The Unit of Toxicology and Chemotherapy originated in 1993 from the former Division of Carcinogenesis and Chemotherapy.

The objective of this unit is to better understand mechanisms of toxicity and carcinogenesis of drugs and chemicals and to develop improved methods for diagnosis and treatment of cancer.

In this context the mechanism of action as well as the spectrum of antitumor and anti-protozoan activities of alkylphosphocholines was studied. In addition, colorectal micro-metastases as well as disseminated tumor cells (DTC) were identified by using sensitive assays. These included a PCR-RFLP assay for K-ras mutations in DTC that were shed from K-ras mutated colon carcinoma, as well as RT-PCR assays for cytokeratin 20 and guanylylcyclase C. Finally, models of liver metastasis of colorectal cancer as well as of bone metastasis of breast cancer were developed and used for evaluating new therapies.

Pharmacological characterization of antineoplastic alkylphosphocholines

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High anti cancer activity in methylnitrosourea-induced primary rat mammary carcinoma, as first described by our group (Berger et al., Cancer Treat. Rev., 1987, 14, 307-317; Muschiol et al., Lipids, 1987, 22, 930-934) and then by others (Hilgard et al. Eur. J. Cancer, 1988, 24, 1457-1461), prompted the clinical use of hexadecylphosphocholine (HPC; INN: miltefosine) in the topical treatment of skin metastases of locally recurring breast cancer. Corresponding to our pre-clinical results, anti-cancer activity was observed in patients, leading to remissions or stable disease in 50% of treated women. Systemic administration of HPC, however, failed to result in tumor responses since gastrointestinal toxicity prevented the administration of sufficiently high dose levels. Nevertheless, the low dose levels administered caused significant increases in platelet and leukocyte counts. Alkylphosphocholines have been a main topic of interest since then and were followed to establish dose/response and structure/activity relationships as well as new pharmacological effects and the mechanisms of action.

a) Structure activity relationships of alkylphosphocholines in mammary carcinoma

Studies on the structure/activity relationships of alkylphosphocholines concentrated on the length of the alkyl chain, the introduction of double bonds into the alkyl chain and on modifications of the polar phosphocholine head. We demonstrated that an elongation of the alkyl chain was associated with increased toxicity, but introduction of a double bond reduced toxicity. A completely different physiological property was obtained with an alkyl chain length of 22 carbon atoms since this structure no longer forms micelles in solution and can be administered i.v. due to the absence of haemolytic properties. Compounds of this chain length, like erucylphosphocholine, have a superior therapeutic ratio and are considered to be second generation alkylphosphocholines (Berger et al. Drugs of Today, 1998, 34, 73-81).

b) Structure activity relationships of alkylphosphocholines in hematopoietic cells

Studies in hematopoietic cells showed that these alkyl phosphocholines caused no toxicity in normal bone marrow cells of rats and mice at concentrations used for therapy. Instead, mild to moderate growth stimulation of platelets and leukocytes was observed. By contrast, alkylphosphocholines inhibited the growth of a panel of leukemic cell lines with their cytotoxicity pending on the length of the respective alkyl chain and induced apoptosis. The
efficacy of these drugs was synergistically increased in combination with gemcitabine and antisense oligonucleotides directed against the gene bcr [1, 2].

**Sensitive detection and quantitation of colon carcinoma cells**

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The main focus was on studies related to early diagnosis of colorectal cancer. To that purpose, colorectal cancers were analyzed for K-ras codon 12 and 13 mutations using a sensitive PCR-RFLP assay. For patients with K-ras mutated tumors this mutation was used as marker for detecting disseminated tumor cells (DTC) in typical target tissues of colorectal cancer metastasis.

The PCR-RFLP assay used was sensitive enough to detect up to one mutant in $10^6$ wild type cells. Based on this assay, a K-ras mutation rate of 46% was found the primary tumor of colorectal cancer patients. The most prevalent mutation at codon 12 was a G→C transversion, followed by a G→A transition which resulted in amino acids alanine and asparagine, respectively, instead of glycine. At codon 13 G→A transitions were found only, resulting in amino acid asparagin instead of glycine. Concomitantly investigated tissues with potential metastatic spread (lymph nodes, liver, bone marrow) were found positive for K-ras mutants even if histologic examinations failed to detect any metastases. Thus, it was demonstrated that K-ras mutated disseminated tumor cells can be detected earlier than with conventional diagnostic means by using the PCR-RFLP assay for tissue samples from critical organs such as the liver [3,4].

In addition, markers of epithelial cells (cytokeratin 20) and gastrointestinal cells (guanylycyclase C) were used as surrogate markers for disseminated colorectal cancer cells as well in order to include patients bearing tumors without K-ras mutation.

**Rat models mimicking metastasis**


In cooperation with: Dr. Matthias Seelig, Klinikum Ludwigshafen, Ludwigshafen; Prof. Dr. G. Golomb, Hebrew University, Jerusalem, Israel

Rat models of metastatic colon cancer (by transplanting tumor cells into the liver) and metastatic mammary cancer (by transplanting tumor cells into the femur and tibia) were developed and used to investigate the anticancer potential of certain new drugs and treatment modes (antisense oligonucleotides encapsulated into nanoparticles, loco-regional administration, chemoembolisation, antibodies).

The number of rat colon cancer cells growing in the liver was determined by assaying the activity of β-galactosidase after transfecting the cell line with this marker gene [5]. The size of osteolytic lesions in the hind-limb of rats was detected and followed by X-rays (fig. 1) and CT-scan. Both models will be used for therapy experiments.

**Fig. 1:** X-ray image of an osteolytic metastasis in rat tibia caused by MDA-MB-231 human breast cancer cells.