spots than those obtained for C-857 under experimental conditions. This technique was used also to demonstrate the DNA adduct formation by C-1748 in human colon carcinoma HT-29 cells. The chromatographic pattern of DNA adducts detected resembled the ones observed in cell-free system. In conclusion, current studies along with interstrand DNA crosslinking demonstrated for a number of 4-substituted analogues suggest that also this new generation of 1-nitroacridines with lowered toxicity are able to bind covalently to DNA. This implies that DNA represents their major molecular target whose covalent modification induces a cascade of biological events eventually leading to apoptosis.

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Oral administration of clofarabine daily × 5 every 4 weeks in patients with advanced solid tumours in a phase I and pharmacokinetic study

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Background: Clofarabine, a next-generation purine nucleoside analogue, inhibits DNA polymerase α and ribonucleotide reductase and disrupts mitochondrial integrity resulting in release of cytochrome C and apoptosis-inducing factor. Several clinical trials examined the activity of an intravenous infusion (IV) of clofarabine in solid tumors and hematological malignancies. However, the oral administration of clofarabine is also possible and may offer advantage over the IV form. For example, superior curative activity was observed with daily oral compared to IV clofarabine administration in HT-29 and colon 36 xenograft models. Therefore, a phase I study to determine the safety and appropriate dose of oral clofarabine is warranted. **Methods:** Pts with advanced solid tumors that failed conventional therapy were treated with clofarabine administered orally for 5 consecutive days every 28 days. Cohorts of pts were dose-escalated according to a modified Fibonacci scheme to determine DLT and the MTD. **Results:** To date 11 pts (M/F: 4/7; median age=64) with advanced solid tumors (kidney n=4, colon n=2, adenoid cystic n=1, bladder n=1, cervical n=1, non-small cell lung n=1, and squamous cell skin n=1) have received 29 cycles (median 3; range 1–4) of oral clofarabine over 4 dose levels (1.0, 1.5, 2.25, and 3.5 mg/m^2). Best response to date: stable disease in 7 pts; progressive disease in 3 pts; and 1 pt pending response assessment. Cycle 1 drug-related grade 1–2 toxicities include: fatigue (n=6), nausea (n=4), abdominal cramping (n=2), anemia (n=2), stomatitis (n=2), leucopenia (n=1), thrombocytopenia (n=1), emesis (n=1), pruritis (n=1), diarrhea (n=1), and myalgias (n=1). One pt with cervical cancer treated at 2.25 mg/m^2 experienced grade 3 diarrhea on cycle 1 day 2, but subsequently was removed from study on day 19 due to obstruction from a large pelvic mass requiring an ileocolostomy. Maximal plasma clofarabine concentrations in the 1, 1.5 and 2.25 mg/m^2 cohorts averaged 5.3 (±1.6), 7.6 (±4.0) and 10.3 (±4.3) ng/mL, respectively. $AUC_{(0-24)}$ averaged 40.7 (±13.9), 59.6 (±16.1), and 87.8 (±27.4) ng*h/mL. Both C_{max} and $AUC_{(0-24)}$ increased with clofarabine dose. The accumulation ratio after 5 days was 1.4 (±0.33). Based on historical IV data, the oral bioavailability of clofarabine was estimated to be >70%. **Conclusion:** Oral clofarabine shows good bioavailability with characteristics of dose-dependent absorption. Accrual continues at 3.5 mg/m^2 to further define the MTD.

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HKH40A, potent agent agaist GI cancers, targets p53 or when that is mutated, Akt

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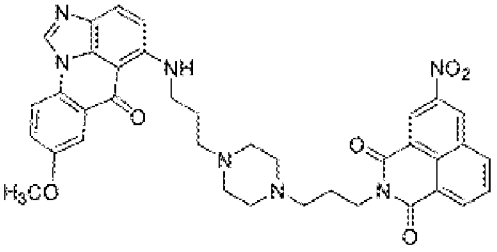
HKH40A is a synthetic agent with very potent but selective activity against gastro-intestinal cancers.

The compound binds to genomic DNA by intercalation of one of the aromatic residues, with the rest of the molecule residing in the minor groove. The operational hypothesis is that the complex "hijacks" critical proteins involved in DNA repair and transcription. Expression array studies have shown that the compound affects the expression of numerous genes in tumor cells, many of them associated with the cell cycle and apoptosis. HKH40A and its closely related des-methoxy analog WMC79 are very toxic to human colon cancer cell lines that express the wild type p53 tumor suppressor gene (LC_{50} = 25nM for RKO and HCT116 cells for HKH40A). Those cells are killed by a p53-dependant apoptotic cascade, initiated by a rapid upregulation of p53, which results in the activation of the FasL pathway, upregulation of the Bax/Bcl2 ratio and the resultant activation of the mitochondrial apoptosis pathway. All these biochemical changes result in activation of caspase 3 that in turn activates pro-apoptotic endonucleases. Upregulation of p53 is frequently a response to DNA damage, which in this case may be the consequence of HKH40A (and WMC79) being potent topoisomerase-1 poisons. However, experiments with topoisomerase-1 deficient cells showed that the enzyme is not the only target for the drugs. HKH40A is also a very potent agent against pancreatic and liver cancer cells (LC_{50} = 80 nM for ASPC-1 and 60 nM for Hep3B). The target for the drug in these tumors is not p53 since that is either mutated or not expressed. The compound arrests the growth of these cells at the G2-M checkpoint (upregulation of cyclin B1 and sustained phosphorylation of cdk1). All of these cell lines overexpress the phophorylated form of Akt, which is a pro-survival protein since it inhibits several key elements of apoptosis. HKH40A is a very potent inhibitor of phospho-Akt and the upstream PI3 kinase in pancreatic adenocarcinoma and hepatocellular carcinoma cells that we examined. The cell cycle effects are consistent with this finding. We conclude, that phospho-Akt is the molecular target for HKH40A in those cancers that express the protein (pancreas and liver). However, in wt p53 cancers the inhibition of topoisomerase 1 and the activation of the p53 cascade appear to be the principal targets for the drug. HKH40A is curative for orthotopic liver cancer in rats, with no evidence of toxicity. HKH40A is a prime agent for clinical development.



550 POSTER

Phase I and pharmacokinetic (PK) study of trabectedin (ET-743) administered as a 1-hour infusion weekly for 3 consecutive weeks every 4 weeks to patients with advanced cancer

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Trabectedin is a tetrahydroisoquinoline alkaloid isolated from *Ecteinascidia turbinata* covalently targets guanine in GC-rich region of DNA minor groove creating DNA bend towards the major groove; interferes transcription factors-DNA interaction; and causes DNA breaks by nucleotide excision repair. Thus cancer cells undergo apoptosis or cell cycle arrest. Trabectedin has been tested in phase I and II studies using different infusion schedules and doses and is well tolerated with preliminary activity in sarcoma, breast and ovarian cancer. To maximize the tolerability, efficacy, and overall therapeutic index of trabectedin, this study evaluated the feasibility, safety, and PK behavior of trabectedin as a 1-h infusion weekly ×3 every 4 weeks. The results of a previous study at our institution indicated a favorable toxicity profile and antitumor activity when the agent was administered over 3 hours weekly ×3 every 4 weeks. To date, 31 pts (median age 45, [23–75]; M:F 17:14; tumor types: sarcoma:ovarian:breast:melanoma 27:2:1:1; 105 Cycles was delivered with a median of 2 [1–14] over 6 dose levels (460 [6], 580 [3], 610 [6], 700 [8], 800 [6 lightly-pretreated (LP) & 1 heavily-pretreated (HP)], 920 mcg/m2 [1]). Dose-limiting toxicities (DLTs) during the first 2 cycles include: gr 4 ANC >5 d [1] at 460; gr 3 myalgia/fatigue [1] at 610; delay of Cycle 2 >2 wk for ANC<1,500, gr 3 neuropathy/fatigue, febrile neutropenia [1 each] at 700; gr 4 neutropenia, followed by rhabdomyolysis and death at 800 in a heavily-pretreated ovarian cancer pt with compromised bone marrow reserve due to repeated carboplatin exposure; treatment held for 2 weeks [1] at 920. Toxicities were mostly mild to moderate except: asymptomatic gr 3 transaminase elevation [22.5%], gr 3/4 CK [6%], gr 3/4 ANC [19%], gr 3 fatigue and myalgia [3%] and gr 3 vomiting [3%], which all occurred at doses ⩾700. PK evaluation up to 700 demonstrated linearity, similar to prior data of other dosing schedules, with $\tau_{1/2}$ 56.5±54.2 h and Vss 2463±1580 L. A confirmed PR was observed for 36 wk in a second-line metastatic uterine leiomyosarcoma; and SD in 4 leiomyosarcoma [24–28 wk], 2 liposarcoma [14, 56 wk] and 1 fibrosarcoma [16 wk] were observed. Clinical activity was seen in selected soft tissue sarcoma subtypes, which failed prior doxorubicin and/ifosphamide-based

chemotherapy. With dose-limiting myelosuppression occurred in only HP pts at 700 and 800 and mainly gr 1–2 toxicity at 610, the recommended phase II dose (RD) for HP is proposed at 610. To further define toxicity profile and RD for LP pts, accrual is ongoing at 800. PK at 800 will be available at the meeting. Weekly 1-h infusion of Trabectedin seems to be convenient, active and well tolerated.

551 POSTER

Irofulven (IROF) enhances the antiproliferative effects of oxaliplatin (oxa) in human colon and breast cancer cells

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Background: IROF (6-hydroxymethylacylfulvene, MGI-114) is a novel DNA-interacting anticancer drug derived from the mushroom natural product illudin S. IROF displays activity against human tumors *in vitro* and *in vivo*, and clinical trials as a single agent and in combination with several other anticancer drugs are underway. This study examined the cytotoxicity by combining IROF with OXA, a DACH-platinum compound demonstrating clinical activity in a wide variety of tumors including colorectal cancers. **Materials and Methods:** Drug interaction studies were performed using the Chou & Talalay method in a panel of human colon and breast cancer cell lines. **Results:** Single agent IROF displayed cytotoxicity against human colon cancer HT29 cells (IC50: 1.3±0.2 μg/mL), HT29 IROF-resistant IF2 cells (IC50: 92±9 μg/mL) and human breast cancer MCF7 cells (IC50: 2.0±0.2 μg/mL), HT29 being the most sensitive. In HT29, the OXA-IROF combination led to clear evidence of synergistic activity (Figure 1).

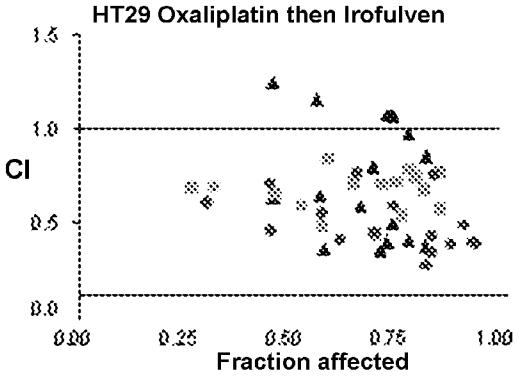

Figure 1. Combination indexes (CI) of IROF-OXA combinations in HT29 colon cancer cells: each spot represents one experiment performed in triplicate (shapes distinguish three separate experiments).

OXA given prior to or after IROF, consistently led to CI<1, demonstrating synergy. Similarly, simultaneous exposure to IROF and OXA was associated with synergy. Similar experiments were done in HT29 IROF-resistant IF2 cells. Acquired resistance to IROF slightly decreased sensitivity in this cell line to the IROF-OXA combination. Additive anti-proliferative effects were observed at low concentration IROF-OXA combinations; whereas, synergy was seen at higher concentrations. From our results, the sequence OXA followed by IROF appears to be the most efficient. To validate the experiments in colon cancer HT29 cells, we explored these combinations in MCF-7 breast cancer cells. In this cell line, additive and/or synergistic effects were observed when OXA was given after or concomitantly to IROF. Only additive effects were observed when OXA was given prior to IROF. **Conclusion:** IROF displays synergistic anti-proliferative effects when combined with OXA over a broad range of concentrations in human colon and breast cancer cells. Acquired resistance to IROF has limited impact on the effects of the combination. Based on these data, the IROF-OXA combination will be further explored in clinical trials, preferably using an OXA prior to IROF schedule.

552 POSTER

Genetic alterations and histology are related to the distinct responses of xenografted gliomas to different alkylating agents

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Background: Response of gliomas to chemotherapy varies widely according to their histological subtype and grade. GBM being resistant while oligodendrogliomas are more chemosensitive. Recent studies showed that, in oligodendrogliomas, the double loss of chromosome 1p and 19q was related to an overall better prognosis. In contrast, several studies have identified genetic alterations related with a poor prognosis, such as EGFR amplification, PTEN mutation or 10q loss, CDKN2A homozygous deletions. Nevertheless, no clear correlation with chemoresistance has been established.
To further study the molecular alterations underlying response to chemotherapy, a series of 12 human gliomas, derived from surgical specimens, was established as xenografts in *nude* mice and used to evaluate *in vivo* the relationship between histology, genetic parameters and response to alkylating drugs generally used in malignant gliomas, BCNU, Carboplatin (CP), Ifosfamide (IFO), and Temozolomide (TMZ).
Material and methods: Of the 12 xenografts used, 8 were high-grade oligodendroglial tumors and 4 were GBM. They were characterized for their genetic alterations, including those considered as "early" alterations, namely chromosome 1 loss +/− chromosome 19q loss, TP53 mutation, and those considered as "late" alterations, namely chromosome 10 loss, chromosome 9p loss, EGFR genomic amplification, PTEN mutation, CDKN2A homozygous deletion and telomerase reactivation. Chemosensitivity to 4 alkylating agents, TMZ {42 mg/kg, d1–5 per os (p.o.)}, BCNU {(5 mg/kg, d1 intraperitoneal (i.p.)}, IFO {90 mg/kg, d1–3, i.p.}, and CP {66 mg/kg, d1, i.p.} was tested.
Results: Although each tumor presented an individual response pattern, GBM had a lower chemosensitivity than oligodendrogliomas and TMZ was the most effective drug. Deletion of 1p+/−19q was associated with higher chemosensitivity, while late molecular alterations, particularly EGFR amplification were associated with chemoresistance.
Conclusions: These results suggest that the combined use of histology and molecular markers should eventually be helpful to select the most appropriate agents in malignant oligodendrogliomas and astrocytomas.

553 POSTER

Activation of trans geometry in bifunctional mononuclear platinum complexes by combining aliphatic and aromatic amines. Mechanistic studies on antitumor action

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The global modification of mammalian and plasmid DNAs by novel platinum compounds, *trans*-[$PtCl_2$(Am1)(Am2)], where Am1 = isopropylamine and Am2 = 3-hydroxymethyl or 4-hydroxymethyl was investigated in cell-free media using various biochemical and biophysical methods. These modifications were analyzed in the context of the activity of these new compounds in several tumor cell lines including those resistant to antitumor *cis*-diamminedichloroplatinum(II) (cisplatin). The results showed that the replacement of both ammine group in clinically ineffective trans isomer of cisplatin [*trans*-diamminedichloroplatinum(II) (transplatin)] resulted in a radical enhancement of its activity in tumor cell lines so that these analogues were more cytotoxic than cisplatin and exhibited significant antitumor activity including activity in cisplatin-resistant tumor cells. Importantly, this replacement also markedly altered DNA binding mode of transplatin. The results offer a strong experimental support for the view that one strategy how to activate trans geometry in bifunctional platinum(II) compounds and to circumvent resistance to cisplatin consists in a chemical modification of the conventional transplatin which would result in their increased efficiency to form in DNA interstrand cross-links. The present work also suggests that such a modification may be accomplished by the replacement of both ammine groups by aliphatic amine ligands, such as isopropylamine and 3-hydroxymethyl or 4-hydroxymethyl. Moreover, the analogues of transplatin apparently represent a novel class of platinum anticancer drugs acting by a different mechanism than "classical" cisplatin.
Acknowledgments
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