lesions, adequate organ functions, written informed consent. L-OHP was administered over 2-hour intravenous infusion at a dose of 100 mg/m^2 (level 1) or 130 mg/m^2 (level 2) on day 1 every 3 weeks, and S-1 (<1.25 m^2, 80 mg/day; 1.25–1.5 m^2, 100 mg/day; >1.5 m^2, 120 mg/day) was given twice daily for 2 weeks followed by 1-week rest. L-OHP was escalated from 100 mg/m^2 (level 1, 3 patients) to 130 mg/m^2 (level 2, 6 patients).
Results: No dose-limiting toxicities were observed at levels 1 and 2. Level 2 (130 mg/m^2) was determined as the RD. A total of 9 patients (6 at RD) were enrolled in the phase I part. Male/female = 9/0, PS 0/1 = 8/1, median age 60 (range, 47−65), colon/rectum = 6/3. The median number of cycles was 5. Peripheral neuropathy was observed in all of the 9 patients without functional impairment. At each level, a patient developed Grade 3 or 4 thrombocytopenia, respectively. Neutropenia was the most commonly observed toxicity (44.4%). The overall response rate was 55.6% (PR5, SD3, NE1, n = 9). Collected PK samples are being analyzed now.
Conclusions: The SOX regimen was well tolerated. Efficacy and safety are comparable to those of FOLFOX 4. We have moved on to the phase II clinical study and are accumulating patients.

Patient	Level	Primary	Stage	L-OHP (mg/m^2)	S-1 (mg/day)	No. of cycles received
#1	1	Colon	−	100	120	8
#2	1	Colon	IV	100	120	12+
#3	1	Rectum	II	100	120	10+
#4	2	Rectum	IV	130	120	4
#5	2	Colon	IV	130	120	8
#6	2	Colon	IV	130	120	3
#7	2	Colon	II	130	120	5+
#8	2	Rectum	−	130	120	2
#9	2	Colon	IV	130	120	4+

Apoptosis

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ABT-263: an orally bioavailable small molecule inhibitor of Bcl-2 family proteins

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One of the hallmarks of cancer cells is their ability to evade death signals and survive cellular stress. Death signals originating from a multitude of sources converge on the mitochondria where Bcl-2 family proteins act as critical regulators of programmed cell death. Dynamic binding interactions between the pro-apoptotic (Bax, Bak, Bad, Bim, Noxa) and anti-apoptotic (Bcl-2, Bcl-xL, Mcl-1) family members act as a rheostat that controls commitment to apoptosis. The anti-apoptotic proteins act as checkpoints in this signaling cascade by binding and sequestering pro-apoptotic proteins thereby protecting cells from apoptosis. Cancer cells frequently over-express Bcl-2 and/or Bcl-xL to suppress the apoptotic signal in order to promote survival or confer resistance to chemotherapy. Inhibition of these anti-apoptotic Bcl-2 family members has therefore become an attractive target for cancer therapy.
Here we describe ABT-263, an orally bioavailable, small molecule Bcl-2 family protein inhibitor under clinical development at Abbott Laboratories. ABT-263 binds with high affinity (Ki < 1 nM) to multiple anti-apoptotic Bcl-2 family proteins including Bcl-xL, Bcl-2 and Bcl-w, and displays potent cytotoxicity against human tumor cell lines derived from small cell lung carcinomas (SCLC) and lymphoid malignancies. Furthermore, ABT-263 potently enhances the cytotoxicity of both chemotherapy and radiation in cells derived from multiple, major tumor types independent of potent single agent activity. When dosed orally in xenograft models of established SCLC, ABT-263 induces complete (100% tumor shrinkage) or partial (>50% tumor shrinkage) responses in eight of the nine models evaluated. ABT-263 also exhibits single-agent activity in models of B-cell lymphoma, and significantly enhances the effects of multiple chemotherapeutics. Using a panel of human tumor cell lines, we have identified patterns of Bcl-2 family protein expression that are indicative of sensitivity or resistance to ABT-263 consistent with its mechanism of action. These profiles provide information useful in the development of biomarkers for potential patient stratification, and will also guide the design of rationale-based combination therapies for clinical trials. These findings strongly support clinical development of ABT-263 in SCLC and lymphoma, as well as part of a combination regimen for other tumor types.

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Induction of lysosomal membrane permeabilization by compounds that induce p53-independent apoptosis

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Background: The p53 protein activates cellular death programs through multiple pathways. The high frequency of p53 mutations in human tumors is believed to contribute to resistance to commonly used chemotherapeutic agents, and it is therefore important to identify drugs that induce p53-independent cell death.
Material and Methods: We screened the NCI mechanistic set (879 compounds with diverse mechanisms of actions) and for compounds that induced apoptosis in p53 wild-type and p53null HCT116 colon cancer cells at <5 μM. Apoptosis by selected compounds were studied in detail.
Results: Of 175 apoptotic compounds identified, most were found to elicit a stronger apoptotic response in cells with functional p53. However, significant apoptosis was observed also in p53null cells. Interestingly, a number of "p53-independent compounds" were found to induce a lysosomal apoptosis pathway (Erdal et al., PNAS 102, 192–7). A number of such compounds have been studied in detail. Translocation of cathepsin-D into the cytosol was observed after treatment with these drugs.
Apoptosis was found to be inhibited by an inhibitor of the lysosomal protease cathepsin-D and by cahepsin-D siRNA. In contrast, inhibition of cathepsin-B by CA-074-Me or by siRNA was found not to inhibit apoptosis. Both the kinetics and extent of cathepsin release was found to vary between drugs, and such differences were found to be reflected in the degree of mitochondrial involvement and kinetics of apoptosis. Despite these differences, cell death by all compounds investigated has been found to be caspase-independent.
Conclusions: Our studies suggest that a large number of compounds that induce the lysosomal apoptosis pathway can be identified in cell based screens. Strategies to identify compounds that may be of therapeutic interest in vivo need to be developed. We have developed a technology to measure the levels of a caspase-cleaved apoptosis product which can be applied not only for 2-D in vitro cultures, but also for 3-D spheroid cultures and ex vivo tumor organ cultures. The method can also be used to assess tumor apoptosis in blood from experimental animals and patients (Kramer et al., Cancer Res. 64, 1751–6). We are currently using this method to evaluate the therapeutic efficiencies of various compounds that induce lysosomal membrane permeabilization.

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In vivo imaging of apoptosis induction by BI 2536, a small-molecule inhibitor of human Polo-like kinase 1 (Plk1), in human cancer xenograft models

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BI 2536, a potent and selective small-molecule inhibitor of the serine/threonine kinase Plk1, blocks proliferation and induces apoptosis in human cancer cell lines of diverse tissue origin and oncogenome signature. In vivo efficacy studies of BI 2536 in multiple human tumor xenograft models have demonstrated broad anti-tumor activity of well-tolerated intravenous (i.v.) dose regimens. In treated tumors, cells arrest in pro-metaphase within 24 hours, followed after 48 hours by a surge of apoptosis, detectable by immunohistochemistry.
This study was designed to assess the induction of apoptosis by BI 2536 in human tumor xenograft models by means of in vivo imaging methods. For analysis by near-infrared fluorescence (NIRF) imaging, nude mice (n = 4) bearing subcutaneously (s.c.) established NCI-H460 lung carcinomas (approx. 100 mm^3) were treated i.v. with BI 2536 at a dose of 60 mg/kg or with vehicle only. Binding of the imaging probe Cy-Annexin V to phosphatidylserine was monitored 48 hours after initiation of treatment. For measurement of the apparent diffusion coefficient of tissue water (ADCw) by magnetic resonance imaging (MRI), nude mice (n = 6) bearing s.c. HCT116 colon carcinomas (approx. 50 mm^3) were treated i.v. with 50 mg/kg BI 2536 or with vehicle only on two consecutive days. MRI was performed before treatment and at defined intervals after initiation of treatment.
Cy-Annexin V binding to phosphatidylserine 48 hours after treatment as quantified by NIRF imaging (average photon counts per tumor nodule per second) was approx. 100% higher ($p < 0.05$) in BI 2536 treated animals compared with controls. MRI experiments showed no difference of ADC_w between the study groups before treatment. Afterwards, diffusivity remained constant in the vehicle-treated tumors whereas it increased by approx. 15% ($p < 0.05$) in the BI 2536 treated tumors on day 4 after treatment initiation. In