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.

598 POSTER

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

from the daily arm, 28 patients had a decrease in ANC post-treatment; with the neutrophil-fall being 3.62 (+2.64) cells/μl and a mean SF of 42% (2–87%). The mean, observed days to neutrophil and platelet nadir are 18 days.
Conclusion: Half of patients with AUCs above 750 (4 of 8) had neutrophil SF of less than 25% (3 of which were the only neutropenic DLTs); as well as platelet SF of less than 35%. With in vitro evidence suggesting B-ol toxicity to be 40−49% that of B in glioma cell lines, further study of hematologic toxicities linked to both B and B-ol PK parameters is warranted and ongoing.

Vaccines

601 POSTER

Cross-trial analysis of immunological and clinical data resulting from phase I and II trials of MVA-5T4 (TroVax®) in colorectal, renal and prostate cancer patients

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Background: The attenuated vaccinia virus MVA has been engineered to deliver the tumour antigen 5T4 (MVA-5T4; TroVax®). 5T4 is a surface glycoprotein expressed by most solid tumours. MVA-5T4 has been tested in two phase I/II and seven phase II clinical trials in colorectal (4 trials), renal (4 trials) and prostate (1 trial) cancer patients. All trials demonstrated that MVA-5T4 was well tolerated when administered alone (2 trials) or in combination with cytokines (5 trials) or chemotherapies (2 trials). Antibody and/or cellular responses specific for 5T4 were induced in the majority of patients and these responses were associated with clinical benefit in each of 6 trials. We have now collated data from all nine TroVax trials and investigated the incidence and kinetics of immune responses across trials and looked for associations with improved survival.
Methods: Antibody responses specific for the 5T4 tumour antigen and the MVA viral vector were monitored by ELISA. Survival data were collated from each hospital site. Immunological and survival data were analysed using proportional hazards regression adjusting for age and gender.
Results: Both survival and immunological response data were available for 189 patients (median age 62), with colorectal (n = 73), renal (n = 89) and prostate (n = 27) cancer. The median number of TroVax vaccinations received was 5 (range 1–12). TroVax was safe and well tolerated across trials and in combination with both chemo- and cytokine-therapies. Of 189 patients analysed prior to treatment with TroVax, 20 (10.5%) had weak positive 5T4-specific antibody responses and 23 (12%) had MVA-specific antibodies. Of 180 patients tested for antibody responses post-vaccination, 159 (88%) and 176 (98%) showed positive responses for 5T4 and MVA respectively. Peak median antibody titres were detected following 2 vaccinations for MVA and 4 vaccinations for 5T4. Exploratory analyses demonstrated significant associations between immune responses and overall survival across trials in patients with colorectal cancer alone (4 trials), renal cancer alone (4 trials) or colorectal, renal or prostate cancer (9 trials).
Conclusions: MVA-5T4 induced 5T4-specific immune responses in the majority of patients irrespective of cancer type or the addition of co-meds. Generally, two to three vaccinations were required to induce 5T4-specific antibody responses in most patients. Although the studies described here were uncontrolled, there were encouraging signs of activity which associated with the presence of 5T4-specific immune responses. These observations will be tested more thoroughly in an ongoing randomized, placebo-controlled phase III trial in renal cancer patients.

602 POSTER

An anti-idiotypic HER2 vaccine can reverse immunological tolerance to HER2 and induce anti-tumor immunity in huHER2 female transgenic mice

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Background: Breast cancer is a widely spread women's disease. Inspite of progress in the field of surgery and adjuvant therapies, the risk of breast cancer metastatic relapses remains high. Thus, it is important to develop adjuvant therapies to decrease mortality related to this type of cancer. In this context, the development of antitumor vaccines takes an important place. The oncoprotein HER2, which is an over-expressed antigen for different human carcinomas, represents a valuable target for the design of such a vaccine. However, an immunological tolerance against HER2 antigen exists representing a barrier to effective vaccination against this oncoprotein. We have selected two human ScFv antibody fragments (named ScFv 40 and ScFv 69) able to mimick the human HER2 antigen and to induce an anti-HER2 response in sera of immunized BALB/c mice (1).
Material and Methods: Production, purification and characterization of anti-Id ScFv 40 and ScFv 69 have been described. The huHER2-Transgenic mice FVB-MMTV.f.huHER2(Fo5) were obtained from Genentech and have been decribed (2). These mice overexpress human HER2 (huHER2) under the murine mammary tumor virus promoter and were used as a model of huHER2-overexpressing breast cancer. The anti-Id scFv vaccines were injected subcutanously (s.c.) after emulsion with Complete Freund Adjuvant (CFA). This injection was followed 2 weeks later by a second s.c. administration with Incomplete Freund Adjuvant (IFA). Two additional injections were given, in combination with IFA, intraperitonally (i.p.) at 21 and 35 days after the initial immunization.
Results: We demonstrated that the sera of immunized BALB/c mice are able to inhibit in vitro and in vivo the growth of human HER2-overexpressing cancer cells. Furthermore, following our vaccination schedule, all the transgenic FVB-MMTV.f.huHER2(Fo5) mice immunized with ScFv 69 were protected from the developpement of spontaneous mammary tumors whereas all control mice immunized with PBS and CFA/IFA developed tumors.
Conclusion: Our results demonstrated the remarkable efficacy of the ScFv vaccine candidate in protecting mice from the developpement of HER2-positive mammary tumors and allow us to consider that ScFv 69 fragment could be used as an anti-idiotype based vaccine for adjuvant therapy for patients bearing HER2 positive tumors.

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