inhibition of PtdCho-PLC and cPLA2. These alterations could have potential as MRS detectable biomarkers for Hsp90 inhibition in vivo.

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332 Poster

Treatment of murine Acute Myeloid Leukemia by 17DMAG, a geldanamycin derivative

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Inhibition of heat shock protein 90 (HSP90) is emerging as a target therapy in cancer either as primary treatment or secondary to chemotherapy in drug resistant cases. HSP90 is a molecule with physiologic roles as regulator of correct folding of nascent proteins and importantly, it is not linked to the multiple cellular circuitries. In solid cancers HSP90 is over-expressed and it was reported that geldanamycin and 17AAG and 17DMAG derivatives disrupt HSP90/oncoproteins complexes.

In the present study we report on 17DMAG effects in C57Bl mice using a murine acute myeloid leukemia (AML) experimental model by inserting subcutaneously leukemic cells embedded in small agar discs (single disc per mouse). The C-1498 AML cells provided by NCI, Frederick Institute, MD, USA was used for the experiments. In untreated control mice, large and vascularized tumor formations are produced locally as well as secondary bone marrow leukemia dissemination. Treated mice were administered 3 courses of i.p. 17DMAG injections (20mg/kg body weight, per each injection in saline solution) consisting of a daily injection over a period of three days followed by five days interval without treatment between the "3-day-drug-administration-course". Each animal received a total of nine injections. 17DMAG treatment of leukemic mice resulted in the shrunken tumors of whitish appearance and decreased bone marrow leukemic load. By immunohistochemistry of tumors we observed high expression of HSP90 and moderate expression of HIF1α (Hypoxia Inducible Factor 1α) and VEGF (Vascular Endothelial Growth Factor) in untreated mice, decreasing after 17DMAG treatment. We conclude that 17DMAG interferes with HSP90/vasculogenic proteins complexes (HIF1α and VEGF), positively effecting murine AML.

The mice have been handled abiding by the regulations of the Ethic Committee of the Hebrew University Hadassah Medical School, Jerusalem, Israel.

333 Poster

Evaluation of the effect of new vitamin D3 derivatives, BGP-013 and BGP-015, administration on human carcinomas

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This study examines the effect of new calcipotriol-based compounds, BGP-013 and BGP-015, administration on different types of human carcinoma cell lines.

The roll of 1α,25-dihydroxyvitamin D_3 [1α,25(OH)$_2$$D_3$, Calcitriol] in cancer prevention and its potential as an anti cancer therapeutic agent has been well established in variety of human tumors in vitro and in vivo. Calcipotriol is a well known Vitamin D_3 analogue which is considered a highly effective topical therapy available for hyperproliferative skin diseases such as psoriasis. Also, calcipotriol is known to be at least 100 times less involved than calcitriol in calcium (Ca^{2+}) metabolism - causing less hypercaliuria, hypercalcemia and bone calcium mobilization. BGP-013 and BGP-015 are new calcipotriol-based compounds synthesized in our laboratory. We tested the effect of the administration of those new compounds on the viability of different types of human carcinoma cell lines: LNCaP– human prostate carcinoma, MCF-7– human breast carcinoma and HT-29– human colon carcinoma, using MTT and Neutral-Red viability assays. The treatment of LNCaP cells with 30μM (a high concentration) of BGP-013 or BGP-015 for 24 hours showed a significant increase in cell death (around 60% mortality), similar to the increase following treatments with calcipotriol and calcitriol ($p<0.01$). The treatment of MCF-7 and HT-29 cells with 30μM of BGP-013 for 24 hours showed a significant increase in cell death (around 50% and 30% respectively), similar to the increase following treatments with calcipotriol and calcitriol ($p<0.01$). Treatments of all cells with 5μM (a low concentration) substances for up to 7 days also showed a significant increase in cell death - around 50% mortality in LNCaP and HT-29, and up to 80% mortality in MCF-7 ($p<0.01$). In addition, the molecular mechanism of cell death following treatments with the compounds compared to calcipotriol and calcitriol was examined using a non-specific pan-caspase inhibitor and flow-cytometry analysis of cell-cycle condition and apoptosis. Those results indicate that an apoptotic cell death mechanism is involved in cytotoxic effect of the new compounds.

All human carcinoma cell lines tested in this study showed a high susceptibility to the new calcipotriol-based compounds, BGP-013 and BGP-015, partially as a result of apoptosis induction. This data indicates that BGP-013 and BGP-015 are potential new therapeutic agents efficient for human carcinoma treatment.

334 Poster

Synergistic activity of 2-deoxyglucose, an endoplasmic reticulum stress inducer, and efrapeptins, dual inhibitors of proteasome and Hsp90, in breast cancer cells

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Efrapeptins (EF) is a family of small, naturally occurring oligopeptides with a potent antitumor activity. Their ability to inhibit tumor growth has been attributed to: a. suppression of Hsp90 chaperone function, and b. inhibition of the chymotrypsin-like and caspase-like activities of 26S proteasome. EF-treatment of breast cancer cells results in the upregulation of glucose-regulated proteins Grp78 and Grp94 with a concomitant downregulation of P-PERK, a common sensor of endoplasmic reticulum (ER) stress. Here, the effect of combining EF with 2-deoxyglucose (2DG) on the growth of breast cancer cells was examined. 2DG induces ER stress by preventing glucose metabolism. An EF-sensitive (MCF-7; IC_{50}=25 nM) and an EF-resistant (MDA-MB-231; IC_{50}=4,000 nM) cell line were employed. Cytotoxicity was determined by MTT viability assays and the data were analyzed using the Median Effect Analysis (Chou and Talalay, Adv. Enzyme Regul. 1984; 22:27-55). Combination of EF with 2DG had a strong synergistic cytotoxic effect in both cell lines. The Combination Index (CI) value was 0.340±0.137 for the MCF-7 and 0.251±0.082 for the MDA-MB-231 cells. In the presence of 2DG, the IC_{50} value of the inhibitory action of EF was reduced 8- (MCF-7) to 30- (MDA-MB-231) fold. Western immunoblotting showed that simultaneous exposure of both cell lines to EF and 2DG led to a larger increase in the protein levels of Grp78 and Grp94 than single drug treatments. MDA-MB-231 cells treated with both drugs also possessed higher levels of the glucose transporter Glut-1 than cells treated with EF or 2DG alone, which indicates that the presence of EF results in an increased uptake of 2DG. Furthermore, the presence of 2DG did not alter the reduction in levels of P-PERK found in cells treated with EF alone. It appears that EF-treatment renders breast cancer cells vulnerable to 2DG treatment while increasing the uptake of 2DG, thus, accelerating the demise of the cells. Synergism was also observed with the ER stress inducers tunicamycin (a protein glycosylation inhibitor) and A23187 (a Ca^{2+} ionophore), although the decrease in the IC_{50} value of the inhibitory action of EF was not as dramatic as in the case of 2DG. This synergism validates the hypothesis that the in vivo antitumor activity of EF may partially be attributed to a reduction in the ability of the tumor cells to deal with environmental conditions that promote ER-stress such as hypoxia and lack of nutrients.

335 Poster

Alpha-particle emitters targeted by specific antitumor antibodies

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Background: Alpha-particle emitters as part of hybrid nanoparticles hold great promise as therapeutics for micrometastatic disease. Here we describe a new therapeutic nanoparticle's design, which consists of three parts: targeting, effecter and linker.

Materials and methods: Targeting part: an anti-HER2/neu mini-antibody-barnase fusion protein (4D5 scFv-barnase-His5). The anti-HER2/neu mini-antibody could be used to deliver barnase to HER2/neu-positive cells and provide its penetration into the target cells, as HER2/neu is a ligand-internalizing receptor. This expression vector has potential applications to both gene and antibody therapies of cancer, because many tumor cells are HER2/neu-positive, breast cancer for example.

Effecter: Tumor targeted alpha-particles can result in high cancer-cell killing with minimal normal-tissue irradiation because of their high energy deposition and short range. Actinium-225 is used in present work as a generator for alpha-particle therapy: it decays with a 10-day half-life and generates three alpha-particle-emitting daughters.

Linker: synthetic strategies for construction of hybrid nanoparticles under study based on chelating agents.

Results: 1. It was proven by experiments with breast cancer cells in-vitro, that anti-HER2/neu mini-antibody created do conjugate effectively with

tumor cells. 2. Stability of chelating agents chosen complex with different metals was proven.

3. Stability of chelating agents complex with mini-antibody created was also proven (in-vitro). Hybrid nanoparticles designed are being evaluated by in-vivo biodistribution studies in animal (rats) models.

Conclusions: Tumor-targeted nanoparticles with conjugated specific antitumor antibodies are promising tools for the reduction of malignant tumors. Our results form basics for creation of new targeted radiopharmaceuticals.

336 Poster

Dolichyl phosphate dependent P-glycoprotein expression in Doxorubicin resistant MCF-7 breast cancer cells

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Introduction: Multidrug resistance (MDR) in breast cancer cells during chemotherapeutic course restricts the possibilities of Doxorubicin (Dox) application. The investigations reveals that MDR correlates accumulation of P-glycoprotein (Pgp) in plasma membrane. The resent results are in favour of the idea that glycoprotein synthesis in malignant tissues is limited by Dolichyl Phosphate (DolP). The aim of the present study is to investigate the effect of polyprenol (PP) which provides a DolP substitute in regulation of N-glycosylation on MCF-7 breast cancer MDR.

Experimental procedures: Breast cancer cell lines, MCF-7 and MCF-7 cells with induced resistance to Doxorubicin (MCF-7/ADR) were used. Pol concentration in the culture medium made up 10-2 -10-6 Pgp expression was detected with monoclonal antibodies using flow cytometry and immunohistochemistry. Intermediates of DPC fractions were analysed by HPLC method. Dolichyl phosphate N-acetylglucosamine-1-phosphate transferase (GPT) due to DPAGT1 polymorphism was assessed in T-cells.

Results: Pol in concentration 10-2 -10-3 M induced apoptosis in MCF-7/ADR cells within 3-4 hours. It is confirmed that plasmatic membrans of MCF-7 cells contain 5,6 - 6,4% of Pgp (the total protein amount) as a resistance marker. Resistant MCF-7/ADR cells differ from sensitive ones MCF-7 in Pgp content by 10-12 times. The study showed 8,5-fold DPC intermediates decrease in MCF-7/ADR cells. The investigations demonstrate that the situation can be changed by treatment with Pol. The DPC concentration in MCF-7/ADR cells was returned to the normal level. It is established that Pol in the concentration 10-4 M aid 7-9-fold reducing Pgp in membranes of MCF-7/ADR cells. The MCF-7/ADR cells cultivation in medium with Pol proceeded to give lowered Pgp content in membranes no over 0,4-0,6 %, which amount was consistent with the level of Pgp in MCF-7 cells.

Conclusions: These results indicate that noncontrollable accumulation of Pgp, which cause MDR can be overcomed using stimulation of DPC with Pol, which provides a DPC substitute in regulation of Pgp. Pol is a promising new agent which clinical usage can open up possibilities to tackling the problem of Dox resistance in breast cancer chemotherapy. It is, also, a hypothesis, which has suggested that there is a genetic polymorphism of DPAGT1 in MCF-7 breast cancer that could mediate Pgp expression and blunt the response to Dox.

337 Poster

MAPK/ERK signaling mediates melatonin-induced neuroendocrine differentiation in prostate cancer cells

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Prostate cancer has become one of the most frequently observed tumours among men and a major cause of death in Western countries. Although a minor cellular component in the normal tissue, neuroendocrine (NE) cells increase in number and importance as long as prostate tumors develop. It is though that NE cells may secrete some factors that help cancer cells to grow, which explains why the presence of NE cells in prostate tumors is sometimes associated with a bad prognosis. However the exact role of NE cells in prostate cancer is still a matter of debate. Previously we have shown that the endogenous antioxidant pineal indole melatonin is able to reduce cell growth and induce NE differentiation in a human prostate cancer cell line, LNCaP, without modifying intracellular cAMP levels or protein kinase A activity. Thus, the aim of this study was to find out the intracellular pathways involved in NE transdifferentiation induced by melatonin and compare it with other NE-inducing stimuli. For this purpose we cultured androgen-dependent LNCaP cells with melatonin, androgen-withdrawn serum or cAMP analogues in order to induce NE differentiation. To discard the involvement of other mechanisms reported so far, we studied by using ELISA assay the production of IL-6 in NE-like LNCaP cells. Although melatonin is well known by its properties as a stimulator of immune system, we did not find any detectable changes in IL-6 levels when cells were treated with the indole. We found that all, melatonin treatment, androgen-withdrawal or cAMP rise, induced a transient activation of MAPK/ERK phosphorylation. Also, melatonin showed the fastest and higher effect in p-ERK activation. On the contrary, no increment in p38 or SAPK/JNK phosphorylation was observed after treatment. Melatonin and androgen withdrawal but not cAMP analogues also induced AKT activation after 24h. In conclusion, melatonin induces NE differentiation in androgen dependent prostate cancer cells by increasing p-ERK levels. This work was supported by “Instituto de Salud Carlos III (FISS-07-PI061715)”

338 Poster

Mechanisms of tumour-selective apoptosis induced by the histone deacetylase inhibitor vorinostat

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Histone deacetylase inhibitors (HDACi) are new anti-cancer agents demonstrating promise in clinical trials for the treatment of haematological malignancies. Vorinostat, the first HDACi to be approved as a cancer therapeutic agent, inhibits the enzymatic activities of histone deacetylases, resulting in hyperacetylation of histone and non-histone proteins and the induction of various biological processes including cell cycle arrest and apoptosis. We have used a novel system of human cell transformation, in which tumorigenic cells were created from normal counterparts through the introduction of hTERT, SV40 large T and small t antigens and an oncogenic allele of H-RAS (Hahn and Counter et al. 1999), to investigate the molecular basis of vorinostat-mediated tumour-cell-selective apoptosis.

Compared to normal cells, transformed cells were hypersensitive to the apoptotic activities of vorinostat and displayed biochemical characteristics consistent with induction of the intrinsic apoptotic cascade. Vorinostat-mediated apoptosis required de novo protein synthesis and we subsequently employed microarray profiling and quantitative real-time polymerase chain reaction techniques to identify vorinostat-regulated genes that might mediate the tumour-selective effects of the compound. Gene ontology and pathway analysis revealed a dominant vorinostat-induced pro-apoptotic gene expression signature in the tumour cells. Transcripts encoding pro-apoptotic Bad, Bak, Bmf and Bik were selectively induced in transformed cells, while the pro-survival regulator BclA1 was selectively repressed. These transcripts remained largely unaltered in normal cells, consistent with tumour cell-selective pro-death signalling. Ectopic expression of BclA1 protected tumour cells from vorinostat-induced apoptosis. Furthermore, the transcriptional signature was specific for HDACi such that it could be induced by the structurally different HDACi depsipeptide but not the topoisomerase II inhibitor etoposide.

These data suggest that altered expression of transcripts encoding apoptotic regulators following HDACi treatment may underpin the tumour cell-selective apoptotic effect of these agents. We have subsequently extended our functional studies to determine which of the differentially regulated pro-apoptotic genes are necessary and/or sufficient for the tumour-selective activities of vorinostat.

Hahn W.C and Counter C.M et al. (1999) Nature 400: 464-468

339 Poster

N-cadherin as a predictor of brain metastases in NSCLC

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Introduction: We have been screening genes encoding transmembrane/secretory proteins that are up-regulated in lung cancers and their brain metastasis, with cDNA microarrays and tumor cells purified by laser-capture microdissection. To verify the predictive value of these gene products from the point of wiew of brain metastases, we have been