polymerase. Experiments with DNA in vitro confirm the threading binding mode and slow complex dissociation kinetics, and measurements using DNA microarrays have shown these agents to be powerful inhibitors of mRNA synthesis at cytotoxic doses. These findings confirm the importance of linker rigidity in kinetically stabilizing the DNA-ligand complex, and the importance of linker rigidity and slow kinetics in conferring template inhibition of transcription. Currently, we are exploring ways of enhancing these properties within the bis(9-aminoacridine-4-carboxamide) paradigm by structural modifications to the chromophore, the threading side-chain, and the linker itself. In the work described here, we report the synthesis and biological activity of a series of compounds in which a benzene ring has been fused to the acridine chromophore at the 5,6 position. In previous studies with monomeric 9-aminoacridine-4-carboxamides, this substitution has been shown to enhance DNA affinity, and to slow complex dissociation rates: effects attributed to enhanced stacking interactions between the intercalated chromophore and the DNA base pairs. In the dimer series, we find the benzacridine substitution enhances both cytotoxic potency and the life-time of the DNA complex.

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Cytotoxic activity of 4'-hydroxychalcone derivatives against Jurkat cells and their effects on mammalian DNA topoisomerase I

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Chalcones (1,3-diaryl-2-propen-1-ones) are alfa, beta-unsaturated ketones with cytotoxic and anticancer properties. Several reports have shown that the compounds with cytotoxic properties may also interfere with DNA topoisomerase functions. We synthesized five derivatives of 4'-hydroxychalcones and carried out cytotoxicity tests against transformed human T (Jurkat) cells as well as plasmid supercoil relaxation experiments using mammalian DNA topoisomerase I. The compounds synthesized was 3-aryl-1-(4'-hydroxyphenyl)-2-propen-1-one. The aryl part was phenyl, p-methylphenyl, p-methoxyphenyl, p-chlorophenyl and 2- thienyl for the compounds I-V respectively. The order of the cytotoxicity of the compounds was; IV > III > II > I > V. The compound IV, 3-(4-chlorophenyl)-1-(4'-hydroxyphenyl)-2-propen-1-one, had the highest Hammett and log P values (0.23 and 4.21, respectively) and exerted both highest cytotoxicity and strongest DNA topoisomerase I inhibition. The compounds I and II gave moderate interference with the DNA topoisomerase I while the remaining ones did not interfere with the enzyme.

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Biochemical and cellular effects of a novel cyclin-dependent kinase inhibitor

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Cyclin-dependent kinases (CDK) are essential components of the cell-cycle regulatory system and due to their frequent deregulations in cancer cells they have become important targets for drug development. We have recently prepared novel group of potent and selective CDK inhibitors based on pyrazolo[4,3-d]pyrimidine scaffold. The prototype derivative LGR1404 is an isomer of roscovitine, which is a well known CDK inhibitor. We therefore directly compared effects of both compounds in biochemical and cellular assays. As expected, compound LGR1404 was found to potently inhibit cyclin-dependent kinases CDK2, CDK5 and CDK9 in enzyme assays, with IC50 values in submicromolar range. Being more potent CDK inhibitor than roscovitine, the compound also demonstrated much stronger anti-proliferative activities in human cancer cell lines, including standardized NCI60 panel. An average GI50 for roscovitine is 19,3 μM, while LGR1404 has GI50 about 7 μM. Cells treated with LGR1404 show a dose-dependent decrease of phosphorylation of the retinoblastoma protein and cell cycle arrest. Moreover, the compound increases cellular level of the tumor suppressor protein p53, stabilizes its nuclear localization and, subsequently, activates transcription of some p53-regulated genes; this effect probably results from inhibition of CDKs involved in transcription. Finally, LGR1404 causes apoptosis in treated cells, as assessed by activation of caspases, fragmentation of PARP and nuclei condensation. In conclusion, all biochemical and cellular effects of the compound are fully consistent with direct inhibition of CDKs, both cell cycle and transcriptional. The novel prototype inhibitor significantly exceeds activities of roscovitine and, thus, demonstrates the qualities of all other pyrazolo[4,3-d]pyrimidine inhibitors with potential pharmacological applications in oncology.

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Dysregulation of defence systems by 5-fluorouracil in colon cancer HT-29 cells

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A primary cause of cancer treatment failure and patient relapse is an acquired or intrinsic resistance to anticancer therapies. Acquisition of drug resistance can be attributed to various factors including inhibition of apoptosis, altered expression of multidrug resistance-associated proteins, altered drug metabolism or uptake, and/or overexpression of defence systems. Since various anticancer drugs are potential inducers of defence pathways, this could have a marked incidence on cancer cell resistance. Using colon HT29 cells, we found that 5-fluorouracil (5-FU), widely used in the treatment of colorectal cancer, induced the expression of mRNAs encoding glutathione transferases M3 and S1 and antioxidants enzymes such as NAD(P)H:quinone oxidoreductase 1, heme oxygenase-1 and γ-glutamylcysteine synthetase. To further determine the mechanisms involved in 5-FU effects, we investigated whether it activates the Nrf2/antioxidant response element (ARE) pathway which is implicated in the regulation of several genes involved in cell defense systems. Translocation of Nrf2 into the nucleus after 5-FU exposure was demonstrated by immunolocalization and western blot assays. By using an ARE driven-reporter gene (luciferase) assay, activation of the luciferase activity by 5-FU was evidenced and this effect was inhibited by co-transfecting a vector expressing a dominant negative Nrf2. Moreover, transfection of Nrf2 siRNA into HT-29 cells increased 5-FU cytotoxicity. In conclusion, these results demonstrate that 5-FU activates the Nrf2/ARE pathway which modulates the chemosensitivity of colon cancer HT29 cells and might represent a potential therapeutic target in 5-FU treatment.

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Heat shock protein 90 inhibitors modulate choline phospholipid profiles and metabolizing enzymes in human melanoma cells

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Heat shock protein 90 (Hsp90) inhibition is a novel anticancer strategy permitting simultaneous depletion of many oncogenic proteins (eg CRAF & HIF-1α) and many Hsp90 targeted drugs such as 17-AAG and 17-DMAG are now in clinical trial. Here we use magnetic resonance spectroscopy (MRS), a non-invasive technique for studying cell metabolism, to assess whether Hsp90 inhibition in human melanoma cells is associated with metabolic alterations that may serve as biomarkers of target modulation in the clinic.

SKMEL28 human melanoma cells were treated with equipotent concentrations of 17-AAG (100 nM), 17-DMAG (200 nM) or our novel agent CCT018159 (30 microM) for 48h then extracted in methanol, chloroform and water (1:1:1), and aqueous fractions analysed by 31P MRS. Western blotting for expression of CRAF and Hsp70 (known to be induced upon Hsp90 inhibition) was used to confirm drug action.

^{31}P MRS analysis indicated that exposure of cells to 17-AAG resulted in an increase in the level of metabolites involved in membrane phospholipid turnover. Cellular phosphocholine (PC), glycerophosphocholine (GPC), glycerophosphoethanolamine (GPE) content increased by ~3, 4 and 2.6-fold respectively (n=4, $p \leq 0.02$). Furthermore, nucleoside triphosphates (NTP) and PC/NTP were also increased by 2 and 1.7-fold respectively ($p \leq 0.049$), concomitant with CRAF depletion and Hsp70 induction.

Similar changes were seen with 17-DMAG (PC, GPC and PC/NTP up by 2.8, 4.8 and ~2-fold respectively) and CCT018159 (PC, GPC & PC/NTP up by 2.4, 3 and 1.4-fold respectively).

We next assessed the effect of Hsp90 inhibitors on the activity of enzymes involved in the breakdown of the major membrane phospholipid phosphatidylcholine (PtdCho). Amplex Red spectrophotometric assay of PtdCho specific phospholipase C (PtdCho-PLC) showed a decrease in the enzyme's specific activity to 45±19% of controls (n=4, p=0.015) in 17-AAG treated cells. Western blotting showed a marked reduction in phosphorylated (activated) cytoplasmic phospholipase A2 (cPLA2) but not total cPLA2 in cells treated with all three inhibitors.

Our results indicate that inhibition of Hsp90 in human melanoma cells results in altered choline phospholipid metabolism that is associated with

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

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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.

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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.

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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.

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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