Journal of Medicinal Chemistry

Viewpoint

pubs.acs.org/jmc

Novel Promising IAP Antagonist on the Horizon for Clinical Translation

Simone Fulda*

Institute for Experimental Cancer Research in Pediatrics, Goethe-University Frankfurt, Komturstrasse 3a, 60528 Frankfurt, Germany

Evasion of apoptosis represents a hallmark of human cancers and is frequently caused by aberrant expression of antiapoptotic proteins.[1] Since inhibitor of apoptosis (IAP) proteins are present at high levels in many tumors and potently block apoptosis, they are considered as promising targets for therapeutic intervention.[2] IAP proteins comprise eight human analogues, among them XIAP, cIAP1, cIAP2, and ML-IAP.[2] XIAP has been shown to inhibit caspases via binding of the BIR3 domain of XIAP to the small subunit of processed caspase-9, while the linker region preceding the BIR2 domain as well as the BIR2 domain of XIAP is critical for inhibition of caspase-3 and -7.

In an attempt to design pan-selective IAP antagonists that neutralize XIAP, cIAP proteins, and ML-IAP, Flygare et al. used a combined approach of structure-based design and solid-phase library synthesis.[3] This resulted in the production of a series of compounds that mimic the four amino acid N-terminus of the endogenous IAP antagonist Smac (Figure 1). Of particular interest among these compounds was the thiadiazole compound **1** (GDC-0152), which was subsequently characterized in more detail for its binding properties, antitumor activity, and pharmacokinetic profile. Binding studies using X-ray crystallography showed that GDC-0152 interacts with the Smac-binding sites of the BIR3 domain of cIAP1 and the BIR domain of ML-IAP. A fluorescence polarization-based competition assay confirmed that GDC-0152 binds in the low nanomolar range to the BIR3 domains of XIAP, cIAP1, and cIAP2; the BIR2 domain of XIAP; and the single BIR domain of ML-IAP, whereas binding to the BIR2 domains of cIAP1 and cIAP2 was found at the low micromolar range. Subsequent functional activity studies using cell-free systems, in vitro cellular assays, and in vivo xenograft studies in mice demonstrated that GDC-0152 potently disrupts protein–protein interaction of IAP proteins and key proapoptotic molecules such as activated caspase-9 and Smac. Consistently, GDC-0152 as single agent increased enzymatic activity of caspase-3 and -7 and reduced cell viability. It is interesting to note that cytotoxic activity of GDC-0152 was found in the breast carcinoma cell line MDA-MB-231 but not in non-malignant human mammary epithelial cells. Furthermore, GDC-0152 rapidly and potently stimulated proteasomal degradation of cIAP1, whereas its inactive enantiomere was devoid of this activity. This is consistent with recent data showing that IAP antagonists can stimulate a conformational change in cIAP1 that promotes its RING dimerization and E3 ligase activity, resulting in autoubiquitination of cIAP1.[4] In a subcutaneous xenograft model using MDA-MB-231 breast carcinoma cells in nude mice, oral administration of GDC-0152 caused marked tumor regression as a single agent, even at the lowest dose of 10 mg/kg daily. In vivo preclinical pharmacokinetic studies showed favorable solubility properties, moderate hepatic clearance, and moderate plasma–protein binding. Of note, GDC-0152 was well tolerated in all preclinical in vivo studies. In the first in men study, GDC-0152 displayed linear pharmacokinetics, moderate clearance, and a moderate volume of distribution, consistent with preclinical predictions.

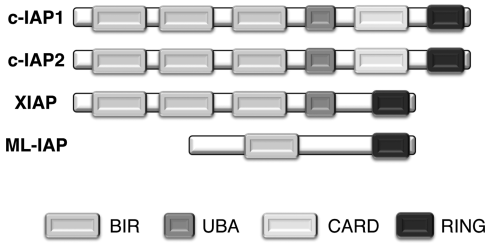

Figure 1. Structures of selected IAP proteins. The pan-selective IAP antagonists that were developed in the study by Flygare et al.[3] target four of the eight human inhibitor of apoptosis (IAP) proteins, namely, XIAP, cIAP1, cIAP2, and ML-IAP. The unifying feature of these IAP proteins is the baculoviral IAP repeat (BIR) domain, while other functional motifs such as the RING, UBA, and CARD domains are expressed in a more selective manner.

Together, these findings indicate that GDC-0152 represents an interesting candidate for further evaluation in clinical trials. However, a couple of questions remain. First, the issue of whether or not GDC-0152 selectively exerts antitumor activity against malignant tumor cells versus nonmalignant normal human cells remains to be addressed in additional studies. While the results of this study point to a selective cytotoxicity of GDC-0152 against tumor cells, the study is so far restricted to one malignant and one nonmalignant cell line. Therefore, additional studies are required to confirm the tumor-selective cytotoxic activity of GDC-0152. Also, the mechanistic basis for this tumor selectivity has not yet been unraveled. Second, additional data on the antitumoral activity of GDC-0152 in a broader panel of cancer cell lines will help to estimate its antitumor activity, as the current study focuses on the breast carcinoma cell line MDA-MB-231, which has been reported to be particularly sensitive to IAP antagonists.[5,6] For example, it will be interesting to explore whether GDC-0152 is active against cancer cells that exhibit high expression levels of ML-IAP, as this compound binds at low nanomolar concentrations to ML-IAP. Third, the fact that tumor regrowth is observed upon termination of treatment with GDC-0152 in the in vivo model raises questions concerning possible underlying mechanisms. Is there insufficient target inhibition by GDC-

Received: April 5, 2012
Published: April 19, 2012

dx.doi.org/10.1021/jm300475b | *J. Med. Chem.* 2012, 55, 4099–4100

0152 in the tumor tissue? Is tumor heterogeneity responsible for a mixed response to GDC-0152 resulting in the outgrowth of resistant tumor cells once treatment with GDC-0152 has been terminated? These questions also underscore that accompanying biomarker studies to investigate target inhibition in the tumor tissue upon treatment with GDC-0152 would be very instructive to better understand the molecular basis of its in vivo activity. Another important question is how the antitumor activity of GDC-0152 can be best exploited in combination treatments. There is ample evidence that the combination of IAP antagonists together with additional cytotoxic principles including anticancer drugs, radiotherapy, or molecular-targeted therapeutics represents a promising strategy to yield synergistic drug interactions. Therefore, further evaluation of GDC-0152 in combination protocols is considered to be a promising future avenue of research.

AUTHOR INFORMATION

Corresponding Author

*Phone: +49 69 67866557. Fax: +49 69 6786659157. E-mail: simone.fulda@kgu.de.

REFERENCES

(1) Hanahan, D.; Weinberg, R. A. Hallmarks of cancer: the next generation. *Cell* **2011**, *144*, 646−674.

(2) Fulda, S.; Vucic, D. Targeting IAP proteins for therapeutic intervention in cancer. *Nat. Rev. Drug Discovery* **2012**, *11*, 109−124.

(3) Flygare, J. A.; Beresini, M.; Budha, N.; Chan, H.; Chan, I. T.; Cheeti, S.; Cohen, F.; Deshayes, K.; Doerner, K.; Eckhardt, S. G.; Elliott, L. O.; Feng, B.; Franklin, M. C.; Reisner, S. F.; Gazzard, L.; Halladay, J.; Hymowitz, S. G.; La, H.; LoRusso, P.; Maurer, B.; Murray, L.; Plise, E.; Quan, C.; Stephan, J.-P.; Young, S. G.; Tom, J.; Tsui, V.; Um, J.; Varfolomeev, E.; Vucic, D.; Wagner, A. J.; Wallweber, H. J. A.; Wang, L.; Ware, J.; Wen, Z.; Wong, H.; Wong, J. M.; Wong, M.; Wong, S.; Yu, R.; Zobel, K.; Fairbrother, W. J. Discovery of a potent small-molecule antagonist of inhibitor of apoptosis (IAP) proteins and clinical candidate for the treatment of cancer (GDC-0152). *J. Med. Chem.* **2012**, DOI: 10.1021/jm300060k.

(4) Dueber, E. C.; Schoeffler, A. J.; Lingel, A.; Elliott, J. M.; Fedorova, A. V.; Giannetti, A. M.; Zobel, K.; Maurer, B.; Varfolomeev, E.; Wu, P.; Wallweber, H. J.; Hymowitz, S. G.; Deshayes, K.; Vucic, D.; Fairbrother, W. J. Antagonists induce a conformational change in cIAP1 that promotes autoubiquitination. *Science* **2011**, *334*, 376−380.

(5) Varfolomeev, E.; Blankenship, J. W.; Wayson, S. M.; Fedorova, A. V.; Kayagaki, N.; Garg, P.; Zobel, K.; Dynek, J. N.; Elliott, L. O.; Wallweber, H. J.; Flygare, J. A.; Fairbrother, W. J.; Deshayes, K.; Dixit, V. M.; Vucic, D. IAP antagonists induce autoubiquitination of c-IAPs, NF-kappaB activation, and TNFalpha-dependent apoptosis. *Cell* **2007**, *131*, 669−681.

(6) Petersen, S. L.; Wang, L.; Yalcin-Chin, A.; Li, L.; Peyton, M.; Minna, J.; Harran, P.; Wang, X. Autocrine TNFalpha signaling renders human cancer cells susceptible to Smac-mimetic-induced apoptosis. *Cancer Cell* **2007**, *12*, 445−456.