

CLIA ID # 99D1030993

CAP ID # 7186701

Patient : Patient X

Collected : 08/20/2007

Date of birth : 04/19/1958

Received : 08/21/2007

Specimen ID : HP07-2410

Physician : Dr. X

Specimen type : Ascitic fluid

Institution : Nashville Oncology Associates

## Clinical

49-year-old male with a diagnosis of metastatic colon cancer since 02/2005. Prior chemotherapy with CPT-11, Avastin and Xeloda resulted in PR, currently progressing, most recent chemotherapy on 08/20/2007.

## Recommendation

Based on the results of this study, the combinations of Cytoxan/epirubicin and Cytoxan/cisplatin are reasonable to consider.

## MiCK Assay Results

Drug tested	Max. Resp. (KU)	Resp. level
4HC(cytosan)	4.3	Moderate
Cisplatin	2.4	Low to moderate
Epirubicin	2.4	
Methotrexate	1.4	Low
Oxaliplatin	0.2	Nonsensitive
Taxotere	0.0	

## Interpretation

Ascitic fluid, drainage:

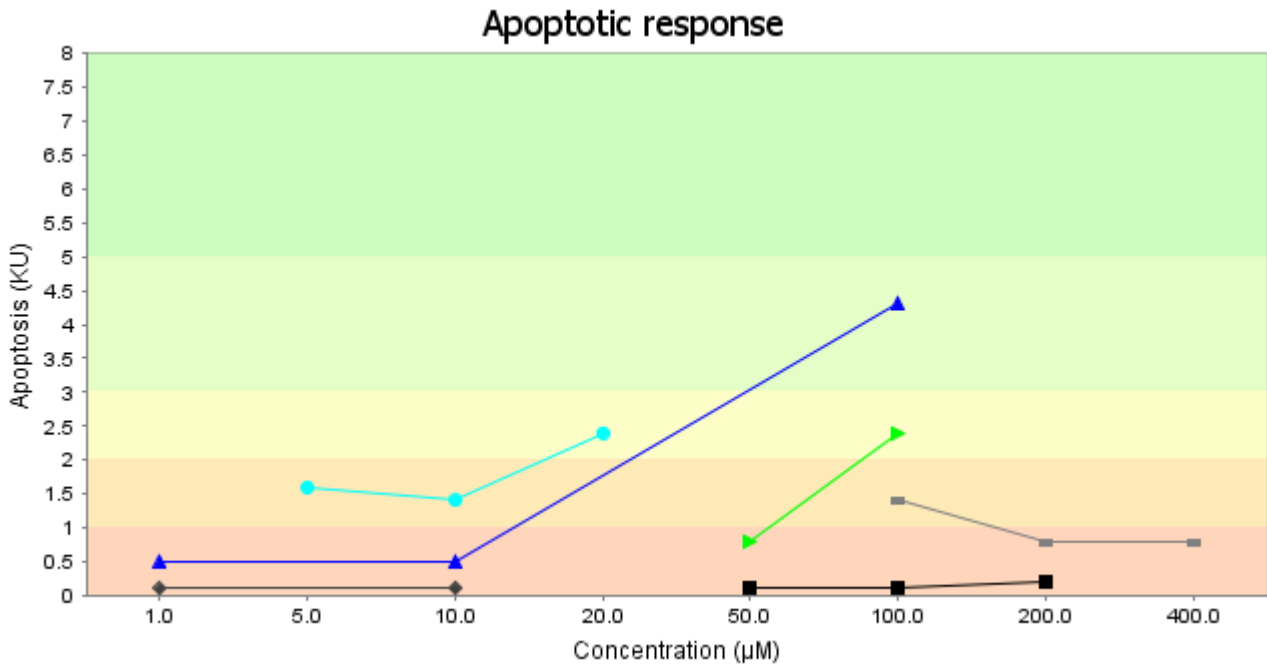
1. Population of cells with morphological and immunocytochemical features consistent with an epithelial neoplasm is identified (see comment).
2. In the MiCK assay, the patient's tumor cells were most sensitive to Cytoxan (see comment).
3. Extent of the response to Cytoxan was consistent with a moderate sensitivity of the tumor cells to this compound (see comment).

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Legend:		ND: data not displayed	NS: not sensitive	
▲	4HC(cytosan)	4.3	●	Epirubicin
▲	Cisplatin	2.4	■	Oxaliplatin
			■	Methotrexate
			◆	Taxotere
				0.0

## Comments

Viable neoplastic cells collected from the specimen were tested for their sensitivity to multiple doses of Cytoxan(4HC), cisplatin, epirubicin, methotrexate, oxaliplatin and Taxotere as single agents, and to a combination of cisplatin/gemcitabine (see chart above). Of note, alkylating agent cyclofosamide requires metabolic transformation by hepatocytes and, thus, cannot be tested in vitro. Synthetic active metabolite of cyclofosamide (4HC) was used in this study.

The MiCK assay identifies drugs most effective in killing patient's tumor cells by apoptosis. Extent of drug induced apoptosis is measured in Kinetic Units (KU). In this study, single agent Cytoxan was the most effective inducer of apoptosis causing 4.3KU maximal response. Of note, responses from 3 to 5 KU are consistent with a moderate drug sensitivity of tumor cells and have been previously seen in patients with partial clinical response to chemotherapy. Responses to epirubicin and cisplatin were consistent with moderately low sensitivity of the tumor cells to these agents. Other tested compounds did not induce any significant apoptosis in the patient's tumor cells. A table in the "Interpretation" section shows maximal apoptotic responses achieved with each of the tested agents.

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In conclusion, results of this study would support including Cytoxan in the treatment protocol if clinically indicated. Based on the pattern of the dose response curve of Cytoxan, use of the drug in its maximal tolerable dose should be considered. If more tumorous material is available, the study can be repeated to test sensitivity of the patient's tumor cells to other agents and comprehensive agent combinations.

All tested chemotherapeutic agents induced apoptosis in a control cell line.

### Microscopic/Immunophenotypic studies

Wright stained cytospin preparations of cells isolated from ascites fluid specimen showed predominantly macrophages, rare granulocytes, probable mesothelial cells and lymphocytes intermixed with a small number of predominantly large sized-atypical epithelioid cells with somewhat irregularly shaped nuclei and moderate amount of cytoplasm, located singly and in small aggregates. Atypical epithelioid cells accounted for approximately 1.5% of the total specimen cellularity. ICC studies showed these atypical cells were positive for CEA. In appropriate clinical settings, these findings could be consistent with involvement by a metastatic colon adenocarcinoma.

Attending pathologist  
Medical Director  
DiaTech Oncology, LLC

Electronically signed on 11-29-2011

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The pathologist's signature on this report indicates that the case was personally reviewed and the findings confirmed by the attending pathologist. This test was performed at DiaTech Clinical Pathology Laboratory. This laboratory is certified under CAP and CLIA-88 and is qualified to perform high complexity clinical testings. The MiCK assay measures drug induced apoptosis and its performance characteristics were determined at Vanderbilt University and at DiaTech Oncology. Clinical use of the MiCK assay is based on a statistically significant increase in CR rate and overall survival of AML patients whose treatment protocol included a drug to which the patient's tumor cells were sensitive in the assay. When used with solid tumors, the MiCK assay is expected to identify drugs most effective in killing patient's tumor cells by apoptosis. This test has not been cleared or approved by the U.S. Food and Drug Administration. The FDA has determined that such approval was not required.

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