mg/m$^2$ over 0.5 h, weekly × 2 q 3 weeks (B). In A, downward dose modification in successive cohorts based on the results of recruitment of cells into S-phase in tumor biopsies (measured by immunohistochemistry for Cyclin A and flow cytometry (FC)) before and after IRN, is used to determine the minimal modulatory dose of IRN. Starting dose (SD) was 80mg/m$^2$, with subsequent exploration of 40 and 60 mg/m$^2$ (present dose). In B, doses are escalated on the basis of toxicity in standard Phase I fashion with 3 pts at each dose level not showing dose limiting toxicity (DLT). Three patients have been entered at each of the doses 20, 40 and 60 mg/m$^2$ (present dose). Pre and post IRN biopsies are performed in some pts. Toxicities > grade 3, with cycle 1 include neutropenia grade 3-4 (6 pts) and hyponatremia (1 pt). Cyclin A index (S+G2) increased by 95-200% (mean 134%) in 4 evaluable pts by IHC and S phase by 265% and 128% in 2 evaluable pts by FC, at 80 mg/m$^2$ of IRN. At 40 mg/m$^2$, increases in Cyclin A index were seen in 2/4 evaluable pts but these increases were <50%. Data indicate marked modulation of cell cycle at a dose of 80 mg/m$^2$ but not at 40 mg/m$^2$ of IRN. Correlation of cell cycle modulation with pharmacokinetics of IRN and SN-38 are in progress.

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## Histone deacetylase inhibitors potentiate breast cancer cell lines to anthracycline-induced apoptosis in a schedule and dose-dependent manner

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Anthracyclines are thought to act by binding to topoisomerase IIá (topo IIá). Covalent binding will stabilize the topo IIá:DNA complex and there by inhibit synthesis of nucleic acids and proteins, resulting in subsequent cell death. Sensitivity to topo IIá inhibitors is dependent on theexpression level of topo IIá in cancer cells. Increased expression of topoIIá has been associated with sensitivity to topo IIá inhibitors. Histone deacetylase inhibitors (HDAC-I) such as sodium butyrate have been shown toincrease topo IIá. The HDAC-I, suberoylanilide hydroxamic acid (SAHA), hasbeen shown to inhibit growth and promote differentiation and apoptosis inseveral transformed cell lines including breast cancer. SAHA is now in earlyclinical trials and appears well tolerated. While HDAC-I may have anti-tumor activity as single agents, synergistic and antagonistic activity has been reported when combined with cytotoxic agents according to preclinical studies. In this study, we examined the effects of SAHA on apoptosis induced by topo IIá inhibitors. We found that cultured breast cancer cells with high levels of topo IIá (e.g.SKBr-3) were sensitive to anthracyclines resulting in growth arrest and apoptosis, whereas epirubicin caused minimal apoptosis in cells with low topo IIá levels (e.g.MCF-7). In SKBr-3 cells, SAHA enhanced epirubicin-induced apoptosis. However, synergistic activity was only observed when cells were preexposed to SAHA for at least 48 hours. Synergistic and additive effects were abrogated when SAHA was administered simultaneously or after exposure to epirubicin. Sensitization correlated with alteration of topo IIá. Furthermore, in cells with low topo IIá, SAHA-pretreatment resulted in sensitization even at concentrations that showed no significant apoptosis by the anthracycline alone. The topo IIá inhibitors, doxorubicin and epirubicin are currently amongst the most active agents for the treatment of breast cancer, but many tumors are resistant to this therapy. Our *in vitro* studies show that the combination of HDAC-I and epirubicin significantly enhances the activity of these agents and/or overcomes resistance in cells with low levels of topo IIá.

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## Specific inhibition of the growth-associated alpha isoform of topoisomerase II by the novel anticancer triazoloacridone C-1305

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Triazoloacridones are new antitumor compounds with potent activity against human leukemias as well as toward solid tumors in animal models. The most active triazoloacridone derivative C1305 is able to stimulate the formation of covalent DNA-topoisomerase II complexes *in vitro*. Topoisomerase II exists as two different isoforms: the alpha isoform which is restricted to proliferating tissues and which frequently is upregulated in human tumors and the beta isoform which is constitutively expressed in all tissues. Unexpectedly, only the alpha isoform of topoisomerase II was covalently associated with DNA following exposure of living cells to C-1305 in marked contrast to amsacrine, a classical topoisomerase II inhibitor which stimulated cleavable complexes with both isoforms. Pulse-field electrophoresis revealed, that the exposure of tumor cells to C-1305 resulted in the formation of 50-200 kbp fragments which correspond to the size of DNA loops. In contrast, amsacrine induced the formation of fragments of about 1,000 kbp. To further clarify the relative importance of the two topoisomerase isoforms in the cytotoxic action of C-1305, different sublines of the DC-3F Chinese hamster cells were studied. Prolonged ellipticine exposure of DC-3F parental cells led to selection of resistant DC-3F/9-OHE cells which have no expression of the beta form and 5-fold decreased expression of the alpha isoform. In addition, two transfectants were used where the expression of one of the two isoforms had been restored by transfection of the resistant cell line with either the alpha or the beta form of topoisomerase II. In agreement with the results presented above, the cytotoxicity of amsacrine was increased in cells transfected with either isoform, while the sensitivity to C-1305 was only altered in cells transfected with the alpha isoform. These results identify C-1305 as the first topoisomerase II inhibitor which selectively targets the growth- and tumor-associated alpha form of topoisomerase II.

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## Phase I and pharmacologic study of the macromolecular topoisomerase-I-inhibitor DE-310 given once every 2 or 6 weeks in patients with solid tumors

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DE-310 is a novel macromolecular drug-delivery system, which comprises the topoisomerase-I inhibitor DX-8951 linked to a biodegrable carrier, carboxymethyldextran polyalcohol, via a peptide spacer. In pre-clinical studies, it has been demonstrated that DE-310 accumulates and is retained preferentially in tumor tissue by an enhanced permeability and retention effect. In the current Phase I study, DE-310 was initially administered as an IV infusion over 3 hours once every 2 weeks (q2w) in a 4-week cycle. Resulting pharmacokinetic (PK) data indicated that the apparent half-life of DE-310 approximated the 2 weeks administration interval, and so the protocol was amended to a 6 weekly schedule (q6w). Dose levels tested to date include: q2w; 1 mg/m$^2$ (dose level I, n=6), 2 mg/m$^2$ (dose level II, n=4) and q6w; 6 mg/m$^2$ (dose level III, n=3), 9 mg/m$^2$ (dose level IV, n=3). Currently, 16 patients (9 male, 7 female), median age 59 years (range, 31-78), median PS 1 (range, 0-2), with a variety of refractory solid tumors are included. The worst hematologic toxicities are: grade 4 neutropenia (n=1), grade 4 leucopenia (n=1), grade 3 thrombocytopenia (n=2), and grade 3 anemia (n=1), all at dose level IV. Non-hematological toxicities are mild to moderate, including: nausea, vomiting, diarrhea, fatigue, anorexia, alopecia, skin reaction, and infusion reaction. At dose level IV, 1 patient experienced DLT due to febrile neutropenia and grade 3 thrombocytopenia. Out of 15 patients assessable for response, 9 achieved stable disease and 2 have major objective tumor regression, which formally qualify as partial responses. To evaluate the PK of DE-310, conjugated DX-8951, DX-8951 and G-DX-8951 were measured in samples taken up to 35 days post first dose. At dose level III, the apparent half-life of conjugated DX-8951, DX-8951 and G-DX-8951 were 13, 10 and 11 days respectively. The AUC0-t ratio (conjugated DX-8951/DX-8951) was approximately 600 and the mean Tmax of DX-8951 was achieved at 75 hours post dose. The active moiety DX-8951 declined in parallel with the carrier-linked molecule, conjugated DX-8951, suggesting that DX-8951 elimination is formation rate-limited. Overall, these data indicate that DX-8951 is released slowly and over an extended period, achieving the desired prolonged exposure. The observed tumor regressions are of interest.