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## Reversal of multidrug resistance by externally added or gene transduced cytokines: An alternative approach

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**Purpose:** Since successful chemotherapy of human cancers is apparently limited by intrinsic or acquired resistance towards cytostatic drugs a variety of reversal strategies mostly aiming at interference with the function of multidrug resistance (MDR)-associated proteins have been proposed. In the present study an alternative strategy acting via modulation of expression of MDR-associated genes following cytokine treatment or cytokine gene transduction was evaluated.

**Methods:** Expression studies were performed by RT-PCR and immuno flow cytometry. Functional data were obtained with drug accumulation assays. Results were correlated with data from a chemosensitization assay towards MDR-associated drugs.

**Results:** Expression of several MDR-associated genes can be affected by either externally added or gene transduced cytokines like TNF $\alpha$ resulting in chemosensitization of the tumor cells towards MDR-associated drugs. However, a panel of MDR-associated genes (mdr1, MRP and LRP) responded in coordinate, or contrastings ways to the modulating agent.

**Conclusion:** Successful MDR reversal strategies should not only focus on one gene such mdr1, but should consider that the MDR phenotype reflects the net effect of expression of a variety of MDR-associated genes.

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## $\alpha$-tocopherol (AT) antagonises the multidrug resistance reversal activity of cyclosporin A, verapamil, clofazimine and B669

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**Purpose:** To investigate the effects of AT on the chemosensitizing interactions of cyclosporin A, verapamil, clofazimine and B669, at 5, 2, 1 and 0.5 $\mu$g/ml respectively, with a P-glycoprotein (P-gp)-expressing multidrug resistant (MDR) human lung cancer cell line (H69/LX4) *in vitro*.

**Methods:** Cell proliferation, vinblastine uptake and P-gp expression were measured by colorimetric, radiometric and flow cytometric procedures.

**Results:** All the chemosensitizing agents restored the sensitivity of H69/LX4 cells to doxorubicin and vinblastine without affecting the level of expression of P-gp. Inclusion of AT (25 $\mu$g/ml) antagonised the MDR-modifying activity of all 5 chemosensitizing agents, effectively preventing restoration of sensitivity to both doxorubicin and vinblastine in these cells. Antagonism of chemosensitisation was not observed with other lipid- and water soluble anti-oxidants or with inhibitors of protein kinase C or of arachidonic acid metabolism, suggesting that the effects of AT may be related to its membrane modulating properties rather than to other biological activities of this agent. AT also prevented the accelerated uptake of $^3$H-vinblastine in the presence of chemosensitizers.

**Conclusion:** High intake of AT may be a potential determinant of unfavourable clinical outcome in patients treated with MDR-modifying agents.

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## Cellular mechanisms of resistance to methotrexate in childhood leukemia

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**Introduction:** In about 25% of patients suffering from acute lymphoblastic leukemia (ALL) treatment failures occur which are most likely due to development of resistance against methotrexate (MTX).

**Methods:** Bone marrow lymphoblasts from 62 patients, suffering from different types of leukemia (acute lymphoblastic leukemia, ALL; acute myeloid leukemia, AML; chronic myeloid leukemia, CML) were examined for uptake of MTX, formation and distribution pattern of intracellulary persisting MTX-polyglutamates (MTXPG), cytotoxicity of MTX and/or activity of thymidylate synthase (TS). MTX and MTXPG were analyzed by high performance liquid chromatography (HPLC) and radiochemical quantification.

**Results:** In blasts from some ALL-patients, we could prove the described positive correlation between the amount of long chain MTXPG and cytotoxicity. Low amounts of MTXPG caused only weak cytotoxicity. Resistance to MTX in blasts from AML-patients is also caused by reduced synthethis of long chain MTXPG. In contrast we also found that ALL-blasts were able to survive MTX-treatment in spite of the formation of high amounts of MTXPG and the very effective or complete inhibition of TS-activity.

**Conclusions:** The results of this study support the assumption that leukemic blasts have the ability to circumvent MTX-and MTXPG-inhibition of TS with a highly enhanced thymidylate salvage pathway.

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## Cellular mechanism of the novel imidazoacridinone antineoplastic agent C1311

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C1311 is a novel therapeutic agent with potent activity against colorectal cancer and has been selected for entry into clinical trial by the EORTC. The compound has previously been shown to have DNA-binding properties and inhibit topoisomerase II in cell-free systems. In this study, cellular uptake and mechanisms by which C1311 interacts with DNA and exerts cytotoxic effects in intact colon carcinoma cells were investigated. The HT29 colon cancer line was chosen to follow cellular distribution of C1311 over a 24-h time course at drug concentrations which just inhibited cell proliferation by 50% or 100%. C1311 co-localization with lysosomal, mitochondrial and nuclear dyes was examined by fluorescence microscopy and effects on these cellular compartments determined by measurement of acid phosphatase levels, rhodamine 123 release or [$^3$H]-thymidine incorporation. The strength and mode of DNA binding was established by thermal melting stabilization, titration and viscometric host duplex length studies. Growth inhibition of HT29 cells by C1311 is concomitant with rapid drug accumulation in nuclei and inhibition of DNA synthesis. C1311 binds to DNA by intercalation and shows a sequence-preferential binding to AT-rich DNA duplexes. Drug uptake is also seen in lysosomes, leading to lysosomal rupture and marked increase of acid phosphatase activity 8 h after drug exposure. The lysosomotropic effect of C1311 is a novel feature for an anticancer agent. As it is unlikely that C1311-induced DNA damage alone would be sufficient for cytotoxic activity, lysosomal rupture might be a critical component for C1311 therapeutic efficacy.

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## In vivo activity of a methotrexat-albumin-conjugate (MTX-HSA) in human tumor xenografts

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**Purpose:** Methotrexate bound to human albumin was investigated for antitumor activity in vivo in 6 human tumor xenografts.

**Methods:** 4 solid and 2 leukemia human tumor xenografts all growing sc in nude mice were treated with MTX-HSA and MTX given ip weekly for 3 weeks.

**Results:** MTX-HSA demonstrated at the MTD (12.5 mg/kg/day free MTX) a higher antitumor activity than MTX (50 mg/kg/day) in 3 models. In the **bladder cancer** BXF 1301 MTX-HSA effected complete durable remissions whereas MTX resulted in a short lasting partial remission only.

In the **prostate model** PC3M MTX-HSA resulted in a T/C of 7% vs 21% for MTX. In the osteosarcoma SXF 1410 the T/C values were 10% vs 15%, respectively. In the large cell lung cancer LXFL 529 both compounds were inactive. In the **acute lymphoblastic leukemia** CCRF-CEM both compounds were similarly active whereas in the promyelocytic leukemia HL60 both compounds were inactive.

**Conclusion:** MTX-HSA is promising novel compound with specific accumulation in tumors which should be developed in solid tumors (e.g. bladder and prostate cancers) as well as in leukemias.