etic/erythropoietic cell ratio. Pharmacokinetic parameters were obtained for each dose level after the first dose, first cycle and last dose, last cycle and indicate dose-proportional drug exposure of all treated animals.

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## Lipid rafts as gateway for antitumor alkyl-lysophospholipids to induce apoptosis

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Synthetic alkyl-lysophospholipids (ALPs), such as 1-O-octadecyl-2-O-methyl-rac-glycero-3-phosphocholine, are antitumor agents known to accumulate in cell membranes. The aim of this study was to understand the mechanism by which ALP enters the cell and induces apoptosis. We demonstrate that in murine lymphoma S49 cells, ALP inhibits de novo biosynthesis of phosphatidylcholine (PC) at the CTP:phosphocholine cytidylyltransferase (CT) step. Exogenous lysoPC providing an alternative route to generate PC (via acylation), rescued the cells from ALP-induced apoptosis. This indicates that a continuous rapid PC turnover is essential for cell survival. To reach CT, ALP needs to be internalized. This internalization did not involve receptor/clathrin-coated pit-mediated endocytosis, nor fluid phase endocytosis. Instead, intact lipid rafts in the plasma membrane were found essential, as ALP was found to accumulate in lipid rafts and artificial disruption of these microdomains resulted in dissociation of ALP from rafts. This led to a reduced ALP endocytosis, and inhibition of apoptosis. Interestingly, an ALP-resistant cell variant, S49AR, showed no impaired PC metabolism after ALP treatment and revealed reduced ALP internalization and reduced levels of sphingomyelin, an essential component of lipid rafts. Therefore, we argue that altered lipid composition of lipid rafts determine raft-mediated endocytosis. For the first time, lipid rafts are recognized as potential targets for anticancer therapy.

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## NADPH oxidase 1 (NOX 1): a novel target for colon cancer therapy

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Recent studies have demonstrated the presence of several novel membrane oxidases in mammalian tissues that share homology with gp91phox, the catalytic moiety of the NADPH oxidase (NOX) found in phagocytic leukocytes. These flavoproteins catalyze the NADPH-dependent reduction of oxygen to superoxide and related reactive oxygen species (ROS). Because the mechanism of ROS generation after exposure of human tumor cells to a wide range of growth factors (including EGF, PDGF, insulin, bFGF, and GM-CSF) remains to be determined, we examined the expression of NOX 1 in a panel of cultured human cancer cells using real-time RT-PCR. NOX 1 mRNA was quantitated as the ratio of the levels of NOX1/18S mRNA using specific plasmids containing either NOX1 or 18S. NOX 1 expression ratios were very high (>130,000) in human colon cancer cell lines (CaCo2, LS174T, and HT-29) and barely detectable (ratios <200) in human MDA-MB468, BT474, and ZR-75 breast cancer cells or DU-145 and LNCap human prostate cancer lines. In a panel of twelve human colon cancers paired with adjacent normal tissues obtained from the City of Hope Frozen Tumor Bank, NOX 1 ratios ranged from 20,000 to 800,000 in 10/12 tumors, were undetectable in 2/12 tumor and normal samples, and were substantially greater in tumor than normal tissue in 7/10 samples. As was the case in cell lines, NOX 1 expression ratios were low (<500) in 12/12 human breast cancer specimens and 6/6 prostate cancers. To assess NOX 1 as a therapeutic target, growth inhibition by the NOX inhibitor diphenylene iodonium (DPI) was examined *in vitro*. The $IC_{50}$'s for DPI were 5, 20, and 40 nM for CaCo2, LS174T, and HT-29 cells that express high levels of NOX 1, and were >2000 nM for DU-145 and MDA-MB468 cells that demonstrate very low level expression of the oxidase. These experiments suggest that growth factor-related reactive oxygen production may play an important role in signal transduction and tumor cell proliferation; and that NOX 1 may be a new target for the development of novel treatments for colon cancer. (Supported by CA 62505)

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## Molecular modes of action of antimalarial artemisinin derivatives as novel anticancer drugs

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Twenty-two chemically characterized compounds derived from Traditional Chinese Medicine were analyzed in drug-sensitive and multidrug-resistant tumor cell lines. The antimalarial artesunate (ART), a semisynthetic derivative of artemisinin from the Chinese plant *Artemisia annua L.*, was among the most active compounds. ART did not exhibit cross-resistance to multidrug-resistant tumor cells overexpressing either the resistance-conferring MDR1, MRP1, or BCRP genes. Isogenic p53 -/- knock out tumor cells were as sensitive as their p53+/+ counterparts indicating that ART was not subject to p53-mediated chemoresistance. The evaluation of ART's anticancer activity in 55 cell lines of the National Cancer Institute, U.S.A., showed that ART was most active against leukemia and colon cancer cell lines. We mined the N.C.I. database and correlated the $IC_{50}$ values with microarray mRNA expression profile of 464 genes. By hierarchical cluster analysis we identified oncogenes and proliferation-regulating genes which were strongly downregulated in ART-sensitive leukemia and colon cancer cell lines. The role of proliferation for ART's response was corroborated using a panel of *Saccharomyces cerevisiae* strains with defined genetic knock out mutations. Furthermore, ART correlated significantly with proliferation parameters (cell doubling times, G0/G1 and S cell cycle phases). We extended our analyses to other artemisinin derivatives, arteether and artemether. Using hierarchical cluster analysis we found that one cluster of genes correlated with the $IC_{50}$ values of all three derivatives, the majority of them being proliferation-associated genes. This speaks again for a general role of the proliferative state for the response of tumor cells towards artemisinin derivatives. Another cluster contained genes correlating specifically with one of the 3 drugs. The correlation to different genes may explain differing anticancer activities of artemisinins.

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## Blockade of endothelin A receptor by ABT 627 suppresses tumor growth, neovascularization and potentiates cytotoxic paclitaxel activity in ovarian cancer cells *in vitro* and *in vivo*

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The endothelin-1 (ET-1)/ETA receptor (ETAR) autocrine pathway is overexpressed in many human tumors, including ovarian carcinoma, and may provide a new target for anticancer therapy. Engagement of ETAR by ET-1 triggers activation of tumor cell proliferation, survival, neoangiogenesis and invasion. In primary and metastatic ovarian carcinomas, ET-1 overexpression is associated with enhanced neovascularization as well as with vascular endothelial growth factor (VEGF) expression. ABT627 (Atrasentan) is a p.o.-active ETAR antagonist that selectively inhibits the ETAR activities and is under clinical develoment in cancer patients. We therefore tested whether ABT627 may potentially block ovarian tumor progression and may affect neovascularization and apoptosis. When tested in culture ABT627, inhibited tumor growth in both primary cultures (PMOV1 and PMOV2) and cell lines (OVCA 433 and HEY) of ovarian carcinoma. In contrast, the ETBR antagonist, BQ 788, does not display inhibitory effects. Furthermore ABT627 inhibited VEGF production and enhanced proapoptotic effect of paclitaxel. Extending these studies *in vivo*, we explored the therapeutic effects of ABT627 on HEY ovarian carcinoma xenografts. HEY cells produced high amount of ET-1, expressed high affinity ETAR (Kd=0.1 nM; 35,600 sites/cell) and developed rapidly growing solid tumors in nude mice. ABT627 (2mg/Kg/24h i. p. for 21 days) produced similar inhibition of tumor growth as paclitaxel (20mg/Kg i. v. Q4x3) with a reduction of 65% (p=0.005) and 67% (p=0.006), respectively, compared with control. Similar results were obtained with high dosage of ABT 627 (10mg/Kg/24h). Immunohistochemical evaluation of tumors revealed that the reduced size of ABT627-treated tumor xenografts coincided with reduced neovascularization and with enhanced ovarian cancer cell death. Administration of ABT627 and paclitaxel in HEY tumor xenografts caused a remarkable antitumor effect. Tumor regression was accompained by a significant inhibition of VEGF,

matrix metalloproteinase-2 (MMP-2), microvessels and with enhanced tumor cell apoptosis. Furthermore, 4 of 10 mice bearing well established HEY xenografts had no histological evidence of HEY tumors after treatment withdrawal. The antitumor, antiangiogenic, and apoptotic activities obtained with ABT 627 and the enhanced cytotoxic effect of paclitaxel provide a rationale for its clinical evaluation in ovarian carcinoma. Supported by AIRC, FIRC, CNR and Ministero della Salute.

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## Ras and Rho GTPases: targets of FTase and GGTase I inhibitors for cancer treatment?

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Ras and Rho family proteins are modified posttranslationally by isoprenylation, which is critically required for correct localization and function. With the advent of inhibitors of prenyltransferases (farnesyltransferase inhibitors, FTIs, and geranylgeranyltransferase inhibitors, GGTIs), it was demonstrated that proteins normally modified by farnesylation could become alternatively prenylated by geranylgeranylation in the presence of FTIs. This has triggered renewed interest in understanding whether there are differential consequences of specific isoprenoid modification of these proteins. We have undertaken direct comparisons of the subcellular localization and biological activity of farnesylated and geranylgeranylated forms of Ras and Rho family proteins, using structural mutants with altered CAAX and hypervariable domains. Results to be discussed were generated by imaging living cells expressing GFP-tagged proteins, transcriptional transactivation assays of reporter genes, transformation assays for morphological transformation and anchorage-independent growth, and microarray analysis of genes whose transcription is altered by a change in isoprenoid modification on the Ras and Rho family proteins. In general, oncogenic forms of Ras (normally F) and RhoA (normally GG) are similarly functional regardless of lipid modification, whereas the function of R-Ras and Rac (both normally GG) is more isoprenoid-dependent. Finally, we also determined whether alternatively prenylated forms of these GTPases could protect cells from growth inhibition by FTIs and GGTIs. We find that farnesylated Rac and R-Ras, but not farnesylated RhoA, protect cells from growth inhibition by GGTIs and are therefore candidate targets for mediating the anti-tumor effects of GGTIs. Taken together, these studies further elucidate the antitumor mechanisms of FTIs and GGTIs.

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## A strategy for identification of gene targets by integrating genome and transcriptome data in cancer

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Accumulation of genetic alterations is thought to underlie tumor development and progression. Genes that are altered as a result of somatic genetic alterations are attractive drug targets, since the growth and survival of cancer cells is likely to be dependent on these critical genes. Also, the absence or low-level expression of such genes in normal tissues provides a basis for specific tumor targeting, as indicated by recent clinical success of therapies directed against the BCR-ABL and Her-2 genes. Here, we developed a strategy for a genome-wide search for genes involved in genomic alterations in cancer. Cancer cell lines were first screened by comparative genomic hybridization (CGH) on a microarray with ~14,000 mapped cDNA clones. Expression levels of the corresponding genes were determined in a parallel experiment. A bioinformatic analysis was performed involving integration of genomic and transcriptomic data, and aligning and displaying the results using the 3.2 Bbp human genome sequence as a backbone. Our analyses of breast cancer revealed 270 genes that were highly overexpressed as a result of their location in regions of the genome undergoing amplification. These 270 genes included virtually all previously discovered oncogenes and amplification target genes in breast cancer, including HER-2, EGFR and MYC, and a large number of candidate novel oncogenes with a similar amplification and expression pattern as the known oncogenes. For example, a novel amplicon was found at 17q21.3, corresponding to a base pair position ~48.6-52.2 Mbp from 17pter. This amplification led to the overexpression of HOXB7 gene, a powerful embryonic regulator, whose amplification was subsequently validated in a set of 10.2% of 363 primary breast tumors. In summary, our analyses point to the substantial importance of genetic alterations as a mechanism for deregulating gene expression in breast cancer. Integration of transcriptomic and genomic copy number data is a powerful method for highlighting specific novel genes that may have potential for therapy development.

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## Characteristics of novel non-hydroxamate inhibitors of histone deacetylases

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Gene expression is in part regulated by differential acetylation of nucleosomal histones resulting in either transcriptional activation (hyperacetylation) or repression (hypoacetylation). This phenomenon is tightly regulated by the balance of histone acetyltransferase (HAT) and histone deacetylase (HDAC) activities. HDAC inhibitors induce the hyperacetylation of nucleosomal histones in cells resulting in the expression of repressed genes that cause growth arrest, terminal differentiation and/or apoptosis in carcinoma cells. These pharmacological properties of HDAC inhibitors have generated significant interest in HDACs as targets for anti-cancer therapy. A-161906, a biaryl hydroxamate, was discovered as a TGFb mimetic and is an inhibitor of HDAC, $IC_{50}$ = 9 nM. However, cellular activity in the 3-15 uM range and minimal efficacy *in vivo* necessitated synthetic efforts to increase cellular potency. The succinimide macrocyclic hydroxamate series maintained sufficient HDAC activity, $IC_{50}$ values in the 30 nM range, and improved cellular activity into the submicromolar (i.e. 100-600 nM) range. This series is exemplified by A-173, with an HDAC $IC_{50}$ value of 38 nM and antiproliferative $IC_{50}$ values of 250 and 150 nM for HT1080 and MDA435 cells, respectively. These compounds demonstrated robust hyperacetylation of histones and induction of p21 and gelsolin in carcinoma cells. *In vivo* activity of this series was significantly improved from the original A-161906. The hydroxamate moiety was replaced by an electrophilic ketone. The a-ketoamides were more potent inhibitors of the HDAC enzymes with $IC_{50}$ values in the 5 nM range and maintained cellular activity, $IC_{50}$ values in the submicromolar range. This series is exemplified by A-378 with an $IC_{50}$ value of 1.8 nM for nuclear HDACs and antiproliferative $IC_{50}$ values of 170 and 120 nM for MDA435 and HT1080 cells, respectively. The a-ketoamides produced similar effect as compared to TSA in cellular models of hyperacetylation and induction of p21. This compound also demonstrated anti-tumor activity in the flank tumor growth model at 30 mg/kg, q.d.x2d, i.p. Synthetic efforts have led to the significant improvement in enzymatic, cellular and *in vivo* activity of Abbott HDAC inhibitors in both the hydroxamate and non-hydroxamate series. Compounds with nanomolar potency against nuclear HDACs and submicromolar activity in cell proliferation assays are described.

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## Lung cancer molecular fingerprinting: expression profiling of gene and protein with cDNA microarray and MALDI-TOF MS

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To develop comprehensive molecular fingerprints of lung cancer, we applied both protein mass spec and cDNA printed array technologies to resected non-small cell lung tumors. For gene expression profiling, we initially analyzed 24 surgically resected human non-small cell lung cancers (NSCLC) and 7 other types of tumors by 5k cDNA microarrays. Using gene expression profiles from these samples, groups of genes were identified that were able to differentiate tumor from normal and lung metastases from lung primary tumors as well as the known histological subgroups of NSCLC. The class-prediction model based on the initial cohort successfully classified most of the samples from an independent test cohort of 14 samples. We then applied Matrix-Assisted Laser Desorption/Ionization-Time of flight Mass Spectrometry (MALDI-TOF MS) to the proteomic profiling of 80 surgically resected human lung tumor and normal lung tissues. Hundreds of protein signals were obtained from both tumor and normal tissues and groups of proteins were specifically expressed in one or the other. Hierarchical clustering analysis successfully distinguished tumor from normal and clustered tumors into related groups by histology. We have begun to apply this tech-