TOPOISOMERASE-MEDIATED DNA DAMAGE IS CELL CYCLE DEPENDENT. DM Sullivan, S Smallwood,* P Hodges,* and WE Ross, Departments of Pharmacology and Medicine, University of Florida, Gainesville, FL.

Many antitumor drugs induce DNA strand breaks (SB) via stabilization of a DNA-topoisomerase II "cleavable complex." We have examined the relationship between this phenomenon and cell proliferation using the intercalating agent m-AMSA and the non-intercalating epipodophyllotoxin VP-16. Wild type CHO cells were treated with drug at several points along the growth curve and the resulting SB's quantified by alkaline elution. An inverse relationship between the SB frequency and cell density was observed, i.e., early log phase cells demonstrated the greatest SB frequency, while late plateau phase cells (predominantly G_1 by flow cytometry) showed no drug-induced SB's. Addition of fresh growth media did not alter drug sensitivity. Uptake of [^{3}H]-VP-16 was not significantly different between log and plateau phase cells. Recovery of drug sensitivity by trypsinized plateau cells seeded at low density in new growth media was coincident with DNA synthesis as measured by [^{3}H]-thymidine incorporation. However, inhibition of DNA synthesis by aphidicolin did not affect recovery of drug sensitivity. Plateau phase cells also demonstrated marked resistance to the cytotoxic effects of VP-16 when compared to log phase cells. Our data suggest that the proliferation-dependent cytotoxicity of these agents is conferred by regulation of topoisomerase II activity during the cell cycle. This likely provides the basis for some of the therapeutic selectivity of these drugs. In addition, our data indicate that topoisomerase II and DNA polymerase α are not coordinately regulated as has been suggested for other putative components of the multienzyme replitase complex.

IMPORTANCE OF IMPAIRED rRNA PRODUCTION IN FLUOROPYRIMIDINE CYTOTOXICITY. CH Takimoto*, EC Cadman and RD Armstrong, Cancer Research Institute, Univ. of Calif., San Francisco, CA.

Previous reports have suggested that dThd-nonreversible fluoropyrimidine cytotoxicity reflects the level of fluorouracil (FUra) incorporation into RNA and may be mediated by an inhibition of rRNA processing. However, the precise mechanism of RNA related cytotoxicity has not been defined. Our earlier studies have shown that equitoxicdoses of FUra, fluorouridine (FUrd), fluorodeoxyuridine (FdUrd) and 5'-deoxy-5-fluorouridine (5'-dFUrd), produce different levels of incorporation into RNA and may be channeled into different classes of RNA. To determine if the fluoropyrimidine induced inhibition of rRNA processing correlates with RNA-directed cytotoxicity, we analyzed the effect of equitoxic doses of these 4 drugs on rRNA maturation in S-180 cells. A 6 hour exposure to 1uM, 10uM and 100uM FUra inhibited rRNA production by 23%, 62% and 77%, respectively. FUrd at 0.1uM, 1uM and 10uM resulted in inhibitions of 20%, 64%, and 84%. FdUrd at 25uM and 250uM resulted in 24% and 80% inhibition, while 5'-dFUrd at 10, 100 and 1000uM caused inhibitions of 13%, 68% and 73%. dThd-nonreversible cytotoxicity was measured and the LD_{90} for all 4 drugs resulted in a similar level of rRNA inhibition: 2.5uM FUrd (77% inhibition), 22uM FUra (68%), 250uM FdUrd (80%) and 600uM 5'dFUrd (72%). The total level of FUra incorporation into whole cell RNA did not correlate with either toxicity or rRNA processing. This does not appear to be a general phenomena, since preliminary experiments with other cytostatic agents, e.g. methotrexate show no disruption in rRNA production. We conclude that there is a strong correlation between dThd-nonreversible cytotoxicity and the inhibition of rRNA processing. However, neither of these events appear to correlate with FUra incorporation into whole cell RNA.

BIOPHYSICAL AND ULTRASTRUCTURAL CHARACTERISTICS OF A SARCOMATOID RENAL CELL CARCINOMA. D.A. Terreros, A. Behbehani*, C. King* and F. Cuppage.* Department of Pathology, University of Kansas, Kansas City, Kansas.

Classification of renal neoplasms has been controversial as the kidney is a mesodermally derived organ. A clear distinction between the less differentiated sarcomatoid variant of renal carcinoma and fibrosarcoma is cumbersome. The purpose of this work was to study a primary renal sarcoma-like tumor that developed in the hydronephrotic kidney of an elderly female. The primary tumor and its metastases to bone and skin had a spindle cell sarcoma-like morphology. Cultures were started with cells obtained from cystic and solid tumor areas. The cell lines are presently in passages 59 and 52 respectively. The cells grow well in eagle's media with or without serum supplement. Both cell lines possess proximal tubular intercellular junctions, microvilli polarized towards the nutrient media, and multiple cellular interdigitations. Their karyotype is hypodiploid with variable chromosomal rearrangements and constant C-1 and C-3 monosomy. HSV-1 and 2, Adeno-5, Echo 11 and Cox-B viruses replicate well in both lines. By means of intracellularly placed microelectrodes, a cellular electromotive force of -16 $\pm$ 1mV S.E.M. (N=70) was found. This value is significantly lower than those measured by us and others in non-neoplastic tubular renal cells studied in vitro. Increased electrogenic substrate uptake, decreased relative potassium conductance and confinement of the Na/K ATPase to restricted spaces formed by the cell to cell junctions and the plastic bottom of the culture dish may be germane to this observation. In conclusion our results support Oberling's theory about the proximal tubular cell origin of renal carcinoma (Nature 186:402, 1960), giving further insight into the biology of its sarcomatoid variant.

OVERCOMING COLON CANCER RESISTANCE TO HEPATIC ARTERY INFUSIONAL 5FUdR CHEMOTHERAPY WITH FOLINIC ACID. Glenn Tisman, Victoria Flener, Mary E. Jones, Lynette Buck. Whittier, CA. 90601.

Reduced folate polyglutamates enhance binding of thymidylate synthetase to 5FdUMP. Increasing intracellular reduced folate concentrations is associated with reversal of cell resistance to 5FUdR in some tumor cells. We have demonstrated that patient red blood cell polyglutamates can be elevated by treating with large doses of intravenous folic acid plus Cyanocobalamin. Perry, J., 1979 has shown that Leucovorin can increase intracellular polyglutamates. Two patients with extensive hepatic metastases from colon cancer were treated with intrahepatic arterial infusions of 5FUdR 0.1-0.3mg/kg/d. One patient responded initially and then relapsed; another was resistant from the start. Both patients had a rapid tumor response when Leucovorin was added to the 5FUdR. In one patient ½ of the resistant dose of 5FUdR (0.15mg/kg/d) when mixed with Leucovorin (0.075mg/kg/d) produced a response clearly demonstrating enhancement of 5FUdR activity. The second patient received 0.2mg/kg of 5FUdR for 18 days without response. Two days after adding Leucovorin 0.2mg/kg/d to the infusate the patient had rapid lysis of fever. Repeat angiography 20 days later revealed 60% decrease in tumor size. The above suggests that Leucovorin may potentiate 5FUdR activity when both drugs are given through the hepatic artery.

POSSIBLE POTENTIATION OF FLUOROPYRIMIDINE ANTI-TUMOR ACTIVITY BY PTEROYLGLUTAMIC ACID (FOLIC ACID) AND CYANOCOBALAMIN (B_{12}). Glenn Tisman, Victoria Flener, Mary E. Jones, Lynette Buck. Whittier, CA. 90601.

Laboratory studies confirmed that both folinic acid and folic acid can potentiate 5FU activity against different tumor cells. Our preliminary clinical work with attempts to potentiate 5FU activity with low doses (3mg) of Leucovorin was unsuccessful (Tisman, et.al., AACR, 19, 1978, 217A). Because folic acid may be the preferred substrate for intracellular conversion to polyglutamates (Perry, J., et.al., 1979), and because reduced folate polyglutamates potentiate the binding of 5FdUMP to thymidylate synthetase, we felt that large doses of folic acid might potentiate 5FU oncolytic effects clinically. Folic acid 200 mg/m^2 plus Cyanocobalamin 10,000mcg (used to enhance intracellular transport of folate) (Herbert, Tisman, et.al., Blood 4:465, 1973) in 200ml. of ½N saline plus 20MEq of sodium bicarbonate was infused I.V. over 2 hours daily for 3 days. After the first hour of each infusion a bolus of 5FU 200mg/m^2 was given I.V. Each treatment was repeated weekly. Thus far 3 patients with breast cancer refractory to 5FU containing regimens have received 36 infusions. Two of 3 patients had an exacerbation of bone pain within 12 to 48 hours of initiation of therapy. All patients had subsequent alleviation of bone pain within 1 week. CEA titers decreased in all patients. Hematologic toxicity was not significant in 2 and mild in one. Two of three patients developed diarrhea at the end of 1 and 3 weeks. Red blood cell folate levels after therapy revealed red cell folates (polyglutamates) were 2 to 3 times normal. The above protocol is clearly associated with tumor response to 5FU in 5FU refractory patients.

BONE MARROW TRANSPLANTATION (BMT) AFTER A NEW BUSULFAN (Bu) AND CYCLOSPHOPHAMIDE (Cy) REGIMEN. PJ Tutschka,* EA Copelan,* (intr. by MR Grever), The Bone Marrow Transplant Program, The Ohio State University, Columbus, OH.

In a rat BMT model we had established that addition of Bu to Cy as conditioning permitted the reduction of the dose of Cy without compromising the quality of engraftment. In the present study Bu (16 mg/kg) was combined with low dose Cy (120 mg/kg) to prepare 18 patients for allogeneic MHC compatible BMT. Six patients were transplanted for acute leukemia in remission (5 AML, 1 ALL), 6 patients for chronic myelogenous leukemia (1 in chronic, 2 in accelerated and 3 in blastic phase) and 6 patients for refractory leukemia. These patients were divided into a regular risk group (leukemia in remission and chronic phase, n=7) and a very high risk group (refractory leukemia and accelerated-blastic phase chronic leukemia, n-11). All patients achieved complete remission. One patient with refractory leukemia had recurrence of leukemia and required a second transplant that was successful. As predicted from the animal studies engraftment was completed before day 14, the median time of aplasia being 6 days. Toxicity, stomatitis and bacterial or fungal infections were absent. Most patients (66%) were afebrile and did not require therapeutic antibiotics during aplasia. Amphotericin B was never required. None of the patients showed acute or chronic GVHD and CMV infections were not documented. Median hospitalization time was 22 days. All 7 patients in the regular risk group survive in CR at a median of 104 days and 8/11 patients in the high risk group survive in CR at a median of 164 days. The overall survival rate is 83% at a median of 5 months. These results compare favorably to results after conditioning with Cy and TBI.