patients) has been observed. Pralatrexate has achieved a remarkably high CR rate among patients with select forms of NHL. The goals of this ongoing trial are to identify the ORR in patents with B- and TCL, and to initiate an international registration study for patients with TCL.

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Research and identification of the polymorphisms of the thymidylate synthase gene in the human tumor cell lines panel of the National Cancer Institute (NCI)

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Background: Thymidylate synthase (TYMS) is the target enzyme for 5-fluorouracil (5-FU). It has been shown that TYMS expression is inversely correlated with the activity and/or toxicity of 5-FU in cancer patients. On the other hand, TYMS expression is dependent on TYMS gene polymorphisms (PM). Three distinct PMs have been identified: the 2R/3R PM, consisting of the presence of 2 or 3 tandem repeats of a 28 bp sequence in the gene promoter; the 3C/3G PM, consisting of a C>G SNP in the second repeat of 3R alleles; and the 6ins/6del PM, consisting of the deletion of a 6 bp sequence in the 3′ untranslated part of the gene.
Methods: DNA was extracted from the cell lines of the NCI panel and TYMS PMs were identified using PCR-RFLP techniques. TYMS catalytic activity was evaluated in cell cytosols using a radioactive substrate.
Results: In the NCI panel, the allele frequency of the 2R allele is 53% (19 3R/3R, 17 2R/3R and 23 2R/2R cell lines). Among the 3R allele-containing cell lines, 7 with 2R/3R and 10 with 3R/3R genotype present at least one copy of the 3G allele (allele frequency: 18%). Finally, the allele frequency of the 6del variant is 32% (32 6ins/6ins, 16 6ins/6del and 11 6del/6del cell lines). We have looked for relationships between 5-FU cytotoxicity, as extracted from the NCI database, TYMS expression and catalytic activity in the cell lines of the NCI panel, and the presence of TYMS gene PMs. 5-FU cytotoxicity is significantly related to none of the PMs, and is not related either with TYMS expression or activity. However, the presence of 3G alleles is significantly associated to high enzyme expression and activity ($P = 0.03$), especially in cell lines with mutated p53 ($P = 5 \times 10^{-5}$). There is a linkage disequilibrium between the PMs, the 3G allele being significantly associated with the 6del allele and the 2R allele with the 6ins allele. In addition, there is a deviation from the Hardy-Weinberg distribution, with a smaller than expected proportion of heterozygous cell lines for any PM. This can be attributed to loss of heterozygosity occurring in tumor cell lines.
Conclusion: The NCI panel offers an interesting model for the establishment of relationships between gene PMs and pharmacological data. The absence of relationship between in vitro 5-FU cytotoxicity and TYMS gene expression, activity and polymorphisms could be due to the fact that 5-FU cytotoxicity was measured in the absence of optimal amounts of the cofactor of TYMS.

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Phase I study of sapacitabine, an oral nucleoside analogue, in patients with refractory solid tumors or lymphomas

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Background: Sapacitabine (CYC682, CS-682) is a rationally designed 2'-deoxycytidine-type nucleoside analogue that can be administered orally. Compared with other nucleoside analogues, sapacitabine is unique in its ability to induce G2 cell cycle arrest and cause single-strand DNA breaks that are irreparable by ligation. Following oral administration, sapacitabine is converted by amidases and esterases in the gut, plasma, and liver to its major active metabolite (CNDAC). Sapacitabine had potent anti-tumor activity in animal studies and was superior to gemcitabine or 5-FU in a mouse liver metastasis model. Previous phase I studies had evaluated once daily dosing (qD) × 3 or 5 days/week for 4 weeks every 6 weeks. To maximize drug exposure, this phase I study evaluates twice daily dosing (b.i.d.) × 7 or 14 days every 21 days, using body surface area (BSA)-based or fixed dosing.
Methods: Eligible patients had incurable advanced solid tumors or lymphomas and adequate organ function. At least 3 patients were enrolled at each dose level. Maximum tolerated dose (MTD) was the dose level at which at least 2/3 or 3/6 patients experienced DLT in the first cycle. The recommended phase II dose (RD) was the dose level immediately below MTD. Pharmacokinetic (PK) sampling was performed after administration of sapacitabine with and without food.
Results: 37 patients were treated, 28 on the b.i.d. × 14 days schedule and 9 on the b.i.d. × 7 days schedule. The most common tumor types were non-small cell lung (n = 7), colon (n = 5), breast (n = 5) and ovary (n = 4). The MTD for the14 day-schedule is 40 mg/m^2 b.i.d. (RD = 33 mg/m^2 or 50 mg b.i.d.). The MTD for the 7 day-schedule is 100 mg b.i.d. (RD =75 mg b.i.d.). DLTs were reversible myelosuppression. One patient treated at the MTD of 40 mg/m^2 b.i.d. died of candida sepsis in the setting of grade 4 neutropenia and thrombocytopenia. Non-hematological adverse events (all grades, regardless of causality) were mostly mild to moderate and included nausea, vomiting, fatigue, diarrhea, constipation and anorexia. PK data are being analyzed. The best response to sapacitabine was stable disease in non-small cell lung (n = 3), ovary (n = 3), colon (n = 2), breast, gastrointestinal stromal tumor and parotid adenocarcinoma (n = 1 for each).
Conclusion: The RD of sapacitabine for the b.i.d. × 14 days schedule is 33 mg/m^2 b.i.d. or 50 mg b.i.d. and that for the b.i.d. × 7 days schedule is 75 mg b.i.d. The DLT was myelosuppression.

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Pharmacokinetics of talotrexin (PT-523), a novel aminopterin analogue, in patients with non-small cell lung cancer

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Background: Talotrexin, N^{α}-(4-Amino-4-deoxypteroyl)-N^5-hemiphthaloyl)-L-ornithine (PT-523) is a nonpolyglutamatable antifolate which has demonstrated improved antitumor activity in a broad spectrum of cancer models by targeting DHFR to inhibit tumor growth. Talotrexin binds more tightly (15-fold, Ki 0.35 pM) to DHFR than methotrexate (MTX). In lung cancer cell lines, talotrexin inhibits tumor cell proliferation at sub- to low-nanomolar concentrations and is more potent than MTX in all cell lines tested. We conducted a dose escalation study of talotrexin administered as a 5–10 minute infusion on Days 1, 8, on a 21-day cycle in non-small cell lung cancer (NSCLC). The primary objectives of this study were to determine the maximum tolerated dose (MTD), pharmacokinetic (PK) profile, as well as the safety and efficacy. This report describes the PK behavior of talotrexin in NSCLC patients.
Methods: Plasma samples were obtained prior to infusion, at the completion of the infusion, at 15 and 30 minutes, then at 1, 2, 3, 4, 6, 8, 10, 16, 24 and 48 hrs after completion of the infusion. A validated LC/MS/MS assay was used to measure talotrexin in plasma. PK parameters were estimated by standard noncompartmental methods.
Results: The PK of talotrexin was characterized in 25 patients with normal renal and hepatic function, and a median age of 59 years (range, 48–76 years). Data was obtained from groups of at least three patients receiving doses of 13.5, 27, 54, 90, and 135 mg/m^2. The talotrexin concentration in plasma decreased in a mono-exponential manner following a rapid distribution phase. In the 6 patients who received the MTD dose of 54 mg/m^2, the mean peak drug concentration in plasma (C_{max}) was 17.42 ng/L (13.7−22.3) and the mean plasma concentration 48 hr after dosing was 17.3 ng/ml (range, 17.8−36.7 ng/mL). The apparent biological half-life ($t_{1/2,z}$), total body clearance (CL) and apparent volume of distribution at steady-state (V_{ss}) were all independent of the dose. Mean (range) values of PK parameters for the entire cohort of 25 patients were: CL, 1.4 L/hr/m^2 (3.6−10.3), $t_{1/2,z}$, 6.6 hr (4.7−6.8) and Vd_{ss}, 8.1 L/m^2 (7.4−13.0).
Conclusions: Talotrexin exhibits linear PK with moderate interpatient variability when administered as a short IV infusion at doses of 13.5−135 mg/m^2. In future PK studies, talotrexin major route of elimination will be examined and an evaluation of whether diminished renal or hepatic function warrants dose modification will be conducted.

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Phase I/II study of oxaliplatin (L-OHP) in combination with S-1 (SOX) as first-line therapy for metastatic colorectal cancer (MCRC)

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Background: FOLFOXs are one of the world's established standard therapies for MCRC. S-1 is an oral dihydropyrimidine dehydrogenase (DPD)-inhibitory fluoropyrimidine consisting of tegafur which is a 5-FU prodrug activated by CYP2A6 in the liver, 5-chloro-2,4-dihydroxypyridine of the DPD inhibitor, and potassium oxonate of the orotate phosphoribosyltransferase (OPRT) inhibitor. The response rate of S-1 monotherapy for chemo-naïve MCRC was 35.7%. SOX may provide a new alternative to FOLFOX. This study was designed to determine the recommended dose (RD), to assess the pharmacokinetics (PK), and to evaluate the efficacy and safety of this combination therapy.
Methods: Patients were eligible as follows: unresectable MCRC with no prior chemotherapy, PS (ECOG) 0–1, age 20−75, measurable

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