IAP proteins as targets for drug development in oncology

Laurence Dubrez1,2
Jean Berthelet1,2
Valérie Glorian1,2
1Institut National de la Santé et de la Recherche Médicale (Inserm), Dijon, France; 2Université de Bourgogne, Dijon, France

Abstract: The inhibitors of apoptosis (IAPs) constitute a family of proteins involved in the regulation of various cellular processes, including cell death, immune and inflammatory responses, cell proliferation, cell differentiation, and cell motility. There is accumulating evidence supporting IAP-targeting in tumors: IAPs regulate various cellular processes that contribute to tumor development, such as cell death, cell proliferation, and cell migration; their expression is increased in a number of human tumor samples, and IAP overexpression has been correlated with tumor growth, and poor prognosis or low response to treatment; and IAP expression can be rapidly induced in response to chemotherapy or radiotherapy because of the presence of an internal ribosome entry site (IRES)-dependent mechanism of translation initiation, which could contribute to resistance to antitumor therapy. The development of IAP antagonists is an important challenge and was subject to intense research over the past decade. Six molecules are currently in clinical trials. This review focuses on the role of IAPs in tumors and the development of IAP-targeting molecules for anticancer therapy.

Keywords: Smac mimetics, apoptosis, antitumor therapy

Introduction: IAP family of proteins

The inhibitors of apoptosis (IAPs) constitute a family of proteins highly conserved throughout evolution. IAPs were initially discovered in baculoviruses two decades ago, as potent inhibitors of apoptosis in infected insect cells. The first human homologous neuronal apoptosis inhibitory protein (NAIP) and cellular IAP 1 and 2 (cIAP1 and cIAP2) were characterized 2 years later, followed by X-chromosome linked IAP (XIAP), survivin, Apollon (also called BRUCE), melanoma IAP (ML-IAP) (also called Livin), and IAP-like protein 2 (ILP2). The IAP family is defined by the presence of one to three conserved protein motifs named a baculoviral IAP repeat (BIR). Most of them form a surface hydrophobic groove that specifically binds a conserved tetrapeptide motif, called IAP binding motif (IBM), found in the active subunits of apoptotic protease caspase-3, -7, and -9 and in cellular IAP antagonists, such as the second mitochondria-derived activator of caspases (Smac) (also named direct IAP-binding protein with low isoelectric point (pI) [DIABLO]) and the high temperature requirement protein A2 (HtrA2) (Figure 1). The first BIR of XIAP and cIAPs does not bind IBM but rather, the signaling molecule transforming growth factor beta (TGFβ)-activated kinase 1-binding protein 1 (TAB1) or the tumor necrosis factor (TNF) receptor (TNFR) associated factors (TRAFs), connecting XIAP and cIAPs with the TGF and TNF signaling pathways, respectively. In addition to the BIRs, cIAPs, XIAP, ML-IAP and ILP2 also possess a C-terminal RING (really interesting new gene)
domain conferring an E3 ligase activity in the ubiquitination or neddylation reactions (for review, 20,21).

Numerous partners of IAPs have been identified, including some caspases, 22–24 some signaling molecules, 25,26 some regulators of the NF-κB: nuclear factor of kappa-light polypeptide gene enhancer in B-cell activating pathways, 27 some regulators of the actin cytoskeleton, 28 and some transcriptional regulators. 29,30 Thus, although they were initially characterized as inhibitors of apoptosis, IAPs display additional nonapoptotic functions in the regulation of cell proliferation, cell division, cell differentiation, cell motility, and in proinflammatory and immune response (for review, 22,23), which could contribute to oncogenesis.

Expression of IAPs in tumors

The expression of IAPs or cellular IAP antagonists such as Smac, 11 HtrA2, or the septin-like mitochondrial protein, ARTS, 30,31 were shown to be altered in a number of human tumor samples (Table 1). Overexpression of IAPs or downregulation of the cellular IAP antagonists have been correlated with advanced progressive disease, aggressiveness, and poor prognosis or low response to treatment (Supplementary Table S1). The alterations of IAP expression can be associated or not, with gene mutations. The baculoviral IAP repeat containing protein (BIRC) 2 and BIRC 3 genes, encoding cIAP1 and cIAP2, respectively, are located on chromosome 11q21-22, a region found amplified in human hepatocarcinoma, 32 mammary carcinoma, 33 medulloblastoma, 34 and in pancreatic, 35 cervical, 36 lung, 37 oral squamous cell, 38 and esophageal 39 carcinomas. Some (30%) mucosa-associated lymphoid tissue (MALT) lymphoma are associated with the chromosomal translocation t(11;18) (q21;q21) generating a chimeric protein composed of the N-terminal sequences of cIAP2 fused to the C-terminal sequence of MALT1. 39,40 Conversely, IAPs can also display antitumoral properties in lymphocytes. The BIRC2 and/or

**Figure 1** The inhibition of caspases by XIAP and the regulation by Smac and Smac mimetics.

**Notes:** (A) Among IAPs, XIAP is a potent caspase inhibitor. XIAP is composed of three BIR domains, one UBA domain (which binds ubiquitin chains), and one C-terminal RING domain, which confers to XIAP an E3-ubiquitin ligase activity. The first BIR (BIR1) can bind to TAB1, connecting XIAP to the TGFβ signaling pathway. The BIR2 and BIR3 contain a surface hydrophobic groove allowing the interaction with IBM found in caspase-3, -7, and -9 active subunits and in IAP antagonists, such as Smac or HtrA2. Moreover, the linker region upstream of BIR2 binds across the substrate binding pocket of caspase-3 and -7, and BIR3 binds the dimer interface of caspase-9, which hinder substrate accessibility and hide the catalytic residue of caspase. Smac is released from the mitochondria into the cytosol during apoptosis, after a maturation process that removes the N-terminal mitochondrial import signal and exposes the IBM to the N-extremity of the protein. Once cytosolic, Smac forms a symmetric dimer and binds the BIR2 and BIR3 IBM grooves of XIAP, preventing them from binding caspases. In a similar manner, monovalent and bivalent Smac mimetics efficiently bind the BIR2 and BIR3 surface hydrophobic grooves and abrogate XIAP-mediated caspase inhibition. (B–D) Comparison of the XIAP-BIR3 (blue) bound to the IBM of caspase-9 (ATPFGQ) (orange) (pdb 1nw9); (B) The IBM (AVPI tetrapeptide) of Smac (red) (pdb 2opz); (C) The monovalent Smac mimetic SM-110 (green) (pdb 2j7); and (D) The BIR domains of IAPs are organized in four α-helices and three β-strand sheets maintained by a zinc ion (yellow). IBMs interact with the surface hydrophobic groove of BIRs (constructed using The PyMOL Molecular Graphics System, Version 1.5.0.4 Schrödinger, LLC, New York, NY, USA).

**Abbreviations:** AVPI, Smac N-terminal tetrapeptide; BIR, baculoviral IAP repeat; HtrA2, high temperature requirement protein A2; IAPs, inhibitors of apoptosis; IBM, IAP binding motif; RING, really interesting new gene; SM, Smac mimetic; Smac, second mitochondria-derived activator of caspases; TAB1, TGFβ-activated kinase 1-binding protein 1; TGFβ, transforming growth factor beta; UBA, ubiquitin associated; XIAP, X-chromosome linked IAP; APAF-1, apoptotic peptidase activating factor.
Table 1 Expression of IAPs and IAP antagonists in human tumors

<table>
<thead>
<tr>
<th>IAPs and cellular IAP antagonists</th>
<th>Tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>XIAP overexpression</td>
<td>Acute myeloid leukemia,136 B-cell chronic lymphocytic leukemia,139,140 bladder carcinoma,141 breast carcinoma,142 cervical carcinoma,143 colorectal cancer,144,145 hepatocarcinoma,146,147 melanoma,148 non-small cell lung cancer,149,150 ovarian cancer,151 prostate carcinoma,152,153 renal carcinoma,154-155 thyroid carcinoma144</td>
</tr>
<tr>
<td>cIAP1 overexpression independently from amplicon 11q21-22</td>
<td>MALT myeloma163,164 Multiple myeloma165,166 colorectal carcinoma,167,168 gastric cancer,169 melanoma,170 neuroblastoma,171 osteosarcoma,172 renal cell carcinoma,173,174 testicular cancer175</td>
</tr>
<tr>
<td>cIAP2 overexpression independently from amplicon 11q21-22</td>
<td>XIAP BIR3 binds the dimer interface of caspase-7, by XIAP, inhibits its activity.19 In addition, XIAP is able to directly inhibit the enzymatic activity of caspases (Figure 1). The XIAP BIR3 binds the dimer interface of caspase-9, and the linker region upstream of BIR2 binds across the substrate binding pocket of caspase-3 and -7, which hinder substrate accessibility and hide the catalytic residue of caspases.47-49 The capacity of XIAP to inhibit caspase activity could account for the resistance of cancer cells to antitumor therapy. Indeed, DNA-damaging treatments, such as ionizing irradiations, induce a translational upregulation of XIAP as a consequence of the presence of an internal ribosome entry site (IRES)-dependent translation mechanism, which results in the resistance of carcinoma cells to radiation-induced apoptosis.50,51 XIAPs can also inhibit cell death at an earlier step, preventing the assembly of caspase-8- or -10-activating platforms. Caspase-8 and -10 are initiator caspases recruited</td>
</tr>
</tbody>
</table>

BIRC3 genes were found to be mutated in some multiple myeloma samples,41,42 and the BIRC4 encoding XIAP in X-linked lymphoproliferative disease.43 The expression and functions of the atypical IAP survivin in tumors, and the development of specific survivin-targeted therapy were recently reviewed by Coumar et al44 and won’t be discussed here.

Role of IAPs in cancer

IAPs as apoptotic regulators

IAPs were first characterized as inhibitors of apoptosis because of their ability to bind caspases. Indeed, cIAPs, XIAP and ML-IAP can bind caspase-3, -7, and -9 via the BIRs and can induce their ubiquitination or neddylation via the RING domain.19,22-24 The influence of the ubiquitination is still not very well established, triggering degradative or nondegradative consequences,22-24 while the neddylation of caspase-7, by XIAP, inhibits its activity.19 In addition, XIAP is able to directly inhibit the enzymatic activity of caspases (Figure 1). The XIAP BIR3 binds the dimer interface of caspase-9, and the linker region upstream of BIR2 binds across the substrate binding pocket of caspase-3 and -7, which hinder substrate accessibility and hide the catalytic residue of caspases.47-49 The capacity of XIAP to inhibit caspase activity could account for the resistance of cancer cells to antitumor therapy. Indeed, DNA-damaging treatments, such as ionizing irradiations, induce a translational upregulation of XIAP as a consequence of the presence of an internal ribosome entry site (IRES)-dependent translation mechanism, which results in the resistance of carcinoma cells to radiation-induced apoptosis.50,51 XIAPs can also inhibit cell death at an earlier step, preventing the assembly of caspase-8- or -10-activating platforms. Caspase-8 and -10 are initiator caspases recruited.
by the adaptor Fas-associated death domain protein (FADD) in multiprotein complexes, which provide the proximity required for caspase homodimerization and self-activation (for review).

These molecular platforms are assembled either in response to the engagement of death receptor from the TNFR superfamily (in which case, these are referred to as death-inducing signaling complex [DISC] and complex II) or in response to genotoxic stress, tumor necrosis factor-like weak inducer of apoptosis (TWEAK) engagement, or toll-like receptor (TLR) 3 stimulation (in which case, they are referred to as Ripoptosome).

Complex II and Ripoptosome; and XIAP inhibits, through a direct interaction via BIR2 and BIR3, the activity of caspase-3, -7, and -9. SMs bind to the BIR domains of cIAPs and stimulate their E3-ubiquitine ligase activity. This results first, in the ubiquitination of RIP1, leading to the canonical NF-κB activation, and second, in the rapid autoubiquitination and subsequent proteasome-mediated degradation of cIAPs. Depletion of cIAPs releases NIK, resulting in the noncanonical activation of NF-κB, and NF-κB target gene expression, including TNFα, MCP-1, and IL-6. TNFα engages TNFR1 via an autocrine pathway. In the absence of cIAP1, stimulation of TNFR1 triggers the assembly of the secondary RIP1-containing cytoplasmic complex (complex II), leading to cell death. SM-mediated IAP depletion can also favor the formation of the Ripoptosome, leading to cell death.

Abbreviations: BIR, baculoviral IAP repeat; cIAP, cellular IAP; IkBα, nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha; IAPs, inhibitors of apoptosis; IL, interleukin; IKK, IkB kinase complex; LUBAC, linear ubiquitin chain assembly complex; MCP-1, monocyte chemoattractant protein; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NIK, NF-κB-inducing kinase; RIP1, receptor interacting protein 1; SM, Smac mimetic; Smac, second mitochondria-derived activator of caspases; TAK, TGFβ-activated kinase; TGFβ, transforming growth factor beta; TNF, tumor necrosis factor; TNFR1, tumor necrosis factor receptor 1; TRADD, TNFR1-associated death domain; TRAF, TNFR associated factor; UBA, ubiquitin proteasome system; XIAP, X-chromosome linked IAP; TAK1, transforming growth factor beta; NIK, nuclear factor-κB (NF-κB) essential modulator; UPS, ubiquitin-proteasome system.

Notes: IAPs are important regulators of NF-κB-activating signaling pathways. Upon TNFα stimulation, cIAPs and RIP1 are recruited to TNFR1 via the adaptors TRADD and TRAF2. cIAPs trigger self-ubiquitination and ubiquitination of RIP1. These ubiquitin chains serve as a scaffold for the recruitment of IKK, TAK, and LUBAC. Once activated, IKK complex triggers the phosphorylation of IκBα, which is then degraded by the UPS and releases NF-κB dimer, which promotes the transcription of target genes. In the noncanonical pathway of NF-κB activation, cIAPs promote the degradative ubiquitination of NIK and associated TRAF proteins and prevent the activation of the IKK complex required for transcription factor activation. On the other hand, cIAPs and XIAP prevent the assembly of RIP1-containing initiator caspase-activating complexes, named complex-II and Ripoptosome; and XIAP inhibits, through a direct interaction via BIR2 and BIR3, the activity of caspase-3, -7, and -9. SMs bind to the BIR domains of cIAPs and stimulate their E3-ubiquitine ligase activity. This results first, in the ubiquitination of RIP1, leading to the canonical NF-κB activation, and second, in the rapid autoubiquitination and subsequent proteasome-mediated degradation of cIAPs. Depletion of cIAPs releases NIK, resulting in the noncanonical activation of NF-κB, and NF-κB target gene expression, including TNFα, MCP-1, and IL-6. TNFα engages TNFR1 via an autocrine pathway. In the absence of cIAP1, stimulation of TNFR1 triggers the assembly of the secondary RIP1-containing cytoplasmic complex (complex II), leading to cell death. SM-mediated IAP depletion can also favor the formation of the Ripoptosome, leading to cell death.
activity) the assembly of the caspase-activating platforms that leads to cell death.\textsuperscript{56,62} (Figure 2). Thus, cIAPs inhibit RIP1-containing caspase-activating platform assembly, either by promoting the ubiquitin-proteasome-mediated degradation of the components of the Ripoptosome\textsuperscript{54} or by inducing a nondegradative ubiquitination of RIP1, which inhibits the cell death complex assembly and promotes survival-signaling pathway transduction.\textsuperscript{56,59,63}

**IAPs as cell-signaling regulators**

The role of IAPs in the regulation of the NF-κB-activating signaling pathways is well documented (for review,\textsuperscript{25,26}). NF-κB is a transcription factor induced by the stimulation of antigen or cytokine receptors, by the recognition of microbiological patterns by the TLRs, the nucleotide-binding oligomerization domain-containing proteins (NODs), or the NOD-like receptors (NLRs), or in response to intracellular injuries, such as DNA damage or reactive oxygen species. NF-κB contributes to the adaptive response of cells, by mediating the expression of the proinflammatory molecules that counter microbial invasion and by promoting the expression of genes involved in cell survival, cell differentiation, and cell proliferation.\textsuperscript{64} The transcription factor consists of heterodimers formed by one Rel subunit (RelA [also called p65], RelB, or c-Rel) and one NF-κB subunit (the p50 subunit of NF-κB1 or the p52 subunit of NF-κB2). In resting cells, the p50/RelA dimer is sequestered into the cytoplasm by the inhibitor of κB (IκB) proteins. Upon stimulation of the cell surface or intracellular receptors, or DNA damage, p50/RelA is released as a consequence of the degradation of NF-kappa-B inhibitor alpha (IκB-α) and translocated into the nucleus to stimulate proinflammatory gene transcription and cell carcinomas and bladder cancers\textsuperscript{36,82,83} (Supplementary Table S1). The influence of IAPs on cell proliferation can be explained by their capacity to stimulate the activity of the c-Myc and E2F1 transcription factors, which are important regulators of cell cycle progression and cell proliferation with oncogenic properties.\textsuperscript{28,29} IAPs have also been involved in the regulation of the invasive properties of mammalian cancer cells, as recently reviewed.\textsuperscript{84}

**Cell proliferation and migration**

cIAPs are positive regulators of cell proliferation, a function correlated with the nuclear localization of the proteins.\textsuperscript{29,81} Interestingly, the nuclear expression of cIAP1 has been associated with advanced disease stages and poor patient prognosis in human cervical and esophageal squamous cell carcinomas and bladder cancers\textsuperscript{36,82,83} (Supplementary Table S1). The influence of IAPs on cell proliferation can be explained by their capacity to stimulate the activity of the c-Myc and E2F1 transcription factors, which are important regulators of cell cycle progression and cell proliferation with oncogenic properties.\textsuperscript{28,29} IAPs have also been involved in the regulation of the invasive properties of mammalian cancer cells, as recently reviewed.\textsuperscript{84}

**Targeting IAPs in cancer therapy**

Targeting IAPs in tumors is an important challenge and several strategies have been explored, including the use of antisense oligonucleotides and antagonist molecules. A synthetic antisense oligonucleotide to XIAP, named AEG35156, was developed by Aegera Therapeutics Inc (Montreal, QC, Canada).\textsuperscript{85} It demonstrated promising efficiency in the preclinical studies. It induced a decrease of XIAP expression in tumor cell lines and tumor xenograft models, and sensitized cells to various standard chemotherapeutic agents and Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand
(TRAIL) receptor agonists. AEG35156 entered into clinical trials (http://www.clinicaltrials.gov/) in 2005, and to date, ten Phase 1, 2, or 1/2 clinical trials have been completed in solid tumors and in acute myeloid leukemia (AML) (Table 2) for review. In the trials, AEG35156 appeared to accumulate in the liver and to have efficiently downregulated XIAP messenger ribonucleic acid (mRNA) in peripheral blood mononuclear cells and hepatocytes. AEG35156 is generally well tolerated except when administered in repeated high doses. Promising results were obtained with AEG35156 used as a single agent in solid tumors and in combination with cytarabine andidarubicin in AML in the Phase 1 studies, but it failed to show any significant antitumoral activity in the randomized Phase 2 studies in pancreatic adenocarcinoma, when combined with Gemcitabine, or in AML, when it was given in combination with cytarabine and idarubicin. The structural characterization of the interaction of XIAP with caspases, or with Smac, or the drosophila Smac homologs has provided very potent tools for the design of synthetic IAP antagonists aiming to inhibit the capacity of XIAP to neutralize caspases. The surface hydrophobic groove of IAP BIRs binds the IBM found in the N-terminal motif of Smac and opens a protein pocket and abrogates XIAP-mediated caspase inhibition. The N-terminal peptide was also derived to produce cell permeable peptides and was shown to mimic the activity of Smac and to sensitize human cancer cell lines to diverse chemotherapeutic agents, including etoposide, teniposide, cisplatin, paclitaxel, 7-ethyl-10-hydroxycamptothecin (SN-38), and TRAIL agonists. In xenograft models, a Smac-derived peptide, made permeable by linking to the shuttle trans-activation of transcription (TAT) from HIV, enhanced the antitumoral effect of TRAIL in glioma, and a polyarginine-conjugated Smac peptide was shown to sensitize non-small cell lung carcinoma cells to cisplatin, with little toxicity to normal tissues. The pharmacological

Table 2 AEG35156 XIAP antisense oligonucleotide in clinical trials (http://www.clinicaltrials.gov/)

<table>
<thead>
<tr>
<th>Phase</th>
<th>Drug combination</th>
<th>Start date</th>
<th>Condition</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase I</td>
<td>Docetaxel</td>
<td>07/2006</td>
<td>Adult solid tumor</td>
<td>• Generally well tolerated</td>
</tr>
<tr>
<td>Phase 1/2</td>
<td>Cytarabine and idarubicin</td>
<td>08/2006</td>
<td>Refractory or relapsed acute myeloid leukemia (24 patients)</td>
<td>• Toxicity included two cases of neuropathy in patients having received multiple AEG35316 doses</td>
</tr>
<tr>
<td>Phase I</td>
<td>Docetaxel</td>
<td>09/2006</td>
<td>Adult solid tumor</td>
<td>• Well tolerated</td>
</tr>
<tr>
<td>Phase I</td>
<td>Single agent</td>
<td>10/2006</td>
<td>Advanced cancer (22 patients)</td>
<td>• Evidence of efficiency (decreased XIAP mRNA in peripheral blood mononuclear cells)</td>
</tr>
<tr>
<td>Phase 1/2</td>
<td>Gemcitabine</td>
<td>11/2007</td>
<td>Metastatic pancreatic adenocarcinoma (14 patients)</td>
<td>• Clinical evidence of antitumoral activity in patients with refractory lymphoma, melanoma, and breast cancer</td>
</tr>
<tr>
<td>Phase 1/2</td>
<td>Paclitaxel</td>
<td>11/2007</td>
<td>Mammary carcinoma</td>
<td>• Toxicities include neutropenia, thrombocytopenia, peripheral neuropathy, fatigue, ascites, and nausea/vomiting</td>
</tr>
<tr>
<td>Phase 1/2</td>
<td>Carboplatin and paclitaxel</td>
<td>11/2007</td>
<td>Non-small cell lung carcinoma</td>
<td>• Failed to show significant antitumoral activity</td>
</tr>
<tr>
<td>Phase 1/2</td>
<td>Single agent</td>
<td>10/2008</td>
<td>Refractory chronic lymphocytic leukemia and indolent B-cell lymphomas</td>
<td>• Well tolerated</td>
</tr>
<tr>
<td>Phase 1/2</td>
<td>Sorafenib</td>
<td>04/2009</td>
<td>Advanced hepatocellular carcinoma</td>
<td>• Did not improve rates of remission</td>
</tr>
<tr>
<td>Phase 2</td>
<td>Cytarabine and idarubicin</td>
<td>11/2009</td>
<td>Refractory or relapsed acute myeloid leukemia (27 patients)</td>
<td>• Generally well tolerated</td>
</tr>
</tbody>
</table>

Abbreviations: RNA, ribonucleic acid; XIAP, X-chromosome linked IAP; IAPs, inhibitors of apoptosis; mRNA, messenger RNA.
properties of such Smac-derived peptides were not good enough to merit consideration of these molecules as therapeutic agents; however, they provided the bases for the structure-based design of IAP antagonists named Smac mimetics (SMs). Several approaches were used, including the screening of peptide or peptidomimetic libraries, and the structure-based design of conformationally constrained SMs (Figure 3). Considerable efforts were invested to improve the affinity of the compounds to the IAP BIR domains, to improve their ability to antagonize IAPs, to improve cellular delivery and activity (ie, their capacity to induce apoptosis or to sensitize to apoptotic agents), and to improve their in vivo stability and bioavailability. The preclinical assays demonstrated their capacity to inhibit tumor growth in multiple solid tumors, acute lymphoblastic leukemia (ALL), and multiple myeloma xenograft models and to sensitize cells to TRAIL, proteasome inhibitors, B-cell lymphoma protein 2 family-targeting compounds, and more conventional therapeutic agents, such as radiation, melphalan, or cisplatin. Importantly, these compounds were well tolerated by animals and did not display toxicity against normal lymphocytes and bone marrow stromal cells or normal mammary epithelial cells. The analysis of binding affinity revealed that similarly to Smac, SMs can bind to XIAP-BIR2, preventing XIAP-caspase-7 and -3 binding, and to XIAP-BIR3, abrogating the XIAP-mediated inhibition of caspase-9. Structural and biochemical studies of the apoptotic activity of Smac cellular protein revealed, first, that it forms a symmetric dimer, and second, that

\[ \text{Smac N-terminal tetrapeptides AVPI} \]

\[ \text{AT-406 (SM-406)} \]

\[ \text{GDC-0152} \]

\[ \text{LCL-161; LCL161} \]

\[ \text{Birinapant (TL-32711)} \]

**Figure 3** Structure of the Smac N-terminal tetrapeptide (AVPI) and SMs used in clinical trials.

**Notes:** AT-406: CAS RN 1071992-99-8; GDC-0152: CAS RN 873652-48-3; LCL161: CAS RN 1005342-46-0; and Birinapant: CAS RN 1260251-31-7.

**Abbreviations:** CAS RN, CAS Registry Number; SM, Smac mimetic; Smac, second mitochondria-derived activator of caspases.
<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Preclinical assays in animals</th>
<th>Clinical trial</th>
<th>Conditions</th>
</tr>
</thead>
</table>
| AT-406 (SM-406 – Debio 1143) | Monovalent | • Inhibited tumor growth and sensitized cells to carboplatin in ovarian cancer xenograft model\(^{119}\)  
• Inhibited tumor growth in breast tumor xenograft model with no sign of toxicity\(^{20}\)  
• Increased chemo- and radiotherapy sensitivity in head and neck squamous cell carcinoma tumor xenograft model\(^{21}\) | Phase 1 | Advanced solid tumors and lymphoma |
| Ascenta therapeutics/debiopharm SA | Monovalent | • Inhibited tumor growth and sensitized cells to carboplatin in ovarian cancer xenograft model\(^{119}\)  
• Inhibited tumor growth in breast tumor xenograft model with no sign of toxicity\(^{20}\)  
• Increased chemo- and radiotherapy sensitivity in head and neck squamous cell carcinoma tumor xenograft model\(^{21}\) | Phase 1 | Combination with daunorubicin and cytarabine in patients with poor-risk acute myelogenous leukemia |
| Birinapant (TL-3271) | Bivalent | • Tumor growth arrest or inhibition in patient-derived primary pancreatic cancer explant model\(^{199}\)  
• Remission in acute lymphoblastic leukemia xenograft models\(^{200}\)  
• Delayed the tumor growth and increases survival in combination with ionizing radiation in a glioblastoma multiforme model in mice\(^{201}\)  
• Inhibited tumor growth in combination with the immunomodulatory agents IFN\(\alpha\) or GM-CSF in a kidney carcinoma xenograft model\(^{202}\)  
• Prevented the tumor growth and increases survival in combination with ionizing radiation in a glioblastoma multiforme model in mice; the effect was not observed in human\(^{203}\) | Phase 1/2 | Combination chemotherapy (doxorubicin, paclitaxel, carboplatin, gemcitabine, irinotecan, docetaxel) in advanced and metastatic solid tumors |
| Birinapant (TL-3271) | Bivalent | • Tumor growth arrest or inhibition in patient-derived primary pancreatic cancer explant model\(^{199}\)  
• Remission in acute lymphoblastic leukemia xenograft models\(^{200}\)  
• Delayed the tumor growth and increases survival in combination with ionizing radiation in a glioblastoma multiforme model in mice\(^{201}\)  
• Inhibited tumor growth in combination with the immunomodulatory agents IFN\(\alpha\) or GM-CSF in a kidney carcinoma xenograft model\(^{202}\)  
• Prevented the tumor growth and increases survival in combination with ionizing radiation in a glioblastoma multiforme model in mice; the effect was not observed in human\(^{203}\) | Phase 1 | Refractory solid tumors or lymphoma |
| GDC-0917 | Monovalent | • Inhibits tumor growth in breast cancer xenograft without affecting normal mammary epithelial cells\(^{204}\)  
• Induces an increased systemic level of cytokines and chemokines (TNF\(\alpha\) and MCP-1), a systemic inflammatory response and hepatic injury when IV administered in dogs; such effects were not observed in human\(^{205}\) | Phase 1 | Well tolerated, no dose limiting toxicities, potent and sustained target inhibition, apoptotic pathway activation in tumor and antitumoral activity in colon cancer and melanoma\(^{206}\) |
| GDC-0152 | Monovalent | • Inhibits tumor growth in breast cancer xenograft without affecting normal mammary epithelial cells\(^{204}\)  
• Induces an increased systemic level of cytokines and chemokines (TNF\(\alpha\) and MCP-1), a systemic inflammatory response and hepatic injury when IV administered in dogs; such effects were not observed in human\(^{205}\) | Phase 1 | Advanced solid tumors and lymphoma |
| HGS1029 (AEG-40826) | Bivalent | • Delays tumor growth in multiple solid tumor xenograft models as a single agent but is ineffective in acute lymphoblastic leukemia xenograft models\(^{108}\)  
• Antitumor activity in combination with chemotherapy against a range of solid tumors including primary models of breast cancer (Novartis website)\(^{9}\)  
• Inhibits tumor growth in combination with a Bcl-2 inhibitor in hepatocellular carcinoma xenograft models\(^{110}\)  
• Inhibits tumor growth and prolongs survival in combination with adenovirus bacteriophage-TNF\(\alpha\) in melanoma xenograft models\(^{111}\) | Phase 1 | Relapsed or refractory lymphoid malignancies |
| LCL161 | Monovalent | • Delays tumor growth in multiple solid tumor xenograft models as a single agent but is ineffective in acute lymphoblastic leukemia xenograft models\(^{108}\)  
• Antitumor activity in combination with chemotherapy against a range of solid tumors including primary models of breast cancer (Novartis website)\(^{9}\)  
• Inhibits tumor growth in combination with a Bcl-2 inhibitor in hepatocellular carcinoma xenograft models\(^{110}\)  
• Inhibits tumor growth and prolongs survival in combination with adenovirus bacteriophage-TNF\(\alpha\) in melanoma xenograft models\(^{111}\) | Phase 1 | Solid tumors |
| Novartis pharmaceuticals | Monovalent | • Delays tumor growth in multiple solid tumor xenograft models as a single agent but is ineffective in acute lymphoblastic leukemia xenograft models\(^{108}\)  
• Antitumor activity in combination with chemotherapy against a range of solid tumors including primary models of breast cancer (Novartis website)\(^{9}\)  
• Inhibits tumor growth in combination with a Bcl-2 inhibitor in hepatocellular carcinoma xenograft models\(^{110}\)  
• Inhibits tumor growth and prolongs survival in combination with adenovirus bacteriophage-TNF\(\alpha\) in melanoma xenograft models\(^{111}\) | Phase 1 | Well tolerated\(^{15}\) |

**Note:**\(^{9}\)Novartis website: [http://www.novartisoncology.us/research/pipeline/fcl161.jsp](http://www.novartisoncology.us/research/pipeline/fcl161.jsp)

**Abbreviations:** GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; IV, intravenous; TNF, tumor necrosis factor; MCP-1, monocyte chemoattractant protein; Smac, second mitochondria-derived activator of caspases; SMs, Smac mimetics; Bcl-2, B-cell lymphoma 2.
dimerization is essential for Smac function; and third, that the capacity of Smac to abrogate XIAP-mediated caspase inhibition required the binding to both BIR2 and BIR3. Overall, these observations support the conclusion that compounds targeting both BIR domains could be more efficient as XIAP antagonists and lead to the development of bivalent small molecules containing two Smac AVPI IBM motif mimetics. As expected, these compounds appeared to be more potent than their monovalent counterparts, in antagonizing XIAP and in activating caspases. Like the monovalent versions, the bivalent molecules either inhibited tumor growth or sensitized cells to both conventional and nonconventional anticancer therapies in the preclinical assays and did not display toxicity to normal human primary cells; however, unlike the monovalent molecules, the bivalent SMs are not orally bioavailable. To date, more than 50 applications for patents related to IAP antagonists have been filed (for review), and six SMs have entered human clinical trials (http://www.clinicaltrials.gov/) for the treatment of cancer (described in Table 3).

Mechanisms of action of SMs
As expected, SMs abrogate XIAP-mediated caspase inhibition and therefore increase caspase-3 and -7 activities (Figure 1). However, in addition to binding XIAP BIRs, SMs also bind the BIR domains of ML-IAP, cIAP1 and cIAP2. SMs stimulate the E3-ubiquitine ligase activity of cIAPs, which results in the ubiquitination of RIP1, leading in turn, to canonical NF-κB activation and the rapid autoubiquitination and subsequent proteasome-mediated degradation of cIAPs. (Figure 2). Depletion of cIAPs abolishes the cIAP-mediated ubiquitination and degradation of NIK and induces canonical activation of NF-κB. In turn, NF-κB induces the expression of proinflammatory cytokines and chemokines, including TNFα, which can trigger cell death by an autocrine pathway. Furthermore, depletion of cIAPs favors the assembly of the RIP1-containing cytoplasmic cell death complexes, such as complex II and Ripoptosome, resulting in cell death in some sensitive cancer cells, or in the sensitization to TNFα or DNA-damaging chemotherapeutic agents. (Figure 2). SMs exert their activity through XIAP and cIAPs and both effects are required for their maximal antitumoral activity. Indeed, IAP antagonists displaying a high and selective affinity for cIAPs over XIAP appeared less potent than pan-IAP antagonists in promoting cancer cell death and in sensitizing cancer cells to TRAIL. As a consequence of cIAP degradation and NF-κB activation, the administration of SMs such as LCL161, GDC-0152, and HGS1029, resulted in the upregulation of cytokines and chemokines, including TNFα, monocyte chemoattractant protein (MCP)-1, interleukin (IL)-7, IL-6, and interferon (IFN)γ. MCP-1 was used as a clinical biomarker for SMs efficiency in clinical programs. The analysis of the proinflammatory characteristics of cellular Smac-induced cell death suggests that the proinflammatory response elicited by SMs could activate the adaptive antitumor immune response in cancers. In dogs, intravenous (IV) administration of GDC-0152 induced an acute systemic inflammatory response with lung and hepatic injury, which are consistent with TNF-α mediated toxicity, however, a similar TNF-α-driven inflammatory response was not observed in humans. Although the first clinical trials did not reveal extensive toxicity of SMs when orally or intravenously administered, additional analysis of the consequences of cytokine and chemokine secretion are required. Because osteoclast differentiation and function are stimulated by activation of the noncanonical NF-κB pathway and because osteoclasts are susceptible to TNF-α-mediated death, Yang et al analyzed the influence of SMs on bone metastasis and demonstrated that SMs stimulated osteoporosis and specifically enhanced metastasis in bone.

Conclusion
SMs are a very promising new class of anticancer therapeutics. Results from preclinical studies have demonstrated an acceptable safety profile and some signs of antitumoral activity, in their use as a single agent or in combination with conventional or nonconventional therapies, such as dead receptor agonists, Bcl-2, or kinase-targeting therapies. The first clinical trials demonstrated a good tolerance and target inhibition. Ongoing and future clinical trials will determine the safety, appropriate indications, and drugs combinations. It will be important to determine the level and the site of production of TNFα and other cytokines and the consequences of cytokine production for tumoral and non-tumoral cells. Since IAPs are involved in the regulation of various cellular functions, it will be interesting to target specific IAP functions in order to limit possible adverse impacts. The consequences of SMs on the immune system in vivo and the use of cIAPs as potential therapeutic targets for inflammatory or immune disorders are still important questions that need to be addressed.
Acknowledgments
Our work is supported by grants from the “Comité de Côte d’Or de la Ligue contre le Cancer,” from the “Association pour la recherche sur le Cancer (ARC),” and from the “Conseil Régional de Bourgogne.” JB received a fellowship from the “Ministère de l’Enseignement Supérieur et de la Recherche” of France, and VG received a fellowship from the “Ligue Nationale contre le Cancer.”

Disclosure
The authors report no conflicts of interest in this work.

References


84. Fulda S. Regulation of cell migration, invasion and metastasis by IAP proteins and their antagonists. Oncogene. [Epub March 11, 2013.]


## Supplementary material

### Table S1 Role of IAPs in cancer

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>XIAP overexpression</strong></td>
<td></td>
</tr>
<tr>
<td>AML</td>
<td>92(^1) Associated with poor cytogenetics(^1)</td>
</tr>
<tr>
<td></td>
<td>78(^2) Inversely correlated with overall survival(^1,2)</td>
</tr>
<tr>
<td></td>
<td>28(^3) Correlated with sensitivity to anticancer drugs (cytarabine)(^3)</td>
</tr>
<tr>
<td>BCLL</td>
<td>100(^1) Correlated with Ki-67 proliferation index and progressive disease; inverse correlation with overall survival(^1)</td>
</tr>
<tr>
<td></td>
<td>301(^1) Associated with poor clinical outcome(^4)</td>
</tr>
<tr>
<td>Bladder carcinoma</td>
<td>176(^1) Independent prognostic factor for early recurrence of invasive cancers</td>
</tr>
<tr>
<td></td>
<td>192(^5) Correlated with poor differentiation</td>
</tr>
<tr>
<td></td>
<td>38(^6) Inversely correlated with recurrence-free survival</td>
</tr>
<tr>
<td>Breast carcinoma</td>
<td>102(^2) Nuclear expression</td>
</tr>
<tr>
<td></td>
<td>55(^1) Independent negative prognostic factor for overall survival</td>
</tr>
<tr>
<td>Cervical carcinoma</td>
<td>77(^1) Cytoplasmic expression</td>
</tr>
<tr>
<td></td>
<td>38(^1) Associated with resistance to irradiation(^1)</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>96(^7) Independent negative prognostic factor</td>
</tr>
<tr>
<td></td>
<td>38(^8) Correlated with tumor dedifferentiation, invasion, stage, and lower disease-free and overall survival(^8)</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>69(^9) Associated with shorter survival(^10,11) and increased risk of relapse and metastasis(^11)</td>
</tr>
<tr>
<td></td>
<td>192(^1) The cytoplasmic expression is an independent negative prognostic factor(^1)</td>
</tr>
<tr>
<td>Melanoma</td>
<td>55(^1) Correlated with advanced tumor stage and inversely correlated with patient survival</td>
</tr>
<tr>
<td>NSCLC</td>
<td>144(^1) Mainly expressed in the cytoplasm</td>
</tr>
<tr>
<td></td>
<td>55(^14) Inversely correlated with proliferation Ki-67 proliferation index</td>
</tr>
<tr>
<td></td>
<td>38(^1) Correlated with resistance to irradiation(^1)</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>AT-406-induced apoptosis is correlated with its ability to downregulate XIAP expression(^15)</td>
</tr>
<tr>
<td>Prostate carcinoma</td>
<td>226,(^16) 69(^17) Deregulation of XIAP occurs early in the pathogenesis of prostate cancer(^17)</td>
</tr>
<tr>
<td></td>
<td>145(^18) Independent predictor of tumor recurrence(^16)</td>
</tr>
<tr>
<td>Renal carcinoma</td>
<td>145(^18) Independent negative prognostic factor(^18)</td>
</tr>
<tr>
<td></td>
<td>66(^19) Correlated with tumor grade and advanced tumor stage(^18,19,20)</td>
</tr>
<tr>
<td></td>
<td>109(^20) Inversely correlated with patient survival(^18,19,20)</td>
</tr>
<tr>
<td>Thyroid carcinoma</td>
<td>72(^21)</td>
</tr>
<tr>
<td>cIAP1 and cIAP2 overexpression associated with amplicon 11q21-22</td>
<td></td>
</tr>
<tr>
<td>Cervical cancer</td>
<td>70(^22) Nuclear expression correlated with low overall survival</td>
</tr>
<tr>
<td>ESC</td>
<td>42(^23) Correlated with resistance to cisplatin/camptothecin</td>
</tr>
<tr>
<td>Hepatocarcinoma</td>
<td>25(^24)</td>
</tr>
<tr>
<td>Mammary carcinoma</td>
<td>15(^25)</td>
</tr>
<tr>
<td>Medulloblastoma</td>
<td>17(^24)</td>
</tr>
<tr>
<td>NSCLC and SCLC</td>
<td>25(^25)</td>
</tr>
<tr>
<td></td>
<td>55(^14) Cytoplasmic expression</td>
</tr>
<tr>
<td></td>
<td>22,(^26) 33(^27) Inversely correlated with patient survival</td>
</tr>
<tr>
<td>Pancreatic cancer</td>
<td>cIAP1 overexpression independent from 11q21-22 amplicon</td>
</tr>
<tr>
<td>AML</td>
<td>Associated with resistance to several anticancer drugs(^2)</td>
</tr>
<tr>
<td>B-cell CLL</td>
<td>22,(^29) 30(^30) Correlated with resistance to irradiation(^30)</td>
</tr>
<tr>
<td></td>
<td>22,(^29) No correlation with fludarabine sensitivity(^31)</td>
</tr>
<tr>
<td>Bladder cancer</td>
<td>102(^25) Nuclear expression correlated with proliferation index (Ki-67), tumor stage, and grade</td>
</tr>
<tr>
<td></td>
<td>Inversely correlated with overall survival and recurrence free-survival</td>
</tr>
<tr>
<td>Cervical carcinoma</td>
<td>70(^22) Nuclear expression</td>
</tr>
<tr>
<td></td>
<td>Correlated with the resistance to irradiation</td>
</tr>
<tr>
<td></td>
<td>Inversely correlated with overall survival and recurrence-free survival</td>
</tr>
<tr>
<td>CLL</td>
<td>100(^1) Correlated with advanced tumor stage</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>46(^31) Nuclear expression</td>
</tr>
</tbody>
</table>

(Continued)
Table S1 (Continued)

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>HNSCC</td>
<td>Nuclear expression correlated with metastasis, advanced stage, and poor patient prognosis</td>
</tr>
<tr>
<td>NSCLC and SCLC</td>
<td>Nuclear expression</td>
</tr>
<tr>
<td>Prostate carcinoma</td>
<td>No correlation with chemotherapy or radiotherapy</td>
</tr>
<tr>
<td>Squamous carcinoma of tongue</td>
<td>Inversely correlated with refractory disease</td>
</tr>
<tr>
<td>cIAP1/cIAP2 inactivation</td>
<td></td>
</tr>
<tr>
<td>Multiple myeloma</td>
<td>Correlated with a better response to treatment (cladribine, cyclophosphamide, fludarabine)</td>
</tr>
<tr>
<td>c-IAP1/ HtrA2</td>
<td></td>
</tr>
<tr>
<td>c-IAP1/Smac DIABLO</td>
<td></td>
</tr>
<tr>
<td>CLL</td>
<td>Correlated with a better response to treatment (cladribine, cyclophosphamide, fludarabine)</td>
</tr>
<tr>
<td>c-IAP2 overexpression independent of t(11q21)</td>
<td></td>
</tr>
<tr>
<td>Breast cancer</td>
<td>144</td>
</tr>
<tr>
<td>Cervical carcinoma</td>
<td>77</td>
</tr>
<tr>
<td>CLL</td>
<td>Associated with progressive disease</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>46</td>
</tr>
<tr>
<td>Prostate carcinoma</td>
<td>69</td>
</tr>
<tr>
<td>ML-IAP overexpression</td>
<td></td>
</tr>
<tr>
<td>AML</td>
<td>Correlated with overall survival</td>
</tr>
<tr>
<td>Adults ALL</td>
<td>Inverse correlation with relapse-free survival and overall survival</td>
</tr>
<tr>
<td>Childhood ALL</td>
<td>Correlated with relapse-free survival</td>
</tr>
<tr>
<td>Bladder cancer</td>
<td>Correlated with relapse-free survival</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>Correlated with resistance to etoposide, vincristine, 5-fluorouracil</td>
</tr>
<tr>
<td>Gastric cancer</td>
<td>40</td>
</tr>
<tr>
<td>Melanoma</td>
<td>Resistance to etoposide</td>
</tr>
<tr>
<td>Neuroblastoma</td>
<td>68</td>
</tr>
<tr>
<td>Osteosarcoma</td>
<td>Associated with MYCN amplification $\rightarrow$ inversely correlated with patient survival</td>
</tr>
<tr>
<td>Renal cell carcinoma</td>
<td>152, 49</td>
</tr>
<tr>
<td>Testicular cancer</td>
<td>131</td>
</tr>
<tr>
<td>Smac downregulation</td>
<td></td>
</tr>
<tr>
<td>AML</td>
<td>Correlated with response to chemotherapy</td>
</tr>
<tr>
<td>Bladder cancer</td>
<td>173</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>Inversely correlated with advanced tumor stage and tumor grade</td>
</tr>
<tr>
<td>Cervical carcinoma</td>
<td>86</td>
</tr>
<tr>
<td>Colorectal carcinoma</td>
<td>Inversely correlated with metastasis and advanced tumor stage Correlated with patient survival</td>
</tr>
<tr>
<td>Endometrioid endometrial cancer</td>
<td>Inversely correlated with tumor grade and correlated with longer disease-specific survival</td>
</tr>
<tr>
<td>Esophageal carcinoma</td>
<td>86</td>
</tr>
<tr>
<td>Lung cancer</td>
<td>88</td>
</tr>
<tr>
<td>Rectal adenocarcinoma</td>
<td>38</td>
</tr>
<tr>
<td>Smac overexpression</td>
<td></td>
</tr>
<tr>
<td>Bladder cancer</td>
<td>75</td>
</tr>
<tr>
<td>Gastric adenocarcinoma</td>
<td>Correlated with advanced tumor stage</td>
</tr>
<tr>
<td>Renal carcinoma</td>
<td>66, 85</td>
</tr>
<tr>
<td>XIAP/ Smac</td>
<td></td>
</tr>
<tr>
<td>Gastric adenocarcinoma</td>
<td>Low XIAP/ Smac ratio</td>
</tr>
<tr>
<td>Renal carcinoma</td>
<td>High XIAP/ Smac ratio</td>
</tr>
</tbody>
</table>

(Continued)
Table S1 (Continued)

<table>
<thead>
<tr>
<th>HtrA2 overexpression</th>
<th>Cohort</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometrial cancer</td>
<td>13941</td>
<td>Nuclear HtrA2 expression is elevated in poorly differentiated lymph node metastatic cancer</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nuclear HtrA2 expression is an independent prognostic factor for endometrial cancer progression-free survival</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>64</td>
<td>Cytoplasmic HtrA2 expression increased in cisplatin-resistant cells</td>
</tr>
<tr>
<td>Prostate carcinoma</td>
<td>105,64 6146</td>
<td>Correlated with tumor grade and dedifferentiation</td>
</tr>
<tr>
<td>Renal carcinoma</td>
<td>8542</td>
<td>Correlated with recurrence-free and tumor-specific survival</td>
</tr>
<tr>
<td>Stomach cancer</td>
<td>6047</td>
<td></td>
</tr>
<tr>
<td>Thyroid cancer</td>
<td>48</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HtrA2 downregulation</th>
<th>Cohort</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometrial cancer</td>
<td>12449</td>
<td></td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>7970</td>
<td>Correlation with tumor grade and higher rate of apoptosis</td>
</tr>
<tr>
<td>ARTS overexpression</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Astrocytoma</td>
<td>7271</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ARTS downregulation</th>
<th>Cohort</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML</td>
<td>72</td>
<td>Resistance to 5-azacytidine</td>
</tr>
</tbody>
</table>

**Abbreviations:** ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; ARTS, sepin-like mitochondrial protein; BCLL, B-cell chronic lymphocytic leukemia; cIAP1, cellular inhibitors of apoptosis; CLL, chronic lymphocytic leukemia; DIABLO, IAP-binding protein with low pi; ESC, esophageal squamous cell carcinoma; HNSCC, head and neck squamous cell carcinomas; HtrA2, high temperature requirement protein A2; IAP, inhibitors of apoptosis; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; MALT, mucosa-associated lymphoid tissue; ML-IAP, melanoma IAP; SLL, small lymphocytic lymphoma; Smac, second mitochondria-derived activator of caspas; XIAP, X-chromosome linked IAP; pl, isoelectric point.

**References**


