RESEARCH HIGHLIGHT

Activation of necroptosis to overcome drug resistance in leukemia

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The understanding of cell death mechanisms is crucial for the development and application of novel anti-cancer therapies to avoid or circumvent drug-resistance in refractory malignancies. Impairment of apoptotic cell death plays a major role in therapy resistance and relapse of acute lymphoblastic leukemia (ALL) patients. Therefore, efforts are being directed at new agents reactivating apoptosis or inducing alternative cell death pathways such as necroptosis, a regulated form of necrosis. In a recent study published in Science Translational Medicine we show that the IAP (inhibitor of apoptosis proteins) inhibitor birinapant potently induces cell death in patient-derived ALL cells *in vitro* and *in vivo* through a receptor-interacting protein kinase 1- (RIP1) dependent mechanism. To define the cell death modality induced downstream of RIP1, we used a multicolor lentiCRISPR approach that allows simultaneous knockout of multiple genes. We observed that apoptosis and necroptosis are induced simultaneously as the inhibition of both pathways is required to restore cell viability upon birinapant treatment. This induction of dual cell death makes birinapant and other IAP inhibitors interesting agents for the treatment of refractory or drug resistant malignancies.

Keywords: Necroptosis; drug resistance; receptor-interacting protein kinase 1; acute lymphoblastic leukemia

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Eliminating cancer cells is the ultimate goal of standard chemotherapies, radiotherapy, novel targeted therapies and in general all anticancer treatments. Most of those therapies rely on the induction of regulated cell death. Therefore, the understanding of programmed cell death pathways and the mechanisms by which they are induced and regulated is key for the development of such treatments.

Apoptosis is the best described pathway of programmed cell death. It is morphologically characterized by a decrease of the cellular volume, mitochondrial outer membrane permeabilization (MOMP), chromatin condensation, nuclear fragmentation as well as membrane blebbing. It can be classified as intrinsic or extrinsic depending on the signaling cascade mediating it. Intrinsic apoptosis can be induced by various intracellular stress signals, and it is mediated by the disruption of the balance of pro- and anti-apoptotic BCL2 family proteins. This leads to MOMP, the formation of a caspase-9 complex (termed apoptosome) and subsequent caspase-3 activation. On the other hand, extrinsic apoptosis is induced by the activation of death receptors by their extracellular ligands. This leads then to the recruitment of an intracellular death-inducing signaling complex (DISC) which activates the initiator caspases -8 and -10, which subsequently activate the effector caspases -3 and -7. The molecular mechanisms of apoptosis have been reviewed

extensively [1-3].

Evasion of apoptosis is one of the hallmarks of cancer and is tightly linked to drug resistance, therapy failure and relapse. Apoptosis resistance is multifactorial, hence various mechanisms have been described by which cancer cells can escape apoptotic cell death. Some relevant escape mechanisms include de-regulation of the pro- and anti-apoptotic BCL2 family proteins, compensatory activation of autophagy (a regulated process of degradation and recycling of cellular components), and defects on death receptor signaling [4-6]. The inability of malignant cells to undergo apoptosis represents a major clinical challenge for the treatment of childhood acute lymphoblastic leukemia (ALL) among many other malignancies. Despite the increase in the long-term survival achieved in the last decades, around 20% of ALL patients show a poor clinical response to the frontline treatments and suffer relapse. Relapsed patients experience a dismal outcome. Because most anticancer drugs, including the frontline agents used for the treatment of ALL (vincristine, dexamethasone and L-asparaginase, short VXL), act through the induction of apoptosis in cancer cells, defects in the apoptotic pathway can confer resistance to those compounds [7]. Besides classical chemotherapeutic drugs, apoptosis impairment also plays a role in the resistance to many novel targeted therapies. For instance, BCR/ABL+ leukemias can become resistant to the BCR/ABL inhibitor imatinib by modulating the pro- and anti-apoptotic balance [8]. Since the overexpression of anti-apoptotic factors is a common resistance mechanism for many drugs in various malignancies, numerous efforts have been directed to the development of drugs that can restore apoptotic cell death. This is the case of the small molecule BH3 mimetics, which target one or more anti-apoptotic proteins, and have been shown to have strong activity against many cancer entities, especially hematologic malignancies [8-11]. Other drugs designed to restore the apoptotic response include the inhibitors and the inhibitors of the IAPs (inhibitor of apoptosis proteins) called SMAC (second mitochondrial activator of caspases) mimetics (SM) [12].

However, as a general strategy, directly targeting the defects in the apoptotic machinery to reactivate apoptotic cell death is potentially problematic. Given the polyclonality of many cancers, it is likely that different clones dysregulate apoptosis through different mechanisms. This renders several targets responsible for the drug resistance [6]. We and others hypothesize that simultaneously activating alternative cell death pathways independent of apoptosis may be an effective approach to target and overcome resistance.

Necroptosis, or programmed necrosis, is a form of regulated cell death independent on the apoptotic machinery. Its activation may thus represent a promising alternative to induce cell death in resistant cancer cells. Necroptotic cells exhibit specific morphological features such as increase of the cellular volume, swelling of the mitochondria and rupture of the plasma membrane [1, 13]. Mechanistically, necroptosis can be triggered by various stimuli including ligation of death receptors such as TNFR1. After ligation of TNFR1, the activation of necroptosis depends on the cellular context and has been mainly studied in conditions of experimental apoptosis blockade [1, 14, 15]. After TNFR1 activation, multiple proteins are recruited to its intracellular domain, including RIP1, a central player in the control of cell survival, apoptosis and necroptosis. The switch of RIP1 from a pro-survival to a pro-death function is controlled, among others, through its ubiquitination by cIAPs, which ubiquitinate RIP1 to maintain its pro-survival state [16-18]. Upon deubiquitination, RIP1 can form a cytosolic complex with RIP3, FADD (Fas associated death domain) and caspase-8 and initiate apoptosis through caspase-8 activation or necroptosis through RIP1 and RIP3 phosphorylation. For the final execution of necroptosis, RIP3 phosphorylates MLKL (mixed lineage kinase-domain like) which translocates to and disrupts the plasma membrane [19-21]. The molecular mechanisms of necroptosis have been reviewed in detail elsewhere [1, 15, 16].

Since necroptosis can induce cell death bypassing the apoptosis blockade, various efforts are being directed to the development of necroptosis-inducing compounds for the treatment of drug resistant cancer. One of such compounds is the small molecule obatoclax (GX15-070). We previously described that obatoclax sensitizes ALL cell lines and refractory patient-derived cells to dexamethasone *in vitro* and *in vivo* by inducing autophagy and RIP1 dependent necroptosis [22]. Another class of compounds with potential cytotoxic activity in apoptosis resistant cancer cells are SM. These small molecule peptidomimetics target one or more IAPs (mainly cIAP1, cIAP2 and XIAP), which are overexpressed in certain cancers including leukemias [23, 24], and have been shown to facilitate cancer cell survival by inducing constitutive RIP1 ubiquitination in cancer cells [18]. While cIAP1 and cIAP2 are responsible for ubiquitinating RIP1, thus maintaining its pro-survival function [17, 18], XIAP can bind and directly inhibit caspases -3, -7 and -9 [25-27]. Therefore, SM can lead on the one side to the liberation of effector caspases, and on the other side to the deubiquitination of RIP1 with subsequent formation of cell death complexes. SM can act as chemosensitizers and synergize with a variety of compounds including death receptor ligands such as TNFα, cytotoxic drugs or radiotherapy [28-32]. Synergy has also been reported for SM with glucocorticoids in ALL [33], with the targeted therapies against *BCR/ABL*- (nilotinib) and *FLT3*-positive leukemias

(PKC412) [34], and with p38 or MK2 inhibitors in AML (acute myeloid leukemia) [35]. Although generally more effective in combination, some SM such as BV6, LCL161 and birinapant have been described to also induce cell death as single agents in various cancer cell lines including various subtypes of leukemia [30, 32, 34, 36].

We assessed the single agent activity of the SM birinapant and LCL161 both *in vitro* and *in vivo* in a broad range of patient-derived pediatric B- and T-ALL samples with a special emphasis on refractory and relapse cases [37]. Birinapant potently induced cell death in around 30% of B-ALL cases at concentrations in the low nanomolar range and was less potent against T-ALL. The response to LCL161, which has been found by others to induce insufficient responses in B-ALL [38], was remarkably less potent *in vitro* and ineffective *in vivo*. The structural differences between birinapant and LCL161 might account for the observed differences in activity. Other groups have shown similar promising results for SM in other subtypes of leukemia. Richmond *et al*. have shown that Philadelphia-like ALL samples have a particularly high sensitivity to birinapant compared to other subtypes of ALL. Furthermore, birinapant enhanced the response of patient-derived samples *in vivo* to the clinical chemotherapeutic regiment VXL [39]. Similarly, Brumatti *et al*. show that birinapant effectively kills primary cells harboring the MLL (myeloid/lymphoid or mixed-lineage leukemia 1) translocation in AML mouse models and patient-derived AML samples at concentrations in the nanomolar range [40]. A different SM, BV6, has also been shown to be effective against primary MLL [41].

We used lentiCRISPR (clustered regularly interspaced short palindromic repeats) technology to show that the activity of birinapant is completely dependent on RIP1 *in vitro* and *in vivo*. Patient-derived ALL cells were transduced with lentiCRISPR targeting RIP1 and carrying the fluorescent marker EGFP. We then xenotransplanted these ALL cells into immunodeficient NSG (NOD scid IL2 receptor gamma null) mice and monitored the expansion of WT (EGFP-negative) and knockout (EGFP-positive) cells under treatment with birinapant. We observed a strong selection of the RIP1 deficient cells *in vivo* and confirmed also *in vitro* their resistance to SM. The loss of RIP1 did not affect the engraftment kinetics of patient-derived B-ALL *in vivo* [37].

To further investigate the downstream cell death mechanism induced by birinapant in this context, we developed a multicolor lentiCRISPR approach to target multiple cell death genes at the same time. We simultaneously transduced patient-derived cells with lentiCRISPR targeting RIP3, caspase-8, FADD or MLKL, each expressing a different fluorescent marker, which allowed us to monitor the expansion of single, double, triple and quadruple knockout cells under birinapant treatment *in vivo*. Knockout of both an apoptotic (FADD or caspase-8) and a necroptotic (RIP3 or MLKL) gene was necessary to rescue cell viability, and targeting either pathway alone was not sufficient to achieve resistance. In contrast with many studies showing that caspase inhibition is necessary to induce necroptosis, we found that apoptosis and necroptosis were simultaneously activated in around 40% of birinapant-sensitive B-ALL patient-derived samples and in the Jurkat cell line [37]. However, this mixed cell death phenotype was not observed in response to birinapant treatment in AML, where the co-treatment with the pan-caspase inhibitor Z-VAD or the clinical caspase-8 inhibitor emricasan actually increased the response to birinapant by TNFR1-dependent necroptosis. Interestingly, the cell death induced by birinapant+ZVAD in that context was not rescued by neither RIP3 nor MLKL deficiency [40]. This together with our observation that around 20% of birinapant-sensitive B-ALL cases are not rescued by the combined inhibition of apoptosis and necroptosis indicate that other uncharacterized cell death mechanisms besides necroptosis and apoptosis might be activated by RIP1 after SM treatment.

Considering that the current clinical challenge in the treatment of ALL is the appearance of drug resistance and relapse, we wanted to evaluate if birinapant would still be active against heavily pre-treated refractory and relapsed samples. To answer this question we screened CRISPR-generated RIP1 knockout patient-derived B-ALL cells for their response to a panel of relevant anti-leukemic drugs and found that RIP1 is not required for the response to drugs such as vincristine, dexamethasone or doxorubicin among others, indicating that there would be no mutational pressure on RIP1 which could be selected for during the treatment in refractory and relapse patients. Additionally, our initial drug screen included 11 samples from relapse cases, 6 out of which showed IC50s lower than 500nM [37]. Similar conclusions were derived from the studies of Richmond *et al*., who observed that birinapant was still effective in reducing the leukemic burden *in vivo* in patient-derived xenografts that had relapsed after pre-treatment with birinapant or VXL [39].

In contrast to the potent activity observed *in vitro* and *in vivo* against hematologic malignancies, no anti-tumor activity of birinapant as a single agent could be detected in the phase 2 clinical trial for platinum-resistant and -refractory ovarian cancer [42]. Single agent activity of SM seems to be cancer dependent, with hematologic malignancies

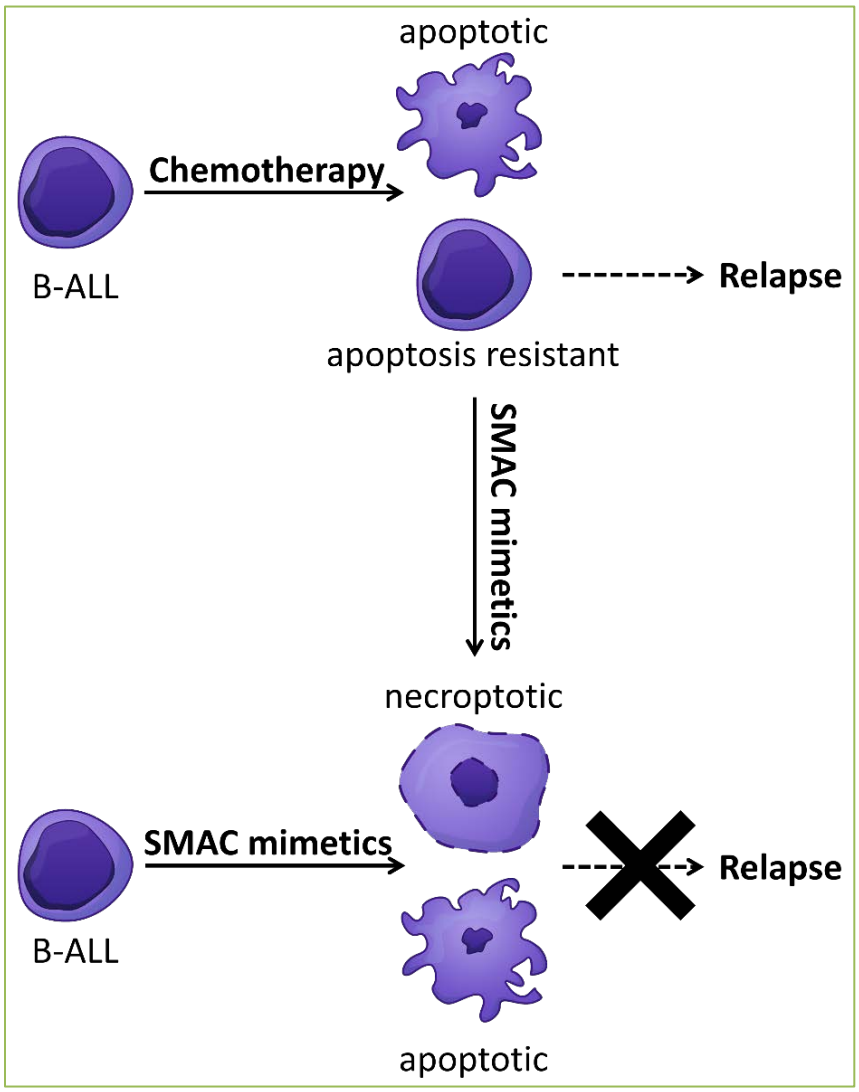

Figure 1. SMAC mimetics induce concurrent apoptosis and necroptosis in precursor B-ALL and are therefore active against apoptosis-resistant cells

representing a subgroup with enhanced sensitivity. Therefore, establishing molecular biomarkers that predict a response to SM is of great importance and many scientists are pursuing this goal. In many cases, especially in the context of cell lines, the production of TNFα in the basal state or after treatment with SM has been shown to correlate with or be necessary for the induction of cell death. In a similar direction, the response to SM has been shown to be dependent on TNFR1 or the TNFR1 signaling pathway, since either treatment with TNFR1 blocking antibodies or TNFR1 knockout confer resistance to SM, and it's expression has been found to be higher in SM sensitive cases [40, 41]. However, we and others have found that either TNFα blockade or TNFR1 targeting were not sufficient to rescue from SM-induced cell death [29, 33, 37, 43, 44]. Furthermore, most of the patient-derived B-ALL samples that we tested had very low expression of TNFα both before and after treatment, with no differences between birinapant-responders and -non responders. Other biomarkers of response to SM have been proposed, including cellular levels and activity of caspases-8 and -10 [45, 46], the ability to form the ripoptosome in response to treatment [47] or cIAP2 upregulation or stabilization [48, 49].

In conclusion, to achieve success in the treatment of cancer, resistance to cell death has to be avoided or overcome. Directly targeting the causes of apoptosis blockade or inducing alternative mechanisms of cell death such as necroptosis are two attractive strategies that have been developed to target such resistance, and SM are compounds which can do both. Our data suggest that the SM birinapant has clinical potential for the treatment of refractory and relapsed ALL by inducing apoptosis and necroptosis in parallel. Simultaneously activating two cell death mechanisms, either by a single compound or by a

combination, may be beneficial for cancer therapy by impairing the appearance of resistance mechanisms (Figure 1). Clearly, a deeper understanding of the molecular mechanisms that govern cell death responses, such as necroptosis, as well as the identification of biomarkers that indicate activation of the pathway will be required to move clinical application of compounds such as SMAC mimetics forward.

Conflicting interests

The authors have declared that no conflict of interests exist.

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Author Contributions

J.A.G and B.C.B. jointly wrote the manuscript. Both authors read and approved the final version of the manuscript.

Abbreviations

ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; BCL-2: B-cell lymphoma 2; BH3: BCL-2 homolog domain 3; BCR/ABL: breakpoint cluster region/Abelson; CRISPR: clustered regularly interspaced short palindromic repeats; DISC: death inducing signaling complex; EGFP: enhanced green fluorescent protein; FADD: Fas associated death domain; FLT3: fms like tyrosine kinase; IAP: inhibitor of apoptosis proteins; IL2: interleukin 2; MK2: mitogen-activated proteins kinase (MAPK)-activated protein kinase-2; MLKL: mixed lineage kinase like; MLL: myeloid/lymphoid or mixed-lineage leukemia 1; MOMP: mitochondrial outer membrane permeabilization; Nec: necrostatin-1; NSG: NOD/Scid IL2 receptor gamma null; RIP1: receptor interacting protein kinase 1; RIP3: receptor interacting protein kinase 3; SMAC: second mitochondria-derived activator of caspases; SM: SMAC mimetics; TNFα: tumor necrosis factor α; TNFR1: TNF receptor 1; XIAP: X-linked IAP.

References

1. Vandenabeele P, Galluzzi L, Vanden Berghe T, Kroemer G. Molecular mechanisms of necroptosis: an ordered cellular explosion. Nat. Rev. Mol. Cell Biol 2010; 11: 700-714.
2. Galluzzi L, Vitale I, Abrams JM, Alnemri ES, Baehrecke EH, Blagosklonny MV, *et al*. Molecular definitions of cell death subroutines: recommendations of the Nomenclature Committee on Cell Death 2012. Cell Death Differ 2012; 19: 107-120.
3. Chipuk J E, Moldoveanu T, Llambi F, Parsons MJ, Green DR. The BCL-2 Family Reunion. Mol. Cell 2010; 37: 299-310.
4. Hu X, Xuan Y. Bypassing cancer drug resistance by activating multiple death pathways - A proposal from the study of circumventing cancer drug resistance by induction of necroptosis. Cancer Lett 2008; 259: 127-137.
5. Mohammad RM, Muqbil I, Lowe L, Yedjou C, Hsu HY, Lin LT, *et al*. Broad targeting of resistance to apoptosis in cancer. Semin. Cancer Biol 2015; 35: Supplement, S78-S103.
6. Igney FH, Krammer PH. Death and anti-death: tumour resistance to apoptosis. Nat. Rev. Cancer 2002; 2: 277-288.
7. Holleman A, den Boer ML, Kazemier KM, Janka-Schaub GE, Pieters R. Resistance to different classes of drugs is associated with impaired apoptosis in childhood acute lymphoblastic leukemia. Blood 2003; 102: 4541-4546.
8. Kuroda J, Pathalakath H, Cragg MS, Kelly PN, Bouillet P, Huang DCS, *et al*. Bim and Bad mediate imatinib-induced killing of Bcr/Abl+ leukemic cells, and resistance due to their loss is overcome by a BH3 mimetic. Proc. Natl. Acad. Sci 2006; 103: 14907-14912.
9. Kang MH, Kang YH, Szymanska B, Wilczynska-Kalak U, Sheard MA, Harned TM, *et al*. Activity of vincristine, L-ASP, and dexamethasone against acute lymphoblastic leukemia is enhanced by the BH3-mimetic ABT-737 in vitro and in vivo. Blood 2007; 110: 2057-2066.
10. Fischer U, Forster M, Rinaldi A, Risch T, Sungalee S, Warnatz HJ, *et al*. Genomics and drug profiling of fatal TCF3-HLF-positive acute lymphoblastic leukemia identifies recurrent mutation patterns and therapeutic options. Nat. Genet 2015; 47: 1020-1029.
11. Sarosiek KA, Letai A. Directly targeting the mitochondrial pathway of apoptosis for cancer therapy using BH3 mimetics - recent successes, current challenges and future promise. FEBS J. 2016; doi:10.1111/febs.13714
12. Fulda S. Smac mimetics as IAP antagonists. Semin. Cell Dev. Biol 2014; 39: 132-138.
13. Vanden Berghe T, Grootjans S, Groosens V, Dondelinger Y, Krysko DV, Takahashi N, *et al*. Determination of apoptotic and necrotic cell death in vitro and in vivo. Methods 2013; 61: 117-129.
14. Hitomi J, Christofferson DE, Ng A, Yao J, Degterev A, Xavier RJ, *et al*. Identification of a Molecular Signaling Network that Regulates a Cellular Necrotic Cell Death Pathway. Cell 2008; 135: 1311-1323.
15. Wu W, Liu P, Li J. Necroptosis: An emerging form of programmed cell death. Crit. Rev. Oncol. Hematol. 2012: 82: 249-258.
16. Christofferson DE, Li Y, Yuan J. Control of Life-or-Death Decisions by RIP1 Kinase. Annu. Rev. Physiol 2013; 76: 129-150.
17. Li H, Kobayashi M, Blonska M, You Y, Lin X. Ubiquitination of RIP is required for tumor necrosis factor alpha-induced

NF-kappaB activation. J. Biol. Chem 2006; 281: 13636-13643.

18. Bertrand MJM, Milutinovic S, Dickson KM, Ho WC, Boudreault A, Durkin J, *et al*. cIAP1 and cIAP2 Facilitate Cancer Cell Survival by Functioning as E3 Ligases that Promote RIP1 Ubiquitination. Mol. Cell 2008; 30: 689-700.
19. Cai Z, Jitakew S, Zhao J, Chiang HC, Choski S, Liu J, *et al*. Plasma membrane translocation of trimerized MLKL protein is required for TNF-induced necroptosis. Nat. Cell Biol 2014; 16: 55-65.
20. Murphy JM, Czabotar PE, Hilderbrand JM, Lucet IS, Zhang JG, Alvarez-Diaz S, *et al*. The pseudokinase MLKL mediates necroptosis via a molecular switch mechanism. Immunity 2013; 39: 443-453.
21. Hildebrand JM, Tanzer MC, Lucet IS, Young SN, Spall SK, Sharma P, *et al*. Activation of the pseudokinase MLKL unleashes the four-helix bundle domain to induce membrane localization and necroptotic cell death. Proc. Natl. Acad. Sci. U. S. A. 2014; 111: 15072-15077.
22. Bonapace L, Bornhauser BC, Schmitz M, Cario G, Ziegler U, Niggli FK, *et al*. Induction of autophagy-dependent necroptosis is required for childhood acute lymphoblastic leukemia cells to overcome glucocorticoid resistance. J. Clin. Invest 2012; 120: 1310-1323.
23. Notarbartolo M, Cervello M, Poma P, Dusonchet L, Meli M, D'Alessandro N. Expression of the IAPs in multidrug resistant tumor cells. Oncol Rep 2004; 11: 133-136.
24. Fulda, S. Inhibitor of Apoptosis (IAP) proteins in hematological malignancies: molecular mechanisms and therapeutic opportunities. Leukemia 2014; 28: 1414-1422.
25. Chai J, Shiozaki E, Srinivasula SM, Wu Q, Dataa P, Alnemri ES, *et al*. Structural Basis of Caspase-7 Inhibition by XIAP. Cell 2001; 104: 769-780.
26. Riedl SJ, Renatus M, Schwarzenbacher R, Zhou Q, Sun C, Fesik SW, *et al*. Structural Basis for the Inhibition of Caspase-3 by XIAP. Cell 2001; 104: 791-800.
27. Srinivasula SM, Hedge R, Saleh A, Datta P, Shiozaki E, Chai J, *et al*. A conserved XIAP-interaction motif in caspase-9 and Smac/DIABLO regulates caspase activity and apoptosis. Nature 2001; 410: 112-116.
28. Fulda S, Vucic D. Targeting IAP proteins for therapeutic intervention in cancer. Nat. Rev. Drug Discov 2012; 11: 109-124.
29. Allensworth JL, Sauer SJ, Lyerly HK, Morse MA, Devi GR. Smac mimetic Birinapant induces apoptosis and enhances TRAIL potency in inflammatory breast cancer cells in an IAP-dependent and TNF-α-independent mechanism. Breast Cancer Res. Treat 2013; 137: 359-371.
30. Vince JE, Wong WWL, Khan N, Feltham R, Chau D, Ahmed AU, *et al*. IAP Antagonists Target cIAP1 to Induce TNFα-Dependent Apoptosis. Cell 2007; 131: 682-693.
31. Roesler S, Eckhardt I, Wolf S, Fulda S. Cooperative TRAIL production mediates IFNα/Smac mimetic-induced cell death in TNFα-resistant solid cancer cells. Oncotarget 2016; doi:10.18632/oncotarget.6915
32. Benetatos CA, Mitsuuchi Y, Burns JM, Neiman EM, Condon SM, Yu G, *et al*. Birinapant (TL32711), a Bivalent SMAC Mimetic, Targets TRAF2-Associated cIAPs, Abrogates TNF-Induced NF- B Activation, and Is Active in Patient-Derived Xenograft Models. Mol. Cancer Ther 2014; 13: 867-879.
33. Belz K, Schoeneberger H, Wehner S, Weigert A, Bönig H, Klingebiel T, *et al*. Smac mimetic and glucocorticoids synergize to induce apoptosis in childhood ALL by promoting ripoptosome assembly. Blood 2014; 124: 240-250.
34. Weisberg E, Ray A, Barret R, Nelson E, Christine AL, Porter D, *et al*. Smac mimetics: implications for enhancement of targeted therapies in leukemia. Leukemia 2010; 24: 2100-2109.
35. Lalaoui N, Hänggi K, Brumatti G, Chau D, Nguyen NYN, Vasilikos L, *et al*. Targeting p38 or MK2 Enhances the Anti-Leukemic Activity of Smac-Mimetics. Cancer Cell 2016; 29: 145-158.
36. Schirmer M, Trentin L, Queudeville M, Seyfried F, Demir S, Tausch E, *et al*. Intrinsic and chemo-sensitizing activity of SMAC-mimetics on high-risk childhood acute lymphoblastic leukemia. Cell Death Dis 2016; 7: e2052.
37. McComb S, Aguadé-Gorgorió J, Harder L, Marovca B, Cario G, Eckert C, *et al*. Activation of concurrent apoptosis and necroptosis by SMAC mimetics for the treatment of refractory and relapsed ALL. Sci. Transl. Med 2016; 8: 339ra70.
38. Houghton PJ, Kang MH, Reynolds CP, Morton CL, Kolb EA, Gorlick R, *et al*. Initial testing (stage 1) of LCL161, a SMAC mimetic, by the pediatric preclinical testing program. Pediatr. Blood Cancer 2012; 58: 636-639.
39. Richmond J, Robbins A, Evans K, Beck D, Kurmansheva RT, Billups CA, *et al*. Acute sensitivity of Ph-like acute lymphoblastic leukemia to the SMAC-mimetic birinapant. Cancer Res 2016; doi:10.1158/0008-5472.CAN-16-0523
40. Brumatti G, Ma C, Lalaoui N, Nguyen NY, Navarro M, Tanzer MC, *et al*. The caspase-8 inhibitor emricasan combines with the SMAC mimetic birinapant to induce necroptosis and treat acute myeloid leukemia. Sci. Transl. Med 2016; 8: 339ra69.
41. Lueck SC, Russ AC, Botzenhardt U, Schlenk RF, Zobel K, Deshayes K, *et al*. Smac mimetic induces cell death in a large proportion of primary acute myeloid leukemia samples, which correlates with defined molecular markers. Oncotarget 2016; doi:10.18632/oncotarget.10390
42. Noonan AM, Bunch KP, Chen JQ, Herrmann MA, Lee JM, Kohn EC, *et al*. Pharmacodynamic markers and clinical results from the phase 2 study of the SMAC mimetic birinapant in women with relapsed platinum-resistant or -refractory epithelial ovarian cancer. Cancer 588-597 2015; 122: 588-597.
43. Eckhardt I, Roesler S, Fulda S. Identification of DR5 as a critical, NF-κB-regulated mediator of Smac-induced apoptosis. Cell Death Dis. 2013; 4: e936.
44. Greer RM, Peyton M, Larsen JE, Girard L, Xie Y, Gazdar AF, *et al*. SMAC Mimetic (JP1201) Sensitizes Non-Small Cell Lung Cancers to Multiple Chemotherapy Agents in an IAP-Dependent but TNF-α-Independent Manner. Cancer Res 2011; 71: 7640-7648.
45. Raulf N, El-Attar R, Kulms D, Lecis D, Delia D, Walczak H, *et al*. Differential response of head and neck cancer cell lines to TRAIL or Smac mimetics is associated with the cellular levels and activity of caspase-8 and caspase-10. Br. J. Cancer 2014; 111: 1955-1964.
46. Polanski R, Vincent J, Polanska UM, Petreus T, Tang EKY. Caspase-8 activation by TRAIL monotherapy predicts responses to IAPi and TRAIL combination treatment in breast cancer cell lines. Cell Death Dis 2015; 6: e1893.
47. Maas C, Tromp JM, van Laar J, Thijssen R, Elias JA, Malara A, *et*

al. CLL cells are resistant to smac mimetics because of an inability to form a ripoptosome complex. Cell Death Dis 2013; 4: e782.

48. Petersen SL, Peyton M, Minna JD, WangS X. Overcoming cancer cell resistance to Smac mimetic induced apoptosis by modulating cIAP-2 expression. Proc. Natl. Acad. Sci 2010; 107: 11936-11941.
49. Lee EW, Seong D, Seo J, Jeong M, Lee HK, Song J. USP11-dependent selective cIAP2 deubiquitylation and stabilization determine sensitivity to Smac mimetics. Cell Death Differ 2015; 22: 1463-1476.