Targeting survivin for therapeutic discovery: the past, the present, and the future promises

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Abstract

Survivin, the smallest member of the Inhibitor of Apoptosis Protein (IAP) family, is over-expressed in cells of almost all cancers but not most adult normal tissues. Survivin expression is required for cancer cell survival and knocking down its expression or inhibiting its function using molecular approaches all cause spontaneous apoptosis. Thus, survivin is an attractive and perhaps an ideal target for cancer drug discovery. However, an FDA-approved drug targeting survivin has yet to emerge. In this review, we examine and evaluate various strategies that have been tested in targeting survivin and the stages of each survivin inhibitor to help understand why we have not yet been successful. We also provide future perspectives moving forward in targeting survivin for drug discovery.

Teaser

This review provides a comprehensive analysis of drug discovery targeting survivin and evaluate the advantages/disadvantages of survivin inhibitor as well as insights on moving forward in targeting survivin for drug discovery.

Introduction

Resistance to traditional chemotherapy and new targeted therapies is a classical hallmark of many cancers and remains a pressing challenge to a successful clinical outcome for a significant population of cancer patients [1]. The need for new specific molecular cancer targets to combat drug resistance remains high. One such molecule, survivin, has garnered substantial attention as a potential cancer specific target. Survivin (also called BIRC5) is a member of the Inhibitor of Apoptosis Protein (IAP) family (Table 1) [2,3] and plays a critical role in both the inhibition of apoptosis and in cell cycle progression [4]. Survivin has been shown to be overexpressed in almost every type of cancer but is undetectable in most normal adult tissues [5]. Survivin's aberrant expression, dual roles in cancer cell survival, and its correlation with resistance to chemotherapies and poor patient outcome make it a promising target for discovery of new cancer therapeutics. The aim of this review is to discuss the distinct strategies that have been used to target survivin or its expression and the validity and potential of each as a relevant clinical cancer chemotherapeutic moving forward. We will also provide future perspectives in targeting survivin for potential therapeutic discovery.

Survivin structure and function

Survivin consists of 142 amino acid residues with a single zinc finger fold Baculovirus Repeat (BIR) domain, a dimerization domain at two different locations in the linear sequence, and a C-terminal domain for protein-protein interaction and nuclear export (see Figure 1A and 1B). Survivin exists as a stable bow-tie-shaped homo-dimer (Figure 1C) formed through interactions of the N-terminal region, linker region, and the N-terminus of alpha helix four [6]. The homo-dimeric interface is mostly hydrophobic with greater than 75% of the residues being nonpolar [7]. The buried accessible area in the interface of a monomeric survivin is 550 Å^2 and occupies only 6% of

the total accessible area of a monomer (9,044 Å^2) compared with ~20% in most other dimeric or oligomeric proteins [8].

Ectopic over-expression of survivin inhibits both extrinsic and intrinsic apoptosis pathways in cell lines and animal models [9-12]. However, the exact mechanism in apoptosis inhibition by survivin remains unknown with the binding of survivin to caspases being proposed as a possible mechanism (Figure 2). Survivin has been suggested to directly bind to and inhibit caspase 3 and 7 while contradictory evidence exists that brings this observation under scrutiny [13,14]. Although myc-tagged survivin in HEK293 cells co-immunoprecipitated with active caspase 3 and 7, survivin seemingly lacks the additional structural moieties such as the BIR2 domain that have been demonstrated to be necessary for caspase 3 and 7 binding by other IAP family members [15-17]. It has also been suggested that survivin can bind to caspase 9 and inhibit its activation [18]. However, it was shown later using purified proteins that survivin alone does not bind to caspase 9 and it may require the presence of hepatitis B X-interacting protein (HBIXP) to bind to and inhibit caspase 9 [19]. The interaction between survivin and HBIXP may be responsible for binding to pro-caspase 9 to prevent its recruitment to the apoptosome and, thus, inhibit its activation [19]. It has also been postulated that an IAP-IAP complex between survivin and X-linked IAP (XIAP) may form to stabilize XIAP and lead to inhibition of caspase 9 [20]. Finally, survivin may prevent the induction of apoptosis by interaction with intermediate apoptotic proteins to indirectly halt caspase activation. SMAC/DIABLO is a proapoptotic protein that promotes cytochrome c dependent apoptosis by binding to and antagonizing IAPs [21]. Survivin can bind SMAC/DIABLO and prevent SMAC/DIABLO inhibition of caspases [22]. Survivin has been shown to co-localize with SMAC/DIABLO and disruption of their physical association induces apoptosis [23]. Thus, it is possible that survivin inhibits apoptosis via interaction with multiple

proteins in the apoptosis pathway.

In addition to the inhibition of apoptosis, survivin acts as a key mitotic regulator (Figure 2) and is essential for proper completion of mitosis and cell division [24]. Survivin expression is tightly regulated during the cell cycle progression, peaking in the G2/M phase [25]. During mitosis, survivin localizes and interacts with microtubules to regulate microtubule formation during cell division by altering microtubule dynamics and nucleation [26]. Additionally, survivin is a critical component of the chromosomal passenger complex (CPC) that also consists of INCENP, Borealis, and Aurora B Kinase [27]. INCENP acts as a scaffold to stabilize the entire complex, while Borealis promotes the attachment of survivin to the complex [28]. Survivin appears to function in targeting of the CPC to the centromere and midbody during mitosis. The CPC is a critical regulator of several mitotic events [29] and, when localized to the midbody, it facilitates proper chromosome alignment during metaphase [30]. The CPC is also vital for correction of chromosome-microtubule attachment errors [31] and activation of the spindle assembly checkpoint [32].

Survivin as a target for therapeutic discovery

For over a decade, survivin has drawn a considerable attention as a potential novel drug target in a variety of human cancers. The attractiveness of survivin as a potential cancer drug target has been in large part due to its dramatic dysregulation of expression between normal adult tissues and most cancers. Survivin is expressed to a high extent in fetal tissues [33], but is undetectable in most normal adult tissues [34]. Interestingly, survivin is overexpressed in almost all human cancers including cancers of lung, breast, colon, stomach, esophagus, pancreas, prostate, liver, and ovary [35].

Survivin has been consistently demonstrated to be an important contributor to both

radiotherapy [36] and chemotherapy resistance [37,38]. High level of survivin expression is predictive of poor clinical outcome and correlates with relapse in bladder cancer [39], non-small cell lung cancer [40], gall bladder carcinoma [41], locally advanced rectal cancer [42]. renal cell carcinoma [43], and breast cancer [44,45].

Expressing recombinant dominant negative survivin produced pro-apoptotic and anti-proliferative effects on human cancer cells and suppressed tumor growth in vivo [46-48]. Cleavage of survivin mRNA by ribozyme increased cell death by caspase dependent apoptosis [49]. Down regulation of survivin expression by antisense oligonucleotides [50] and siRNAs inhibited cancer cell proliferation and increased chemosensitivity [51]. These studies using molecular probes along with the expression profile of survivin in cancer and its clinical relevance have clearly established survivin as a target for anticancer drug discovery.

Therapeutics targeting survivin

Despite the promise and interest in survivin as a target for anticancer drug discovery, at present time there exists a small portfolio of anti-survivin agents, which can generally be classified in to three broad categories of inhibitors targeting (1) regulators of survivin expression, (2) survivin interaction with ligand proteins, and (3) survivin homo-dimerization. In addition, survivin has also been considered and tested as a cancer vaccine for immunotherapy.

1. **Inhibitors targeting survivin expression.** Because survivin has no known enzymatic activities and it is considered "undruggable", the initial effort of targeting survivin was not on the survivin protein itself, but rather on inhibiting survivin expression to avoid targeting survivin protein directly. This approach includes the use of antisensense oligonucleotides and small-molecule inhibitors.

1.1. Antisense oligonucleotides. Antisense oligonucleotides such as LY2181308 and

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SPC3042/EZN-3042 (Table 2) have been developed to inhibit survivin expression as anticancer therapeutics. These oligonucleotides have been tested in clinical studies with mixed findings.

LY2181308 is a 2'-O-methoxymethyl-modified single strand antisense oligonucleotide targeting survivin mRNA to limit survivin expression developed by Eli Lilly [52]. LY2181308 inhibited the expression of survivin gene at both mRNA and protein levels in a panel of cell lines and it significantly inhibited growth of human tumor xenografts [52,53]. The positive pre-clinical activity of LY2181308 led to its clinical testing as a single agent and in combination with other chemotherapeutics. However, the outcome of these clinical studies are mixed. LY2181308 was well tolerated as a single agent and did not appear to cause additional toxicity to cytarabine and idarubicin in refractory or relapsed AML patients [54]. With this cohort of 16 AML patients, it appeared that the combination of LY218308 with cytarabine and idarubicin showed synergistic benefits. LY218308 also inhibited survivin expression in patients with high survivin level although the correlation between survivin inhibition and response was not studied. It is also noteworthy that the cohort size in this study was small and a bigger cohort size is needed to validate the findings on the synergism.

The findings from clinical study of LY218308 on solid tumors are not encouraging. Neither the phase I trial of LY218308 as a single agent for solid tumors [55] or in combination with docetaxel/prednisone in a phase II trial for castration-resistant prostate cancers [56] showed any benefit of LY2181308. The authors noted that lack of response might be due to the lack of survivin inhibition by LY2181308 in these solid tumors although survivin expression was not measured in these studies and they were unable to achieve the required pharmacokinetic levels necessary for survivin inhibition.

SPC3042 is a locked antisense oligonucleotide that targets the stop codon of the open reading

frame in exon 4 of the survivin transcript and was under preclinical development by Santaris Pharma [57]. SPC3042 displayed improved potency compared to LY2181308. However, unlike LY2181308, SPC3042 had a significant effect also on the mRNA and protein levels of Bcl-2 in addition to that of survivin. Down regulation of survivin expression by SPC3042 sensitized PC3 prostate cancer xenograft to taxol treatment in vivo. In 2006, Santaris Pharmaceuticals licensed the developmental rights of SPC3042 to Enzon-Pharmaceuticals and it was rebranded as EZN-3042. In primary ALL cells, EZN-3042 synergized with chemotherapy and eliminated ALL cells in vitro [58]. EZN-3042 also demonstrated success in vivo as it achieved ~60% down regulation of survivin mRNA in Calu-6 lung xenografts and ~40% tumor growth inhibition. In combination with paclitaxel, EZN-3042 achieved >80% tumor growth inhibition [59]. However, the phase I trial of EZN-3042 was terminated since EZN-3042 produced several dose-limiting toxicities and it was unable to be safely administered with other chemotherapeutics [60]. As such, further clinical development of this oligonucleotide has been halted by Enzon Pharmaceuticals.

In summary, the success in clinical trials has been limited by targeting survivin expression using antisense oligonucleotides, particularly for solid tumors. The lack of response in solid tumors suggests that this strategy of inhibiting survivin expression may have limited potential in targeting survivin and treating the difficult-to-treat and aggressive solid tumors. One contributing factor to the ineffectiveness of antisense oligonucleotides may be the intrinsic disadvantages of oligonucleotides including stability and availability issues. These disadvantages may also account for the dose limiting toxicities that have been evident in previous trials and are consistent with the currently limited portfolio of FDA-approved antisense oligonucleotide therapeutics.

1.2. Small-molecule inhibitors. In addition to antisense oligonucleotides, small-molecule inhibitors (Table 3) have also been developed by targeting transcription of the survivin gene. These

inhibitors include YM155 and EM-1421, which have been tested in clinical studies, and other compounds such as SF002-96-1 and FL118, which are still at the pre-clinical stage.

YM155 (sepantronium bromide) is the first small-molecule inhibitor targeting the expression of survivin, identified via high throughput screening (HTS) using a survivin-promoter-luciferase reporter assay aiming to identify small-molecule inhibitors that may bind to the promoter sequence of the survivin gene [61]. YM155 potently inhibited survivin-promoter-driven luciferase expression without effect on the expression of other antiapoptotic proteins. It also effectively inhibited growth of human prostate PC3 ectopic xenograft tumors. In a follow-up study, YM155 induced cell death with an average IC50 of 15 nM in a panel of 119 human cancer cell lines [62]. Continuous 3- or 7-day YM155 infusion in xenograft models also showed significant tumor-suppressing activity on different tumors including melanoma and cancers of breast, lung, and bladder without significant toxicity as indicated by little body weight loss [62,63].

Due to the promise of YM155 in cell-based and preclinical studies, it has been investigated in clinical trials as a single agent and in combination with other anticancer therapies (for a review see [63]). While YM155 is well tolerated with a MTD of 4.8 mg/m^2 [64] and it has shown some efficacy against blood cancers, mixed results were generated when tested against various solid tumors with modest efficacy at best for some tumors [63]. For example, no improvement in response rate was observed for non-small cell lung cancer patients in multiple phase II trials of YM155 as a single agent and in combination with carboplatin and paclitaxel [65,66] while a phase II trial of castration resistant prostate cancers (CRPC) showed modest activity with 25% of patients displaying prolonged stable disease [67]. While the mixed successes of YM155 in clinical studies suggest that targeting survivin may help identify a novel approach to cancer therapy, clinical development of YM155 is now questionable. One confounding factor is that YM155 cannot be

used for bolus dosing and has to be continuously infused 24 hrs a day in 3- or 7-day dosing cycles. Currently there are no ongoing clinical trials with YM155.

Since the discovery of YM155, there have been large undertakings to determine its precise mechanism of action. While it has been shown to inhibit survivin transcription, it does not work via binding to survivin promoter sequence as anticipated but rather by inhibiting the survivin upstream transcription factor Sp1 [68]. Evidence also exist to suggest that YM155 may inhibit survivin expression by disrupting interleukin enhancer-binding protein factor 3 and p54^{nrb} complex, a critical complex for transcription of survivin [69]. There is also compelling evidence to suggest that YM155 may be a DNA damage-inducing agent [70] and its inhibition of survivin expression may be secondary to this event. Considering that Sp1, the target of YM155, is a ubiquitous transcription factor and that its inhibition of survivin expression may be a secondary event to DNA damages [70], YM155 unlikely inhibits survivin expression specifically. Hence, designating YM155 as a survivin inhibitor is inappropriate and misleading.

EM-1421 (terameprocol) is another small-molecule transcriptional repressor of the survivin gene under development as survivin inhibitor by Erimos Pharmaceuticals that acts to inhibit the ubiquitous transcription factor Sp1 [71]. In addition to inhibiting survivin expression, EM-1421 has been shown to also inhibit the expression of cdc2, another Sp1-controlled gene [72]. EM-1421 induced G2 cell cycle arrest in a variety of solid tumor cell lines [73] and systemic treatment with EM-1421 suppressed the growth of human tumor xenografts including breast, prostate, colorectal, and liver cancers [74]. Using clonogenic survival assays, it was found that EM-1421 was able to induce radiosensitization in non-small cell lung cancer cells [75]. Similar as YM155, EM-1421 has been tested in clinical trials. For advanced leukemia patients, EM-1421 demonstrated a safe profile and showed partial responses in a few patients with CML or AML in a phase I study [65,76].

However, in a phase I study for high grade gliomas, EM-1421 did not display any response and stable disease was observed in 32% of patients [77]. Most recently, the safety profile of EM-1421 was tested as a vaginal ointment for treatment of cervical intraepithelial neoplasia caused by human papilloma virus [78] in a Phase I/II trial using healthy adult women. The ointment with 1% and 2% EM-1421 showed a promising safety profile with no severe adverse effects in healthy subjects.

SF002-96-1 is a natural drimane sesquiterpene lactone isolated from *Aspergillus* that was identified to potentially inhibit survivin promoter activity using survivin-promoter-driven reporter assay in Colo320 cells [79], similarly as described above for identification of YM155. SF002-96-1 decreased Colo320 cell viability and induced apoptosis as evidenced by increased caspase 3 activity and cleaved chromosomal DNA by DAPI staining. Further studies using promoter-reporter assay, SF002-96-1 was shown to inhibit the activity of transcription factors STAT3 and NF-κB but not TCF/β-catenin, which all regulates survivin transcription, and also decreased survivin mRNA and protein levels. Thus, SF002-96-1 may inhibit survivin expression by inhibiting multiple upstream transcription factors such as STAT3 and NF-κB. As with other nonspecific strategies, unexpected dose-limiting toxicities may result from the disruption of other genes besides survivin, which may limit the utility of SF002-96-1 going forward.

FL118 is a nonselective small-molecule inhibitor of survivin expression that structurally resembles the topoisomerase I inhibitor, irinotecan. FL118 appears to inhibit survivin expression by disrupting survivin promoter activity. However, FL118 also down regulates the expression of Mcl-1, XIAP, and cIAP2. Strikingly, FL118 displayed greater anti-tumor activity in squamous cell carcinoma and colon cancer cell line-derived xenografts, as well as a patient-derived head and neck cancer xenograft as compared to leading first line chemotherapeutics [80]. A detailed molecular mechanism of FL118 action is currently lacking but warranted with the promising in-

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vivo data. However, based on the studies of YM155, it is tempting to speculate that FL118 may also inhibit upstream transcription factors that regulate survivin expression. It may also be possible that the effect of FL118 on survivin expression is a secondary effect of DNA damage potentially induced by FL118 inhibition of topoisomerase (see discussion above on YM155).

GDP366 is a small-molecule compound that has been found to reduce the mRNA and protein levels of both survivin and oncoprotein Op18. Treatment with GDP366 inhibits cancer cell proliferation both in vitro and in vivo [81]. Little data are available to evaluate GDP366 and more mechanistic studies are needed to elucidate the mechanism of action by which GDP366 produces its effects on survivin expression and its potential as a cancer therapeutics.

While targeting the transcription of survivin gene has gained progresses with YM155 and EM-1421 tested in clinical trials, the common issues with all these inhibitors are that they are not selectively targeting survivin expression. Most of these inhibitors act on one or more upstream transcription factors that regulate the expression of survivin as well as many other downstream genes. Effectively, these inhibitors are not survivin inhibitors but rather transcription factor inhibitors. The lack of specificity of these inhibitors in targeting survivin transcription as identified using survivin promoter activity assay and designating these compounds as survivin inhibitors are indeed troubling.

Although both YM155 and EM-1421 have generally been tolerated in clinical trials despite the fact that they both inhibit a ubiquitous transcription factor Sp1, their limited clinical efficacies suggest that targeting the survivin upstream transcription factors such as Sp1 to limit survivin expression may unlikely represent an appropriate strategy to target survivin for therapeutic development. It is also unclear if the other downstream target genes of these transcription factors may work against the inhibition of survivin expression in suppressing cancers in the clinical

setting. These findings also indicate that targeting Sp1 may not lead to effective cancer therapeutics for development.

2.   **Inhibitors targeting survivin interaction with other proteins.** Because survivin is known to work by binding to and interacting with many important cellular proteins, there have been various efforts in targeting survivin interaction with its binding partners (see Table 4).

The first in this category of inhibitors is called shepherdin, a peptide with a sequence ($K^{79}H$ SPGCAFL^{87}) of survivin that inhibits Hsp90 interaction with survivin and destabilizes survivin [82]. Tagging the amino terminus of sheperdin to either helix III of the Antennapedia or HIV-/Tat sequence led to a cell permeable shepherdin that was able to accumulate in cells and inhibit survival of HeLa, PC3, and DU145 cells by inducing apoptosis without apparent effect on normal cells. The cell permeable shepherdin also effectively inhibited PC3 xenograft growth in vivo. Because shepherdin binds to Hsp90 and may destabilize many proteins including survivin, the cell death induced by shepherdin could be due to multiple factors regulated by Hsp90. It would be of interest to determine if a peptide with a sequence from Hsp90 that inhibits Hsp90 binding to survivin has similar effects as shepherdin.

In another study searching for peptide mimetics interacting with survivin using yeast two-hybrid system, a peptide derived from heavy chain 1 of ferritin was identified to interact with survivin [83]. Interestingly, this peptide, when cloned into a disabled thioredoxin for purification of recombinant protein, was able to bind to survivin and suppress survivin expression and induce apoptosis in breast and glioma cancer cells. The reduced survivin expression appears to be proteasome dependent. However, these authors have also shown that the full-length heavy chain 1 of ferritin does not interact with survivin. Thus, the peptide-induced survivin loss may not be via inhibition of survivin interaction with ferritin. It remains to be determined how this peptide derived

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from ferritin would induce loss of survivin and induce apoptosis of cancer cells.

Using a shape-based structural screening for the second mitochondria-derived activator of caspase (SMAC) mimetic that would inhibit interaction between SMAC and IAP proteins, a novel hit compound, UC-112, was identified that significantly activate caspases in melanoma and prostate cancer cell lines [84]. Continuous treatment of A375 melanoma xenograft tumors with UC-112 for 3 weeks significantly inhibited tumor growth in a dose-dependent manner with little reduction in the body weight of the mice. Interestingly, UC-112 dose-dependently inhibited survivin expression as well as expression of other IAPs albeit to a less extent. Currently, it is unknown if UC-112 disrupts the interaction between SMAC and survivin or other IAPs. It is also unknown how UC-112 suppresses the expression of survivin and other IAPs. However, it appears that UC-112 may reduce their stability via proteasome-dependent manners. Computational docking analysis predicts that UC-112 may bind to the BIR domain of survivin [85] although experimental evidence is needed to prove this binding. It also remains to be determined why disrupting the interaction between SMAC and IAPs by UC-112 would lead to degradation of IAPs. Does UC-112 have other unknown activities that would facilitate the degradation of survivin and other IAPs, perhaps by changing the conformation of these proteins? Many questions remain to be answered in order to further develop UC-112 as a SMAC mimetics to inhibit survivin and/or other IAPs.

In a follow-up structure-activity relationship study, analogs of UC-112 were synthesized and analyzed [85]. One of the analogs, 4g, has an iso-propyl group substituted on the C-linker of UC-112 (Table 4) with a 4-fold increase in activity in growth inhibition of cancer cell lines and increased selectivity to survivin as compared to UC-112. 4g also proved effective in inhibiting the A375 melanoma xenograft in vivo similar as the parent compound UC-112. The successes of

these studies certainly warrant further mechanistic investigation of SMAC mimetics as survivin inhibitor and for potential development. SMAC mimetics represent an approach that inhibit survivin interaction with other proteins by binding to survivin and, thus, eliminate other effects by binding to its partners as in the case of shepherdin. However, modification and optimization of SMAC mimetics are likely needed to increase its specificity to survivin over other IAPs, which may prove to be difficult since their binding site, the BIR domain, is the consensus signature domain of IAPs.

Withanone is a herbal ligand from the plant *Withania somnifera*, which was predicted to bind to the BIR domain of human survivin using computational docking analysis and to interfere with the caspase inhibitory function of survivin [86] similar as UC-112. However, no experimental testing has been conducted on withanone to determine if it inhibits or binds to survivin although withanone has been shown previously to have mild activity in inhibiting proliferation of various cancer cell lines [87-89]. Clearly, more studies are need to investigate if this natural product hold any promise in binding to survivin and inhibiting its interaction with SMAC.

In another study, the protein-protein interaction sites in survivin were analyzed in-silico and hotspot residues were identified and used to generate a potential pharmacophore that may interfere survivin interaction with its binding partner chromosome passenger complex (CPC) [90]. HIV protease inhibitors, particularly Indinavir, was found to match the pharmacophore. Although Indinavir was able to inhibit the survival of MDA-MB-231 breast cancer cells with an IC_{50} greater than 500 μM, it is unknown if Indinavir binds to survivin and inhibits its interaction with its binding partners such as those involved in the formation of the CPC. Interestingly, Indinavir decreased the XIAP protein level and increased caspase 3 cleavage. It remains to be determined if this effect was via binding to and inhibiting survivin. It is noteworthy that another HIV protease

inhibitor, Nelfinavir, has previously been shown to be able to potentiate Imatinib cytotoxicity in meningioma and inhibited survivin expression [91] while Indinavir did not affect survivin expression. Thus, it is unclear if these HIV protease inhibitors actually bind to and inhibit survivin interaction with its ligand proteins. Clearly, more studies remain to be conducted on these HIV protease inhibitors to determine if they bind to or act on survivin and can be repurposed as potential new anti-cancer therapeutics by binding to and inhibiting survivin. However, because of its high IC50 at 500 µM, Indinavir will unlikely be repurposed as a survivin-targeting anticancer drug.

Due to lack of known enzymatic activities and, thus, lack of high throughput assays for survivin, Abbot Laboratories conducted an NMR- and affinity-based screening of their libraries for compounds binding to survivin and identified Abbot 8 that binds to a pocket in survivin, with a Kd of 75 µM, that may affect protein-protein interaction [92]. Via structure-activity analysis, modification of Abbot 8 increased its affinity in binding to survivin. However, Abbot 8 and its analogs were not tested for their activity in suppressing cancer cells or in inhibiting survivin interaction with its binding partners in this initial report.

Using Abbot 8 as a lead compound targeting a site that may affect protein-protein interaction, three compounds (LLP3, LLP6, and LLP9) were synthesized and tested as survivin modulators [93]. While LLP6 has no effect on cancer cells, LLP3 and LLP9 were able to delay mitosis and inhibit proliferation of HUVEC and PC3 cells. When tested against two isogenic glioma cell lines, LLP3 has an IC_{50} of 13.6-38.1 µM [94]. LLP3 was shown to be able to disrupt the interaction between survivin and its binding partner Ran [94], which may be responsible for the anticancer activity of LLP3. Thus, Abbot 8 and the LLP derivatives may work by inhibiting survivin interaction with its binding partners. Additional studies are clearly needed to investigate the fate of survivin following the binding of LLP compounds and survivin release from its binding partners

in the CPC.

Similar to LLP3, S12 was identified as a survivin-targeting molecule capable of binding to a cavity in survivin that may induce conformational changes in the protein structure that disrupt normal functions of survivin [95]. S12 treatment alters microtubule dynamics in cancer cells, resulting in disruption of spindle formation, misalignment of chromosomes during metaphase, and G2/M cell cycle arrest. S12 also inhibits cancer cell proliferation in vitro and suppresses growth of pancreatic xenograft tumors in a dose dependent manner without effecting the overall expression of survivin. In an additional study, S12 also inhibited the proliferation and growth of sonic hedgehog driven medulloblastoma cancer cells by inducing cell cycle arrest and apoptosis [96]. The detailed mechanism of action of S12 and whether it affects survivin interaction with its binding partners is awaiting to be investigated.

In summary, it is clear from the above discussions that the strategy targeting survivin interaction with its binding partners helps circumvent the issues encountered in targeting upstream regulators of survivin expression. This strategy may result in true survivin inhibitors that bind directly to survivin protein. However, limitations also exist with this approach. Firstly, inhibitors such as shepherdin does not bind to and inhibit survivin. Instead, it binds to and inhibits survivin's binding partner Hsp90, which also regulates the stability of other proteins in addition to survivin. Thus, inhibitors such as shepherdin are not specific to survivin and may represent Hsp90 inhibitors. Secondly, inhibitors such as UC-112 bind to the BIR domain, which is a consensus signature domain of IAPs. Thus, it may prove to be challenging to generate a true survivin-selective inhibitor that does not affect other IAPs. Thirdly, it is currently unknown what other or all protein partners of survivin would be affected by the inhibitors such as LLP3 designed to inhibit survivin interaction with other protein partners. Finally, the data on the mechanism of action of this

category of inhibitors, especially LLP compounds and S12, are limited for detailed evaluation. More studies are needed to determine the value of the strategy in targeting survivin interaction with its binding partners.

3. Inhibitors targeting survivin homo-dimerization. Because survivin has no known enzymatic activities but works as a homo-dimer, targeting its homo-dimerization for identifying and developing survivin inhibitors has recently been attempted [8]. This approach not only helps circumvent some of the issues encountered in targeting upstream regulators of survivin expression and result in true survivin inhibitors that bind directly to survivin proteins, it may also help eliminate survivin protein, resulting in spontaneous apoptosis due to survivin elimination. Because the homo-dimerization interface of survivin is unique, the inhibitor to this site is likely to be selective to survivin over other IAPs.

As discussed above, the buried accessible area in the homo-dimeric interface of a monomeric survivin is 550 Å^2 and occupies only 6% of the total accessible area of a monomer (9,044 Å^2) compared with ~20% in most other dimeric or oligomeric proteins [8]. Thus, targeting the homo-dimerization interface to disrupt survivin homo-dimerization would be feasible. Furthermore, disrupting survivin homo-dimerization will lead to exposure of the hydrophobic dimeric interface, which would target the protein to proteasome for degradation. In a recent in-silico screening study targeting the critical dimerization residues following detailed computational analysis of the dimerization interface, a hit molecule, LQZ-7, was identified [8] (see Table 5). LQZ-7 not only was able to specifically disrupt survivin dimerization and inhibit cancer cell survival, it was also able to induce survivin degradation in a proteasome-dependent manner. However, it had no effect on survivin transcription or synthesis nor the expression of other members of the IAP family (unpublished observations).

Further analysis of LQZ-7 analogs led to identification of superior compounds including LQZ-7F that can more effectively disrupt survivin dimerization, cause proteasome-dependent survivin degradation, and inhibit cancer cell survival. The IC_{50} of LQZ-7F against a panel of human cancer cell lines ranged from 0.4-4.4 μM and it induced spontaneous apoptosis in these cancer cells. Using pull-down assay with immobilized LQZ-7F and purified survivin, it was found that LQZ-7 can directly bind to survivin, possibly to the interface for homo-dimerization (Figure 3). LQZ-7F also showed promising in-vivo activity. It effectively inhibited the growth of PC3 xenograft tumors and reduced survivin protein in the xenograft tumors as expected. LQZ-7F was also well tolerated without affecting the body weight of the mice [8]. Future studies of LQZ-7F and other LQZ-7 analogs as well as further optimization may prove fruitful in targeting survivin homo-dimerization for developing novel true survivin-targeting cancer drugs.

Docking LQZ-7 and LQZ-7F in survivin revealed how they interact with the dimerization residues (Figure 3A and 3B). LQZ-7 has three important interactions with survivin via (a) H-bond between an aniline NH group of LQZ-7 and Glu[94] of survivin; (b) interaction between the substituted aniline in LQZ-7 and Phe[93], Phe[101], and Leu[98] in the hydrophobic pocket of survivin via π-π stacking and hydrophobic interaction; and (c) H-bond between the carboxylic acid of LQZ-7 and Trp[10] of survivin. LQZ-7F has two key interactions with survivin via (a) H-bond between the primary amine group of LQZ-7F and Glu[94] of survivin and (b) the tetracyclic furazanopyrazine ring of LQZ-7F interacts with Phe[101], Phe[93], Leu[98], and Trp[10] via π-π stacking and hydrophobic interactions. These interactions may effectively inhibit survivin homo-dimerization.

Based on the above pre-clinical studies, targeting survivin by disrupting its homo-dimerization may be a good strategy going forward. Although it has been shown that LQZ-7 and LQZ-7F bind to survivin, disrupt survivin homo-dimerization, and induce survivin degradation, it

is yet unknown if they truly bind to the homo-dimerization interface of survivin as intended. Since this study is the only one that has shown targeting the homo-dimerization domain of survivin, caution needs to be taken when considering targeting survivin by disrupting its homo-dimerization. Additional independent studies are clearly needed to demonstrate the feasibility of this strategy in targeting survivin and possibly other homo-dimeric proteins for therapeutic discovery. It is also noteworthy that targeting homo-dimerization is more challenging than targeting survivin interaction with other proteins. It will be very perplexing to establish a high throughput assay to investigate and target homo-dimerization. In-silico screening may have to be used for such studies as demonstrated by the study discussed above.

4.   Survivin vaccine and immunotherapy. The fact that survivin is over-expressed in many cancers but not expressed in most adult normal tissues has led many to believe that it may have potential to serve as a cancer vaccine for immunotherapy. Although this concept is relatively new, there have been therapeutics fast tracked to clinical trials (Table 6).

In one study, it was found that an 8-amino acid peptide survivin-2B80-88 (A^{80}YACNTSTL^{88}), derived from survivin 2B, a splicing variant of survivin, binds to HLA-A24 and is recognized by CD8$^+$ cytotoxic T lymphocytes [97]. Cytotoxic T lymphocytes induced by survivin-2B80–88 ex vivo are capable of recognizing and acting on cancer cells expressing HLA-A24 and presenting endogenously processed survivin-2B peptide in tumor cells. Also, HLA-A24$^-$ restricted cytotoxic T lymphocytes from peripheral blood mononuclear cells of HLA-A24$^+$ breast, colorectal, and gastric cancer patients can be induced by the survivin-2B80–88 peptide and they effectively cause cytotoxicity against HLA-A24$^+$ but not HLA-A24$^-$ lung adenoma A549 cell line [98]. These data formed the basis for a phase I clinical trial using the survivin-2B80–88 peptide in patients with advanced or recurrent breast and colorectal cancers [99,100]. Although survivin-

2B80-88 was well tolerated, it unfortunately did not elicit significant clinical response alone or in combination with incomplete Fruend's adjuvant in these patients.

In a similar study, another survivin peptide mimetic SVN53-67/M57-KLH or SurVaxM that can be presented by MHC class I to induce CD8^{+} cytotoxic T lymphocytes [101]. Immunization of C57BL/6 mice with this peptide resulted in rejection of orthotopic GL261 glioma xenografts. SVN53-67/M57 also stimulated cytotoxic T lymphocyte responses against human tumor cells of several different MHC class I haplotypes ex vivo. Currently, two ongoing clinical trials for newly diagnosed glioblastomas and multiple myelomas are actively recruiting participants to test the efficacy of SVN53-67/M57 (https://clinicaltrials.gov).

Cancer immunotherapies targeting survivin certainly offer an exciting approach for treating cancers. The ability to direct the immune system to recognize and kill cancer cells is revolutionizing cancer treatment. While survivin peptides and mimetics have yet to demonstrate significant clinical efficacy in immunotherapy, it is promising that they appear to be well tolerated and do not produce apparent toxicities. Also, it may not induce aberrant immune reactions as some other immunotherapeutics. Clearly, this approach is exciting as it may be a way to produce a robust immune response specifically against cancer cells that overexpress survivin.

Conclusion and Perspectives

Survivin has consistently been demonstrated to be a critical factor in tumor progression and resistance to chemotherapy. The fact that survivin is overexpressed in almost every cancer but not expressed in most adult normal tissues has positioned it as a strong tumor marker with a robust correlation to poor patient prognosis. Despite the limited response of currently available therapeutics targeting survivin in clinical trials, targeting survivin for cancer intervention remains a highly attractive option. The lack of efficacy and dose-limiting toxicities in clinical trials despite

strong pre-clinical data for some inhibitors may be attributable to a deficiency in true survivin-specific inhibition. Strategies that target survivin directly by methods such as immunotherapy or direct homo-dimerization inhibitors may hold the key to overcome some of the inadequacies of previous attempts in targeting survivin moving forward. Considering the fact that inhibiting survivin enhances the apoptotic response of tumor cells induced by chemo or radiotherapies, there remains an opportunity to be exploited for combinational therapy including direct survivin inhibitors and current standard of care treatments. This combinational approach may shed light on potential synergistic effects and may overcome the drug or radiation resistance paradigm in many cancers.

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Conflict of interest

The authors declare no conflict interest.

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Figure Legends

Figure 1. Structure of survivin. **A**. Schematic linear structure of survivin with domains highlighted in different colors. DD, dimerization domain; BIR, Baculovirus Repeat domain; PBD, protein-binding domain; NE, nuclear export. **B**. Atomic structure of survivin (PDB 1F3H) with domains color in correspondence with that shown in the linear structure in panel A. The zinc finger residues (Cys^{57}, Cys^{60}, His^{77}, and Cys^{84}) are shown in ball and stick symbols with the zinc ion shown as a red ball. **C**. Atomic structure of dimeric survivin (PDB 1F3H) with one subunit shown in pink and the other in blue.

Figure 2. Participation of survivin in regulating apoptosis and cell cycle progression. Survivin regulates both cell cycle progression by binding to chromosomal passenger complex in the nucleus and apoptosis by binding to SMAC/DIABLO and caspases in cytoplasm.

Figure 3. Predicted binding modes of LQZ-7 and LQZ-7F in survivin. Both LQZ-7 (A) and LQZ-7F (B) interact with important residues for survivin homo-dimerization including residues Leu^{98} and Phe^{101}.

Table 1. Members of the IAP family

IAP Member	Abbreviation	Mol. Wt.	Reference
Neuronal apoptosis inhibitory protein	NAIP/BIRC1	160 kDa	[102]
Cellular Inhibitor of Apoptosis protein 1	c-IAP1/BIRC2	69 kDa	[103]
Cellular Inhibitor of Apoptosis protein 2	c-IAP2/BIRC3	68 kDa	[103]
X-linked Inhibitor of Apoptosis protein	XIAP/BIRC4	55 kDa	[104]
Survivin	Survivin/BIRC5	16.5 kDa	[105]
BIR repeat-containing ubiquitin conjugating enzyme	BRUCE/BIRC6	528 kDa	[106]
Melanoma IAP/Livin	ML-IAP/livin/BIRC7	33 kDa	[107]
IAP-like protein 2	ILP2/BIRC8	27 kDa	[108]

Table 2. Antisense oligonucleotides suppressing survivin expression

Name	Sequence	Remark	Reference
LY2181308	5'-TGTGCTATTCTGTGAATT-3'	In-vitro IC50 of 10-100 nM in inhibiting survivin expression. Lack of efficacy in phase II trials as a single agent or in combination with docetaxel	[52-56]
EZN-3042	5'-CTCAatccatggCAGc-3'	In-vitro IC50 of 5 nM in inhibiting survivin expression and IC50 of 5-40 µM in cell killing. Phase I trial terminated due to dose-limiting toxicities	[58-60]

Table 3. Small-molecule inhibitors suppressing survivin expression
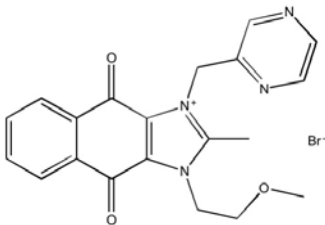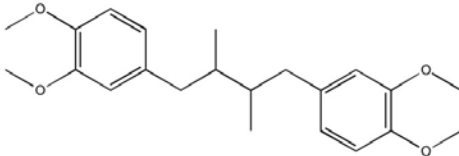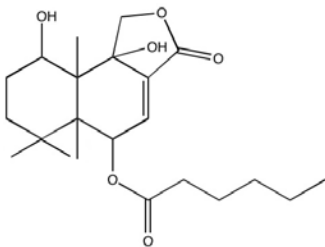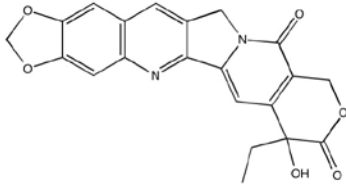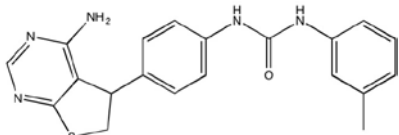
Name	Structure	Remark	Reference
YM155		Inhibitor of Sp1. In-vitro IC50 of ~15 nM. Phase I and II trials with modest efficacy.	[61,62]
EM-1421		Inhibitor of Sp1. In-vitro IC50 of 5-40 µM. Phase I and II trials with partial response.	[71-75]
SF002-96-1		Inhibitor of STAT3 and NF-κB In-vitro IC50 of 3.4 µM in inhibiting survivin promoter activity.	[79]
FL118		In-vitro IC50 of 6-8 nM In-vivo xenograft models of colon and head and neck cancers	[80]
GDP366		In-vitro IC50 of 1 µM (colon cancer cell line) In-vivo xenograft model of colon cancer	[81]

Table 4. Inhibitors targeting survivin interaction with its ligand proteins
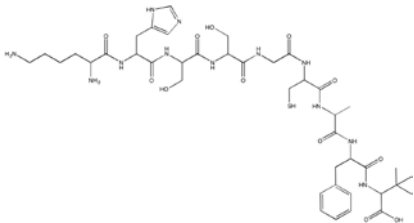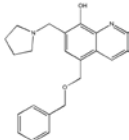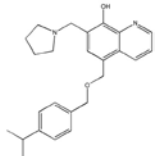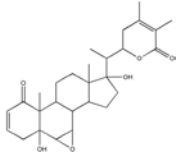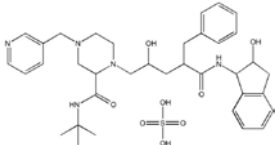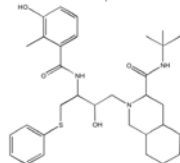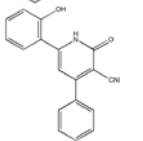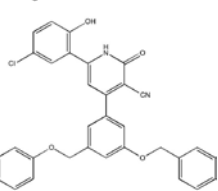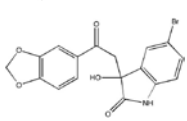
Name	Structure	Remark	Reference
Shepherdin		In-vitro IC50 of 25-75 µM. In-vivo xenograft model of prostate cancer line	[82]
UC-112		In-vitro IC50 of 2.1 (0.7-3.4) µM. In-vivo xenograft models of melanoma	[84]
4g		In-vitro IC50 of 0.5 µM. In-vivo model of melanoma cell line	[84,85]
Withanone		In-silico prediction only. No experimental data	[86-89]
Indinavir		In-vitro IC50 of >500 µM (MDA-MB-231)	[90]
Nelfinavir		Inhibits survivin expression. In-vivo xenograft of meningioma.	[91]
Abbot 8		In-vitro Kd of 75 µM	[92]
LLP3		In-vitro IC50 of 13.6-38.1 µM. In-vivo xenograft model of glioblastoma multiforme	[93,94]
S12		In-vitro IC50 of 6-8 nM (colorectal cancer cells) In-vivo xenograft models of pancreatic and medulloblastoma	[95,96]

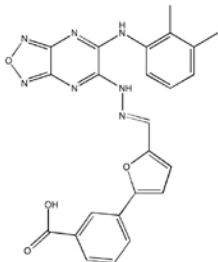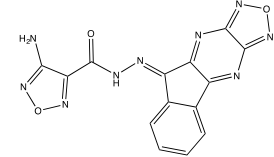**Table 5. Inhibitors targeting homo-dimerization of survivin**

Name	Structure	Remark	Reference
LQZ-7		In-vitro IC50 of ~20 µM for prostate cancer cell lines	[8]
LQZ-7F		In-vitro IC50 of 2.4 (0.4-4.4) µM for multiple cancer cell lines In-vivo xenograft model of prostate cancer	[8]

Table 6. Immunotherapies targeting survivin

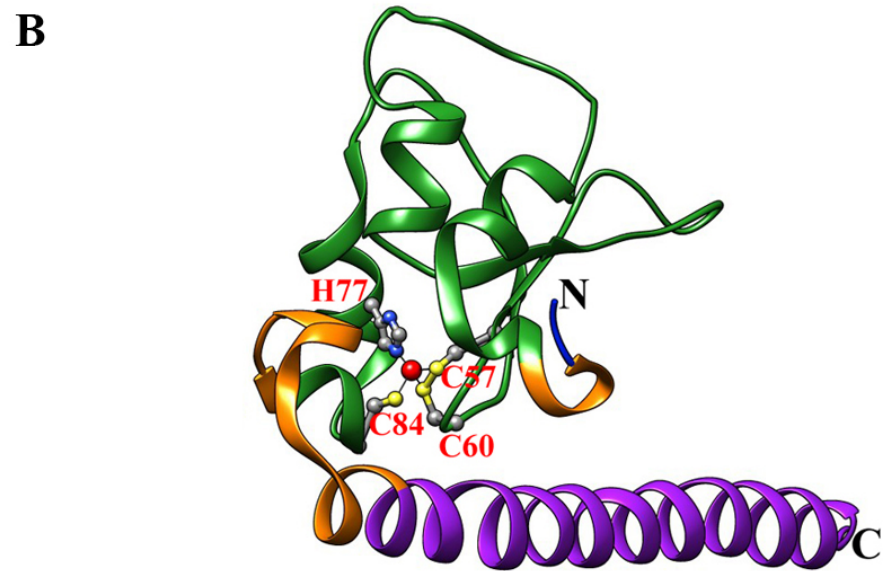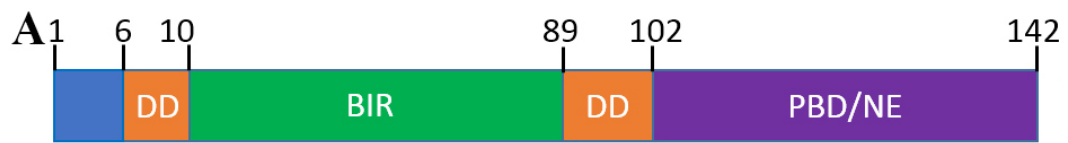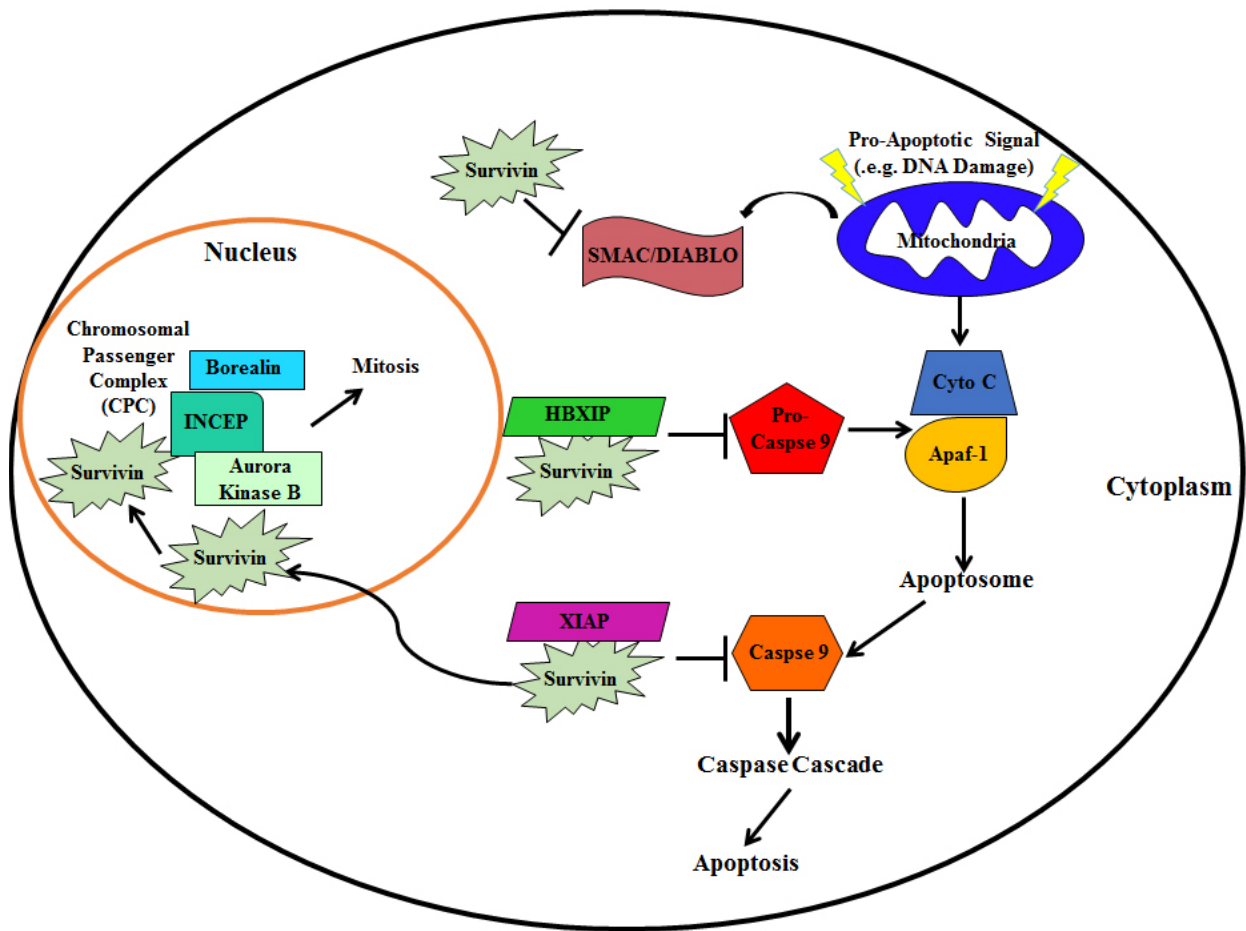| Names	Sequence	Remark	Reference
Sur-2B80-88	AYACNTSTL	Lack of clinical response in phase I trial	[97-100]
SurVaxM	DLAQCFFMFKELEGW	In-vivo glioma xenograft model. Clinical trials ongoing	[101]

Figure 1

Figure 2

Figure 3