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**A phase I trial of LY573636 in patients with advanced solid tumors**

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LY573636 is an acylsulfonamide that induces apoptosis through an unknown and potentially unique mechanism of action. Preclinical studies demonstrate broad activity in both in vitro and xenograft tumor models. LY573636 activates the intrinsic apoptotic pathway, however evaluation using the NCI COMPARE analysis has not revealed any mechanistic similarities to known chemotherapeutic agents. The primary objective of this trial was to evaluate the toxicity of LY573636 given as a flat dose, intravenous, 2-hour infusion every 21 days. Three patients were treated at each dose level, with dose escalation based on the modified Fibonacci schema. The starting dose in humans was 100 mg (1/10 of the MTD in the most sensitive species). To date, 18 patients with various cancers including 5 patients with non-small cell lung cancer (NSCLC) have been treated with a total of 51 doses at 6 dose levels (100mg, 200mg, 400mg, 660mg, 1000mg &1400mg). The mean number of cycles given at each completed dose level is 9. Seven patients have had stable disease allowing for more than 2 cycle of therapy. The maximum number of cycles given to any individual patient is 7 (thymoma). Toxicities have been minimal (Grade 1). We have not yet observed any hematologic or gastrointestinal toxicity to date. No dose limiting toxicities have been observed and therefore dose escalation continues. A secondary objective of this study is to determine the pharmacokinetics(PK) of this drug. PK samples have been analyzed for the first 12 patients following 100–660mg. The total plasma LY573636 concentration increased linearly with dose over this dose range and the terminal half-life appears to correspond to the rate of albumin turnover (approximately 15 days). The total volume of distribution of LY573636 is approximately that of albumin (8–9L). An additional secondary objective has been to evaluate the use of the M30-Apoptosense assay (DiaPharma, Ohio) as a tool to measure in-vivo level of apoptosis in response to treatment with LY573636. Plasma samples for apoptosis measurement have been collected on all patients at doses 400 mg and above, at multiple time points. The analysis is not yet complete but will be presented at the meeting.

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**Activation of caspases in human t-lineage leukemia cells treated with analogs of leflunomide metabolite**

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Caspase activation plays a central role in the execution of apoptosis. There are two major biochemical pathways of caspase activation: the cell surface death receptor pathway and the mitochondria-initiated pathway. In the cell surface death receptor pathway, activation of caspase-8 is the critical event that transmits the death signal. Activated caspase-8 can, in its turn, activate downstream caspases by direct cleavage of caspase-3 or indirectly by cleaving Bid and inducing cytochrome c release from the mitochondria. In the mitochondrial-initiated pathway, caspase activation is triggered by the stress-induced formation of an apoptosome, a complex of apoptosis protease activating factor-1 (Apaf-1), cytochrome c and ATP. This complex activates procaspase-9. Then active caspase-9 cleaves and activates downstream caspase-3. Pro-caspase-3 is present in both the mitochondrial and cytosolic fractions of untreated Jurkat T lymphocytes. Pro-caspase-8 was found only in the cytosolic fraction. In apoptotic cells, caspase-3 was present in the cytosolic, mitochondrial, and nuclear fractions, whereas caspases-8 was still found in the cytosolic fraction only. A drug library of leflunomide derivatives were synthesized as a result of structure-based design models and evaluated for in vivo caspase-3 & 8 activation. In a systematic effort of anticancer drug discovery, we have constructed three-dimensional homology models of the EGF-R kinase domain and the BTK kinase domain to evaluate structure activity relationships and design new analogs of leflunomide metabolite (LFM) with more potent inhibitory activities against EGF-R tyrosine kinase and BTK. We then subsequently prepared more than one hundred compounds (LFM-analog) with different X-groups. The purpose of the present study in this paper was fast screening of the new LFM analogs for their apoptotic effect on cancer cells using active caspase-3 and caspase-8 as a specific markers of apoptosis. Two leading compounds, LFM-A12, a-Cyano-b-hydroxy-b-methyl-N-[4-(trifluoromethoxy)phenyl]propenamide as an inhibitor of the Epidermal Growth Factor Receptor Tyrosine Kinase and LFM-A13, [a-cyano-b-hydroxy-b-methyl-N-(2,5-dibromophenyl)propenamide as an selective inhibitor of Bruton's Tyrosine Kinase (BTK), respectively, emerged as potent activators of caspase activity.

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**Roles of N-cadherin in anoikis and invasion**

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Disruption of integrin-extracellular matrix interactions in normal epithelial cells induces apoptosis, a process known as anoikis. Anoikis plays an important role in blocking the growth of detached cells at inappropriate locations, thus the acquisition of anoikis-resistance is regarded as a critical step during the metastatic transformation of a tumor. In the present study, we used esophageal cancer cell lines as a model system to elucidate the molecular mechanism (s) of anoikis. Cells were cultured on poly-HEMA coated tissue culture plates for 3, 24 and 48 hours. Based on microscopic observations, all the cell lines formed compact multi-cellular aggregates (MCA) by 16 hours post-plating. Lysates were prepared at each time points from each cell line, and western blot immunostaining was performed with antibodies against PARP and pro-caspase 3 and then, the cells were classified as anoikis-sensitive (TE2, TE3) or anoikis-resistant (HCE4, HCE7). Anoikis sensitive cells expressed E-cadherin whereas resistant cells expressed N-cadherin that is expressed only in mesenchymal cells. When N-cadherin is down-regulated by siRNA, anoikis-resistant cells underwent apoptosis but when N-cadherin-mediated cell-cell adhesion is disrupted, the cells remained to be resistant to anoikis suggesting that survival signal could be generated not by N-cadherin mediated cell-cell adhesion but by N-cadherin itself. In addition, N-cadherin expressing HCE4 cells showed higher invasive ability than E-cadherin expressing TE2 cells but down-regulation of N-cadherin by siRNA reduced its invasive ability. Taken together, our results showed that survival signal generated by N-cadherin make cancer cells resist to anoikis as well as invade to surrounding tissues and drugs targeting N-cadherin down-regulation may reduce the chance of metastasis and invasion.

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**Significance of radiation apoptosis in cancer and its modification by beta carotene**

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Apoptosis is the predominant biological response of crypt epithelial cells to low levels of genotoxic and cytotoxic damage that occur chronically in the small intestine. Intracellular oxidation increased by radiation generated free radicals play a major role in apoptotic cell death. The effect of beta-carotene has been studied on the radiation-induced apoptosis in 6 weeks Swiss albino mice exposed to 4.5 Gy of $\gamma$ radiation. Quantitative cellular variations of intestine were studied taking apoptotic Index, post exposure survival fraction of jejunal crypt cells spared from apoptotic death. Number of apoptotic cells, number of mitotic figures and number of total cell population /crypt section, number of total cell population/villus section were taken as endpoints for 30 days study. An inverse relationship of apoptotic index and survival fraction of crypt cells is noticed. Radiation-induced acceleration in apoptosis has been found associated with greater adaptability. The results of our study support the fact that dietary antioxidants can limit epithelial cell death in response to oxidant stress. In the case of retinoids, beta carotene the cytoprotective response exceed their inherent ability to interact with the injurious oxidant which is suggestive of actions on intracellular pathways regulating cell death. It is also clear from the findings that the antioxidants like beta carotene can protect the normal cell against radiation induced apoptosis at the regulatory gene level. The data is indicative of the future prospects and ensures exploitation of oral administration of $\beta$-carotene against radiation in clinical application and radiotherapy for cancer. Taking jejunal crypt cell as model for a fast proliferating tissue, present study also envisages the apoptosis as an effective gene mediated cellular phenomenon with a strategy to increasing probability that any particular crypt epithelial cell will survive injury and acquire the set of multiple regulatory/modulatory mechanisms against mutations which are necessary for malignancy to occur. The high proliferating capacity and a feed back mechanism which can speed up or slow down the production of new cells according to demand should involve regulatory genes, which warrants careful evaluation for an effective radiotherapy.