glioma resulting each in 100% tumor regressions, no synergy was found when administered simultaneously suggesting alternative scheduling for this combination.
Conclusions: Gimatecan in a protracted schedule is highly active against malignant glioma xenografts and has synergistic activity with temozolomide, imatinib and everolimus suggesting this new topoisomerase I inhibitor for the treatment of malignant glioma.

597 POSTER

Novel Topoisomerase 1 mutations in colorectal carcinoma cell lines are involved in SN38 resistance

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Background: DNA Topoisomerase I (Top1) is a nuclear enzyme that catalyzes the relaxation of supercoiled DNA during DNA replication and transcription. The enzyme transiently cleaves a single DNA strand to form a covalent Top1-DNA cleavage complex. TopI is the molecular target of camptothecin and related drugs such as irinotecan and SN38 (the irinotecan's active metabolite). SN38 interferes with the activity of TopI by forming stable covalent ternary complexes which convert the DNA-single strands breaks in double strand breaks and triggers S-phase cell killing. We have previously obtained several HCT116-derived clones resistant to SN38 in order to study drug resistance mechanisms.
Materials and Methods: Four SN38-resistant clones have been analyzed for Top1 mutations, expression and activity. We have then performed functional analysis of these clones when they are challenged with SN38 and specifically monitored the double strands breaks with gH2AX staining and replication activity with molecular combing.
Results: Our results revealed that all the resistant clones displayed a Top1 mutation without modification of Top1 expression or intrinsic activity. However, we observed less Top1-DNA cleavage complex and less double strand breaks in presence of SN38 in the four resistant clones. In addition, using DNA combing, we have looked at replication fork behaviour when cells are treated with SN38. It appeared that the sensitive cells displayed a typical asymmetry of the replication fork. At the contrary, the four resistant clones were less sensitive to the asymmetry induced by SN38.
Conclusion: These results indicate that the Top1 mutations observed in the four clones may be responsible for an altered Top1/SN38 interaction. Moreover, we showed a direct effect of SN38 on replication fork.

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Voreloxin (formerly SNS-595) is a potent DNA intercalator and topoisomerase II poison that induces cell cycle dependent DNA damage and rapid apoptosis in cancer cell lines

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Background: Voreloxin (formerly SNS-595) is a replication-dependent agent that induces DNA damage, irreversible G2 arrest and apoptosis by selective intercalation of DNA and poisoning of topoisomerase II (Stockett et al.; Hawtin et al., AACR 2008). Voreloxin is under clinical investigation in acute myeloid leukemia and ovarian cancer (Lancet et al., ASH 2007; McGuire et al., SGO 2008). Voreloxin is a naphthyridine analog, related to the quinolones, which have not previously been used for cancer treatment. To further define the mechanism of action, induction of DNA damage by voreloxin during different cell cycle phases was investigated. The role of DNA intercalation in the induction of DNA damage was studied with two voreloxin analogs predicted to have enhanced intercalation or to lack the ability to intercalate. The molecular events linking DNA damage with voreloxin-induced G2 arrest and apoptosis were also assessed.
Methods: DNA damage and apoptosis in solid and hematologic cell lines were monitored by gammaH2AX foci formation and annexin V labeling along with PARP cleavage, respectively. DNA repair signaling was evaluated by western blot analysis.
Results: Voreloxin induced dose-dependent DNA damage in S, G2 and M phases of the cell cycle, whereas G1 cells were markedly less sensitive to the drug. These data were consistent with the selectivity of voreloxin towards proliferating cells. No evidence of DNA damage was observed with the predicted non-intercalative voreloxin analog. Induction of DNA damage in non-mitotic cells by voreloxin over the concentration range was biphasic: a dose-dependent increase was observed up to 10 μM; at 20 μM, reduced DNA damage was detected. Voreloxin-induced DNA damage activated ATR signaling, reflected by rapid and sustained phosphorylation of the checkpoint kinases CHK1 and CHK2. Phosphorylation of DNA-PKcs was also observed. Activation of ATR signaling is consistent with the G2 arrest induced by the voreloxin. At cytotoxic concentrations of voreloxin, apoptosis is induced as indicated by annexin V binding and PARP cleavage.
Conclusions: DNA damage induction by voreloxin and certain analog correlates with predicted ability to intercalate DNA. The biphasic induction of DNA damage by voreloxin during replication is consistent with the well-characterized mechanism of action of the fluoroquinolones towards bacterial gyrase (prokaryotic topoisomerase II).

599 POSTER

The iron chelator di-2-pyridylketone-4,4,-dimethyl-3-thiosemicarbazone causes DNA damage in breast cancer cells

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Iron chelators have historically been studied for treatment of iron overload disease and for their potential to alleviate the cardotoxic side effects of anthracycline chemotherapy. Di-2-pyridylketone-4,4,-dimethyl-3-thiosemicarbazone (Dp44mT), is being developed as an iron chelator with selective anticancer activity. We investigated the mechanism whereby Dp44mT kills breast cancer cells, both as a single agent and in combination with doxorubicin. Dp44mT alone induced selective cell killing in breast cancer cell lines (MDA-MB-231 and MCF-7) when compared to healthy breast epithelial cells (MCF-12A), and was also highly toxic to aggressive neuroblastoma cells. It induced a G1 cell cycle arrest and reduced cancer cell clonogenic growth at nanomolar concentrations. Dp44mT, but not the iron chelator desferal, induced DNA double strand breaks quantified as S139 phosphorylated histone foci (gamma-H2AX) and Comet tails induced in MDA-MB-231 cells. Doxorubicin-induced cytotoxicity and DNA damage were both enhanced significantly in the presence of low concentrations of Dp44mT. We will present data highlighting the mechanism(s) of DNA damage-mediated cytotoxicity of Dp44mT in breast cancer cells. Dp44mT may serve as a mechanistically unique treatment for cancer due to its dual abilities to chelate iron and target DNA.

600 POSTER

Hematologic pharmacodynamics linked to the pharmacokinetics of berubicin (B), a blood–brain barrier penetrating anthracycline active against high grade glioma, in phase I/II clinical trials

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Background: Preclinical studies demonstrated that B, a 4′-O-benzyl anthracycline designed to circumvent P-gp/MRP1-mediated efflux, effectively crosses the BBB, is retained in brain & brain tumor tissue for >24 hrs, also demonstrating in vivo activity against glioblastoma multiforme (GBM) in an orthotopic model.
Materials and Methods: A multicenter, phase I dose-escalation study of B was administered as an IV infusion, designed as 2 arms: 3 days, every 3 weeks; or weekly x 4, every 5 weeks. Enrolled patients were adults with recurrent/refractory GBM or other primary brain tumors. Peripheral blood samples were collected at selected timepoints with B and primary metabolite (berubicinol; B-ol) quantified by LC/MS/MS. PK parameters describing B disposition were determined by fitting compartmental models to plasma concentration-time data, and non-compartmental models to B-ol data. Complete blood counts were taken at baseline and several times throughout each cycle. The surviving fraction (SF) and decrease in leukocytes, neutrophils, and platelets were calculated and linked to B and B-ol PK parameters.
Results: Thirty-five patients have been enrolled at daily x 3 doses of B ranging from 1.2 to 9.6 mg/m^2; and another 13 patients enrolled on the weekly regimen, with doses ranging from 7.5 to 13.3 mg/m^2. Mean (range) population terminal t1/2 is 35.0 (11.0–89.2) hrs, plasma CIT is 46.8 (22.1–107.5) L/hr/m^2, and Vss is 1896 (583–4722) L/m^2 for both arms. Percentage of unchanged drug renally eliminated was 3.8% (0.4–14.9). Several PRs and one CR have been noted, even at dose levels below the daily arm MTD of 7.5 mg/m^2/day. Clinical comparisons of B and B-ol AUC show exposures of metabolite ranging 4–19% (mean 9.3%) of that of the parent. Regimen related toxicity has been minimal with the most common adverse event being myelosuppression. Thus far, of 34 evaluable patients