The mRNA was labelled with fluorescent dyes (Cy3 and Cy5) using reverse transcription and hybridised to 5808 cDNA clones arrayed on glass slides. The array slides were prepared in-house and encompass known genes and ESTs known or suspected to be associated with cancer, for example apoptosis genes and cell cycle genes. Cell cycle analysis showed that each cell line was arrested in the G1 phase of the cell cycle and a depletion of cells in S phase was also found. Each cell line was affected to a varying degree. Both 5FU and 5FdUrd treatments cause similar effects upon the cell cycle in these three cell lines. Microarray analysis of 5FU and 5FdUrd treated cells demonstrated that each cell line had its own distinctive pattern of gene expression, which was time and drug dependent. A considerable number of genes exhibited altered expression, either increased or decreased relative to control levels. In excess of 500 genes were altered over the entire course of the experiment within each cell line. No evidence of alteration of genes involved in pyrimidine metabolism or DNA/RNA metabolism was found. No evidence for p53 induced gene expression could be found in wild-type p53 HCT116 cells, in contrast to published data. Within one cell line, a number of integrin related genes appear to be affected by both 5-FU and 5-FdUrd. Whereas another cluster of genes affected within this cell line appear to relate to 5FU mechanism of action compared to 5FdUrd.

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Combination of troxacitabine (troxatyltm) and cytosine arabinoside is beneficial in human leukemia cells

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Troxacitabine (Troxatyltm) is a novel β-L-nucleoside analogue with potent antineoplastic activity both *in vitro* and in several tumor models *in vivo*, and is presently in Phase II clinical trials. Recent results have shown that the combination of troxacitabine and araC in patients with leukemia has resulted in complete responses. To determine the mechanistic basis of this interaction, we have investigated, in the laboratory, the *in vitro* and *in vivo* effect of the combination of araC and troxacitabine against lymphoblastic CCRF-CEM leukemia. We observed that combination of troxacitabine and araC *in vitro* was synergistic with combination indices between 0.1 and 0.7 as calculated with the CalcuSyn software (Biosoft). The combination of troxacitabine and araC had a greater impact on DNA synthesis recovery than either agent alone. Analysis of the effect of troxacitabine on the intracellular metabolites of araC revealed that troxacitabine did not inhibit araC deamination and caused a slight decrease in the overall intracellular accumulation of araCTP. The lower accumulation of araCTP could not be attributed to the feedback inhibition caused by troxacitabine triphosphate on dCK. Furthermore, our *in vivo* experiments demonstrate that combination of araC and troxacitabine is better at slowing down the progression of leukemia in mice than either agent alone without additive toxicities. Injections i.p. qdx5 of 10mg/kg araC in combination with troxacitabine at 5 or 10 mg/kg increased ILS of mice to 43 and 57% compared to 34% for araC alone and 41 and 44%, respectively for troxacitabine alone: this represents an improvement in ILS of 8 and 17%, respectively when compared to araC alone at 10mg/kg. A pharmacokinetic study revealed that troxacitabine did not influence the disposition of araC. Overall, the results show that the anti-leukemic activity of troxacitabine and araC is complementary when both nucleoside analogues are used in combination. These effects appear to be related, at least in part, to their respective cytotoxic characteristics rather than to a pharmacokinetic interaction. These results encourage the use of troxacitabine and araC in combination as a first-line regimen for treatment of leukemia.

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TP53 status may predict pathological complete remission (pCR) to cisplatin + fluorouracil + leucovorin (PFL) in ethmoidal intestinal type adenocarcinoma (ITAC) treated with preoperative chemotherapy

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Background: Intestinal-type adenocarcinoma (ITAC) of the ethmoid sinus is an uncommon tumor. The prognostic value of pCR is well recognized in H&N cancer. The pCR is defined as the absence of any tumor cell at pathological evaluation of the complete surgical specimen. A correlation between TP53 status and pathologic complete remission (pCR) to primary PFL chemotherapy followed by cranio-facial resection was performed within a prospective phase II trial on ethmoid ITAC. Of 37 ITACs included in the study 23 diagnostic specimen biopsies were available and analyzed for TP53 status. Chemotherapy consisted of PFL every 3 weeks for 2-5 courses. Pts not achieving a clinical partial remission after two cycles underwent immediate surgery.

Methods: Genomic DNA was extracted from microdissected tissue obtained from formalin-fixed, paraffin-embedded tissue. TP53 gene from exon 5 to exon 8 of all samples were amplified by PCR and screened by dg-DGGE (double gradient-Denaturing Gradient Gel Electrophoresis) analysis. In cases showing an abnormal dg-DGGE pattern an automated DNA sequencing was performed.

Results: Tumor extension (UICC 97), TP53 status mutated vs wild type, number of chemotherapy cycles and number of pathological complete remission are reported in the cross-table.

N. cycles	T1/T2		T3/T4		Total pts
	TP53mut	TP53wt	TP53mut	TP53wt	
≤ 2	–	1*	5	–	6
3-5	3	2	8	4	17
pCRs	–	2	-	4	6

*this patient received only 1 cycle of PFL for cardiac toxicity and was not in pCR at surgery

Conclusions: Preliminary results indicate that pCR to PFL is strongly related ($p<0.0001$) to TP53 status. TP53 status seems to predict response better than T extension. Number of total cycles seems not to play a role in obtaining a pCR in TP53 mutated tumors. In the future, better selection of potentially responsive patients will allow more individualised treatment approach. Supported in part by AIRC.

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Rapid and complete thymidylate synthase (TS) inhibition in tumors after fluorouracil (5-FU) by methylene-tetrahydrofolate (ch2fh4) preloading

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TS is inhibited by the metabolite of 5-FU, 5-fluorodeoxyuridylate (FdUMP), in formation of a covalent ternary complex with 5,10-methylene-tetrahydrofolate (CH2FH4), that is required in great excess for stable inhibition. High degrees of TS inhibition are needed for effecting thymidine depletion and cytotoxities. Leucovorin (LV) has been used as a folate source in attempts to expand tumor CH2FH4 pools and increasing TS inhibition. We have previously shown that CH2FH4 is stable as a pharmaceutical, with promising results in Phase I-II trial with weekly 5-FU (Cancer J 10:266-297, 1997). We now show that preloading by i.v. bolus administration of pharmaceutical CH2FH4 (50-200 mg total dose) before bolus 5-FU results in immediate and substantial expansion of tumor CH2FH4 levels which are associated with unprecedented degrees of immediate TS inhibition. Serial surgical tumor biopsies were obtained in 17 patients given CH2FH4, in 19 patients given LV (500 mg/sq m), and in 9 patients given 5-methyl-tetrahydrofolate (CH3FH4). Tumoral TS activities CH2FH4 levels were measured by [3H-FdUMP] binding assays. The predominant tumor type was colorectal cancer in all groups. In the LV group, TS levels were over 0.5 pmol/g at 20 min after 5-FU in 8 of the 19 patients, with average (± SD) TS inhibition only 67.1 ± 32.0 percent, and only 5 patients showed over 97% inhibition. Results with CH3FH4 were even less effective than LV. In contrast, tumors of patients receiving CH2FH4 showed over 97% inhibition in 14 of the 17 patients, and all but one had less than 0.5 pmol/g TS activity by 20 min after 5-FU. Levels of tumor folates showed selective increases in CH2FH4, and in CH2FH4/FH4 ratios, by more than two-fold, to an average of 0.50 nmol/g and to 36.3%, respectively. These results provide direct evidence that CH2FH4 is rapidly transported into tumor tissue as the parent molecule, for effecting profound TS inhibition by FdUMP. Supported in part by the Swedish Cancer Society and NCI CA39629.