric and breast cancers, significantly more work is required to therapeutically target these complexes, and resolve the composition, organization and interfaces of JNK complexes that drive tumorigenesis and metastatic disease progression in other tumor types.

## Perspectives

- JNKs represent attractive therapeutic targets for several cancers, including gastric and breast cancers. Various experimental models have demonstrated that direct JNK inhibition is able to block primary tumor growth, impede the tumor-initiating potential of cancer stem cells, modulate the structural and cellular landscape of the metastatic niche, and thereby inhibit metastatic disease progression.
- Although significant headway has been made in improving the specificity and selectivity of ATP-competitive and substrate-competitive JNK inhibitors, these compounds and peptides are unlikely to yield clinical success as anti-cancer therapies due to their indiscriminate inhibitory effects on the physiological and tumor-suppressing functions of JNK. The evidence indicates that direct JNK inhibition is unlikely to work and that alternate approaches are required. In line with this, strategies targeting the scaffold-JNK and JNK-substrate interfaces provide a crucial proof-of-principle that protein–protein interactions within discrete JNK signaling complexes represent effective and clinically viable therapeutic targets.
- For clinical viability, anti-cancer therapies targeting JNK must discriminate between the distinct functions of JNK and specifically inhibit JNKs oncogenic activities. Identifying the scaffold proteins, substrates and protein–protein interaction interfaces that drive oncogenic JNK signaling will be critical for the development of effective JNK-targeting strategies in these disease contexts.

## Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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## Authors Contributions

SLL conceptualized and drafted the manuscript. All authors contributed to figure preparation and edited the manuscript.

## Abbreviations

CSC, cancer stem cell; D-motif, docking motif (JNK interaction partner); D-site, docking site (JNK); ERK, extracellular signal-regulated kinases; HCC, hepatocellular carcinoma; JBD, JNK binding domain; JIP, JNK-interacting protein; JNK, c-Jun N-terminal kinase; MAP2K, mitogen activated protein kinase kinase; MAP3K, mitogen activated protein kinase kinase kinase; MAPK, mitogen activated protein kinase; SAB/SH3BP-5, SH3 domain-binding protein 5; SAPK, stress activated protein kinase; SH3RF3, SH3 domain containing ring finger 3; SYT11, Synaptotagmin 11; TNBC, triple negative breast cancer.

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