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Pharmacokinetics and safety of OSI-7904L (Liposomal Thymidylate Synthase Inhibitor) in patients with advanced solid tumours

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Background: OSI-7904L is a liposomal formulation of OSI-7904, a potent thymidylate synthase (TS) inhibitor, currently in Phase I clinical trials. OSI-7904 does not require polyglutamation for activity and binds noncompetitively to the TS enzyme. Nonclinical studies have demonstrated that liposome encapsulation of OSI-7904 results in increased plasma residence and superior anti-tumour activity compared to either the non-liposomal drug or to 5-fluorouracil.
Methods: In this ongoing Phase I dose escalation study nine patients have received OSI-7904L at 3 dose levels: 0.4, 0.8 and 1.6 mg/m^2 (3 pts/cohort). Drug was administered via a 30 minute IV infusion on a day 1 every 21 day schedule. Plasma and urine samples were obtained over 7 days following the first dose for assessment of OSI-7904 concentrations using a validated LC/MS/MS assay. Pharmacokinetic parameters were determined using noncompartmental analysis.
Results: Eight males and 1 female have been entered so far with a median age of 64 yrs (range 39-67) and the following tumour types: 6 colorectal, 1 each Klatskin, liposarcoma and testicular. The median number of prior chemo regimens per patient was 3 (range 2-4) and 7/9 pts have received prior TS inhibitor therapy. A total of 21 cycles have been administered to date. Plasma concentrations of total OSI-7904 following IV infusion of OSI-7904L decreased in a biphasic manner with a terminal half-life of approximately 60 hours. Substantial interpatient variability was observed apparently due to differences in the amount of total drug cleared in the alpha phase. C_{max} values appeared to increase linearly with increasing dose with median values of 159, 488 and 792 ng/mL for the 0.4, 0.8 and 1.6 mg/m^2 dose groups, respectively. AUC also appeared to increase with increasing dose with median values of 3480, 6720 and 11400 ng hr/mL, respectively. Administration of liposomal OSI-7904L (0.8 mg/m^2) yielded a 17-fold increase in dose normalized AUC relative to administration of non-liposomal OSI-7904 (1.0 mg/m^2) (Schwartz et al. 2001).
Conclusions: These preliminary data indicate that this liposomal formulation (OSI-7904L) appears to alter the disposition properties of the parent drug resulting in a long circulating time and dose-related increase in plasma concentration.

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In vivo anti-tumor efficacy of liposomal OSI-7904L in human tumor xenografts

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OSI-7904L (previously GS7904L) is a liposomal formulation of the thymidylate synthase (TS) inhibitor OSI-7904 (previously GW1843U89, GS7904). The *in vivo* efficacy of OSI-7904L, currently in Phase I clinical trials, has been evaluated in 27 different human tumor xenografts in mice on folate free diet. Of the histiotypes tested, colon-derived lines showed the highest response rate to drug treatment (66% response rate, 8/12; response defined as >45% tumor growth inhibition versus vehicle treated controls) compared to all other types combined (46% response rate, 7/15). In an effort to understand the factors contributing to response to this agent, the colon subset was analyzed for p53 status, TS promoter polymorphism, TS levels, TS inducibility by OSI-7904L, folate receptor status, and tumor doubling time. Tumor TS levels were measured by immunoblot analysis prior to treatment with 25 mg/kg OSI-7904L and 24 hrs after treatment. Both baseline and 24 hr TS levels were inversely correlated with TGI, indicating the importance of TS in response to OSI-7904L. In the literature, the homozygous double repeat sequence (2R/2R) in the TS promoter region has consistently correlated with lower TS levels and better clinical response to 5-FU. Of the colon lines tested here, 9/12 were 2R/2R for the promoter polymorphism, possibly contributing to the high response rate. To confirm the relevance of the TS pathway, paired isogenic colon cell lines, which differ only in levels of TS or the salvage pathway enzyme, thymidine kinase (TK), were utilized to directly compare tumor response. H630-10 human colon carcinoma cells have acquired resistance to 5-FU through gene amplification of the TS gene. Baseline tumor xenograft TS levels were confirmed to be 10-20 fold higher than the H630 parent line. TGI of H630 tumors was 64% after treatment with OSI-7904L (25 mg/kg; qd1-5) compared to 31% TGI in H630-10 which was less responsive to this agent. Thymidine kinase can utilize circulating thymidine to form thymidylate and circumvent TS inhibition. HCT-8 TK(-/-) human colon xenografts responded better to OSI-7904L treatment (96% TGI) compared to the TK-competent HCT-8 parent line (73% TGI). TGI positively correlated with *in vitro* folate receptor levels assessed by flow cytometry but did not correlate with tumor doubling time or p53 status. Taken together, these results confirm the importance of the TS pathway for this class of agents and the selectivity of the TS inhibitor, OSI-7904L.

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Mechanisms of activation of FdUMP[10], by evaluation of intracellular thymidylate synthase inhibition in FM3A cells

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5-Fluorouracil (5FU) and 5-fluoro-2'-deoxyuridine (FUdR) exerts their activity by inhibition of thymidylate synthase (TS) via their metabolite FdUMP. FdUMP, is a suicide inhibitor of TS, but cellular uptake or intracellular activation limit the effect of 5FU. Therefore a multimer of FdUMP, FdUMP[10] was synthesized, acting as a FdUMP-prodrug. FdUMP[10]is stable in cell culture. After cellular uptake, FdUMP is supposed to be released intracellularly to inhibit TS. In H630 cells FdUMP[10] was 400-times more cytotoxic than 5FU. We characterized how FdUMP[10] would exert its cellular effects. For this purpose we used the TS in situ assay (TSIA), which enabled evaluation of intracellular TS inhibition. For this assay we used tritiated deoxycytidine, which, after cellular uptake, was converted to tritiated dCMP, subsequently to tritiated dUMP, and by TS to dTMP, releasing tritiated water. We measured TSIA in parent FM3A cells and its thymidine kinase deficient variant FM3A/TK-. The TS inhibition was evaluated after a 4-hr exposure, a 4-hr exposure followed by incubation in drug-free medium (DFM), and a 24-hr exposure, and compared with equimolar FdUMP concentrations. After 24 hr exposure to 0.5 μM FdUMP and 0.05 μM FdUMP[10] TSIA were 7% and 1% of control, respectively, but at 4 hr only 19% and 7%, respectively, which increased to 61% and 69%, respectively, after suspension in DFM. FUdR at 5 nM was equally active (0% at 4 and 24 hr, increasing to 55% after DFM), and the effect of 1 μM 5FU was 47% at 4 hr, and 20% at 24 hr, increasing to 70% after DFM. In FM3A/TK- cells, TSIA was decreased to only 94 and 86% after FdUMP or FdUMP[10] exposure for 4 hr, and was similar after 24 hr. In addition, FUdR, which needs activation by TK, was inactive, while 5FU was only slightly less active than in FM3A cells. These results indicated that both FdUMP and FdUMP[10] needed degradation to FUdR in order to be active in FM3A cells. Since activation can occur both intra- and extracellularly, we exposed the cells to the drugs in combination with a specific phosphatase and nucleotidase inhibitor. These inhibitors reduced the effect of both FdUMP and FdUMP[10] in FM3A cells after 24-hr exposure to 26 and 56%. In conclusion, FdUMP[10] probably has a dual effect, either as a direct prodrug for FdUMP after intracellular uptake, or as a prodrug of FUdR which is formed extracellularly. This activation seems to be tumor specific because of the better therapeutic efficacy *in vivo*.

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Enhanced DNA-directed effects of FdUMP[10] compared to 5-FU

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Our laboratory is investigating FdUMP[10] as an alternative to 5-FU for fluoropyrimidine chemotherapy for treatment of colorectal cancer and other solid tumors. In the NCI 60 cell line screen, FdUMP[10] was about 400 times more effective at inhibiting tumor cell growth. We have conducted time-lapse video microscopy to investigate the relative pro-apoptotic and anti-proliferative effects of FdUMP[10] and 5-FU in HT-29 cells. FdUMP[10] at 10-8 M concentration induced relatively few pro-apoptotic responses compared to 5-FU at 10-6 M, however, it nearly completely inhibited cell proliferation at this concentration. Flow cytometry demonstrated that HT-29 cells exposed to FdUMP[10] accumulated in S-phase. Comet assays were used to determine if HT-29 cells exposed to FdUMP[10] incurred greater DNA damage. Exposure of HT-29 cells to FdUMP[10] resulted in substantially

greater tail moments than was observed for cells exposed to 5-FU. The results are consistent with FdUMP[10] displaying enhanced DNA-directed effects relative to 5-FU.

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The antitumor activity of OSI-7836 (GS7836, 4'-thio-araC), a nucleoside analog, in combination with cisplatin in human NSCLC xenografts in mice

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OSI-7836 (GS7836, 4'-thio-araC) is a nucleoside analog currently in Phase I trials for the treatment of solid tumors. OSI-7836 has been shown to have antitumor efficacy in a range of xenograft models, including non-small cell lung carcinoma (NSCLC). Cisplatin, a standard agent used in combination in the treatment of NSCLC, was selected for combination studies with OSI-7836 in three NSCLC xenograft models (Calu-6, H460, HOP-92). Each drug was administered alone at its predetermined optimal dose on a day 1,8 schedule (OSI-7836, 1000 mg/kg; cisplatin, 9 mg/kg) and at 60% of optimal dose of each drug for the combination (OSI-7836, 600 mg/kg; cisplatin, 5.4 mg/kg). In the H460 xenograft model, the combination of OSI-7836 and cisplatin demonstrated increased efficacy with a Log Cell Kill (LCK)= 2.4, compared to OSI-7836 alone (LCK=1.4) or cisplatin alone (LCK= 0.6). In addition, the combination produced cures in 2 of 8 animals compared to 0 of 8 animals for either single agent. In the HOP-92 xenograft model, LCK could not be determined for either group dosed with OSI-7836 due to the large number of cures, but the OSI-7836 plus cisplatin combination demonstrated improved efficacy (6 of 8 animals cured) compared to OSI-7836 alone (4 of 8 cures) and cisplatin alone (1 of 8 cures). Similarly, in the Calu-6 xenograft model, the combination of OSI-7836 and cisplatin demonstrated improved efficacy (7 of 8 cures) compared to OSI-7836 alone (2 of 8 cures) and cisplatin alone (1 of 8 cures). Further preclinical OSI-7836 and cisplatin combination studies are ongoing. These data demonstrate that OSI-7836 can be combined effectively with cisplatin to improve the antitumor efficacy in lung xenograft models, supporting the investigation of this combination in the clinic for NSCLC.

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Phase II trial of edatrexate in adult patients with metastatic soft tissue sarcomas, an Eastern Cooperative Oncology Group (ECOG) final report

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Edatrexate (EDX) is a water soluble anti-folate, formed by modification of the N10 position of 4-amino-folate. Based on promising preclinical and clinical data for soft tissue sarcoma (STS) (Casper et al. Cancer 1993;72:766-770), ECOG performed a phase II study of EDX as first line chemotherapy in metastatic STS. Wasserheit, C. et al (ASCO, 1998) reported preliminary data. All types of adult STS were allowed, with the exception of synovial cell sarcoma (competing trial). EDX was given as an intravenous infusion of 80 mg/m^2 weekly for 5 weeks, followed by dosing every other week. Starting week 7, doses were escalated every other week by 10 mg/m^2 unless toxicity occurred. Of the 46 patients entered, two were ineligible. Patients' histologies included 16 leiomyosarcomas, 7 MFH, 5 spindle cell tumors, 4 liposarcomas, and the remainder with various other histologies. For the 44 eligible patients, based on an intent to treat analysis, the response rate was 14% (95% confidence interval 5-27%), including 5 partial responses 11% (95% confidence interval 4-27%), and 1 complete response 2%. The duration of partial responses ranged from 54 to 598 days with the complete response continuing at last follow up. At a median follow-up period of 18.2 months, the median survival was 14.5 months. For the 45 patients with toxicity data, two toxic deaths occurred, which may have been EDX related: hemorrhage; pneumonia. Grade 3 or 4 toxicity occurred in 29% of patients. Most common toxicities were liver (11%), stomatitis (9%), anemia (9%), nausea (7%), arthralgias (7%), and less than 5% included leukopenia, thrombocytopenia, vomiting, diarrhea, and fatigue. In conclusion, response rates and durations, survival and toxicity are comparable with that achieved by chemotherapy considered "standard of care." This study population had a high proportion of patients with unfavorable risk factors for response to chemotherapy, including 36% leiomyosarcoma, 42% older than 60 years and 45% with liver metastases. Further EDX study is justified given these results and the limited efficacy of currently available therapy.

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A pharmacokinetic (PK) phase I (PI) study of ZD9331 and carboplatin in relapsed ovarian cancer (ROC) with a pharmacodynamic (PD) endpoint

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ZD9331 is a rationally designed, specific non-polyglutamatable thymidylate synthase (TS) inhibitor that is active against ROC in phase I/II trials (Plummer et al, Proc. Am. Soc. Clin. Onc., 1999: Rader et al, Annals Oncol, 11: 83; 2000). In light of broad sensitivity of human ovarian tumour cell lines to ZD9331 and non cross resistance to platinum based drugs (Jackman et al, Biochem Biophys Acta, in press) we studied the combination ZD9331/carboplatin in a PI PK & PD study in ROC. Eligible patients were 18 yrs or over, with a histological diagnosis and radiological evidence of ROC, and a platinum treatment - free interval of at least 6 months. Up to 3 prior lines of chemotherapy were permitted. Carboplatin was administered on day 1 of each 21day cycle (60-min IV infusion) at a fixed dose of AUC5. ZD9331 was given on day 1 (2 hrs after carboplatin, 30-min IV infusion) and day 8 of each 21day cycle. Thirteen patients have been enrolled to date (median age of 57), treated with ZD9331 at 4 dose levels, 40 mg/m^2(1), 65 mg/m^2(2), 85 mg/m^2(3)and 100 mg/m^2(4). No grade 3 or 4 toxicities were seen at dose level 1. One patient had grade 3 neutropaenia at level 2, and 1 patient had grade 3 neutropaenia at level 3. Dose limiting toxicities were seen in 2 patients at 100 mg/m^2 (grade 4 neutropaenia $>$ 7 days, and grade 4 fatigue $>$ 7 days). Plasma deoxyuridine (measured by HPLC in 6 patients in dose levels 1 and 2) was elevated indicating TS inhibition to at least day 12. Plasma ZD9331 was measured by ELISA, using a specific ZD9331 rabbit polyclonal antibody. Carboplatin was assayed by atomic absorption spectrometry. There was no PK interaction between the 2 drugs as ZD9331 PK data were similar to previous monotherapy studies, and measured AUC of carboplatin corresponded with that administered (WinNonlin professional, compartmental analysis). Antitumour activity was observed in 4/13 patients. The likely recommended phase II dose (carboplatin AUC5, ZD9331 85mg m^2) is being expanded to 6 patients with further PK and PD evaluation at this dose level. We conclude that this combination is well tolerated, TS inhibition is achieved at doses below the maximum tolerated dose, and antitumour activity is observed.

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Phase I and pharmacokinetic study (PK) of the combination of multitargeted antifolate pemetrexed (ALIMTA) with irinotecan (CPT-11) in patients with advanced malignancies

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Inhibition of key enzymes involved in folate metabolism remains an attractive therapeutic strategy. Pemetrexed, pyrrolopyridimine based antifolate is a potent inhibitor of multiple folate-dependent enzymes including thymidylate synthase, dihydrofolate reductase and glycinamide ribonucleotide formyl transferase and has broad-spectrum antitumor activity. Preclinical evidence of synergy and minimally overlapping toxicity profiles served as the rationale for this clinical evaluation of the combination of pemetrexed and CPT-11. The feasibility of administering this combination is being evaluated in a phase I trial. Pemetrexed is given IV over 10 min followed by CPT-11 as a 90-min IV infusion every 3 weeks. 23 patients received 75 courses at the following pemetrexed/CPT-11 doses (mg/m^2) (patients/courses): 300/175 (3/13), 300/250 (6/22), 400/250 (9/21), 500/250 (5/19). Myelosuppression was the principal toxicity in this group of patients. Febrile neutropenia was seen at 300/250 (1 patient) and grade 4 vomiting at 400/250 (1 patient)(DLT). Other non-dose-limiting toxicities were diarrhea and skin rash. The protocol was then amended to include 'nutritional' quantities of folic acid (FA) (400 mcg/d) and vitamin B12 (1mg/9 weeks) supplements so as to reduce pemetrexed-induced toxicity and allow further dose-escalation. Doses explored with FA and B12 are 450/250 (4/41), 500/250 (9/61) and 500/300 (4/21). Demographics: median age- 58 (30-77); 30 M/10 F; PS 0-1(36) and PS 2(4). Primary tumors: Colorectal (10), Mesothelioma (10), Lung (6), Pancreas (3), Hepatobiliary (3) and others (8). One febrile neutropenia (DLT) was seen at 500/300 and accrual continues at this dose. One partial response in a patient with a 5-FU-refractory metastatic rectal cancer and 3 minor responses in patients with ampullary,