58

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.

59

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.

60

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*.

61

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