pounds were redocked more exhaustively and then subjected to a modified similarity Tanimoto screen based upon the six TPIs. From the resulting ranked similarity and interaction energy scores, 535 ligands were selected for fully flexible ligand docking. The 535 docked solutions were then scrutinized for appealing interactions between the ligand and the active site residues, particularly hydrogen bonds, and packing efficiency in the active site.

Results: The top 40 ranked ligands were then ordered from the NCI repository. 13 were available and are currently undergoing biological evaluation.

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Structural consequences of trans-membrane association and mechanism of molecular recognition at the active site of human estrone sulfatase, a potential target for hormonal breast cancer therapy

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Human estrone (E1)/DHEA sulfatase (ES), along with cytochrome P450 aromatase and 17beta-hydroxysteroid dehydrogenase1 (17HSD1), is responsible for maintaining high levels of the active estrogen, 17beta-estradiol (E2), in tumor cells. ES catalyzes the hydrolysis of E1-sulfate, which is subsequently reduced to E2 by 17HSD1. The presence of ES in breast carcinomas and ES-dependent proliferation of breast cancer cells have been demonstrated. Selective estrogen enzyme modulators that inhibit these enzymes have shown promise as anti-proliferative agents. Rational design of specific ligands requires detailed understanding of molecular structure of the active site. Although the precise sub-cellular localization of the functional ES is not clear, this membrane-bound enzyme is distributed in the rough endoplasmic reticulum (ER). The full-length enzyme has been purified from the microsomal fraction of human placenta in the active form and crystallized. The three-dimensional structure of the enzyme has been determined by X-ray crystallography at 2.6 angstrom resolution. The structure shows a trans-membrane domain consisting of two anti-parallel alpha-helices that protrude from the roughly spherical molecule, thereby giving it a "mushroom"-like shape. These highly hydrophobic helices, each roughly 40 angstrom long and situated between residues 179 and 235, are capable of traversing the membrane, thus presumably anchoring the functional domain to the membrane surface facing the ER lumen. The location of the trans-membrane domain is such that the opening to the active site, buried deep in a cavity in the "gill" of the "mushroom", rests near the membrane surface. Furthermore, a spatially proximal polypeptide segment between residues 468 and 500, consisting of several hydrophobic sidechains and displaying high thermal motion, also presumably associate with the lipid bilayer. The residues from the membrane-associating regions line the entry path leading to the active site. The catalytic amino acid hydroxyl formylglycine 75 is found to be covalently linked to a sulfate moiety. While D35, D36, D342 and Q343 are involved in coordination of the Mg2+ ion, H290, H136, K134 and K368 play important roles in catalysis. Residues V101, F178, V177, L74 and F488 could participate in substrate recognition. Details of steroid-protein and lipid-protein interactions will be presented. This work is partially supported by the grants GM59450 and GM62794 from the NIH (to DG).

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Synthesis, structure and anticancer activity of a novel platinum(II) coordination compound of 4-aminosalicylic acid

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We have previously synthesized binuclear complexes of Pt(II)involving the naphthoquinone bridging ligand,attempting to explore the possible synergistic effect of anthracyclins and cis-platin. The results were encouraging and therefore we extended our research to Pt(II) complexes involving various hydroxyquinonic ligands. Considering that hydroxyquinones are slightly soluble, the selection of smaller ligands exhibiting coordination ability towards Pt(II) metal centers, is a good choice. Along this line, we present preliminary results on a new mixed-ligand Pt(II) complex of 4-amino-salicylic acid with imidazole. The complex was prepared by reacting of sodium 4-aminosalicylate with K2PtCl4 in aqueous medium in a 1:2 molar ratio. To the mixture, excess of saturated imidazole was added. The final mixture was stirred for 24h at RT. The dark yellow precipitate (MT: C10H8O3N3PtCl, MW: 448.5) was filtrated,washed with water and methanol and air-dried. It is soluble in DMSO.

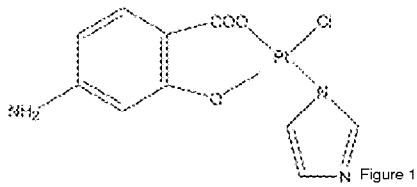

Figure 1

4ASA is coordinated to the Pt(II) central atom in a bidentating way, via the carboxylic and phenolic oxygen donor atoms forming a 6-atom ring. The imidazole and chloride ligands complete the square planar coordination environment of Pt(II). The IR spectrum confirms the above as the band at 3139 cm^{-1} is characteristic for non-coordinated –NH2 groups.Moreover,the ν(C=O) stretching vibration at 1627 cm^{-1} is shifted to lower frequencies compared to the uncoordinated ASA(C=O) group (1638 cm^{-1}).Accordingly,the ν(C-O) vibration is shifted to higher frequencies (1307 cm^{-1}) relative to that of 4ASA (1302 cm^{-1}). Finally,the ν(Pt-O) stretching vibration was found at 334 cm^{-1}. Human cancer cell lines of lung (A549), colon HCT-15), melanoma (SK-MEL-2) and ovaries (A2780) were used for the cytotoxicity test *in vitro* with the SRB assay. The cytotoxic activity was evaluated by measuring the concentration of the complex required to inhibit the protein synthesis by 50% (IC_{50}) compared to cis-platin. Each value is the result of triplicate experiments.

Table 1

Cell	cis-platin	complex
A549	0,275	0,075
HTC-15	1,490	0,650
SK-MEL-2	0,155	0,140
A-2780	0,240	0,148

The present results illustrate that the complex exhibits cytotoxicity against all cell lines tested,with higher rate of activity than that of cis-platin. Nevertheless,the solubility effects are of key importance to the improvement of the complex's toxicity. Therefore, research on the synthesis and study of new, mixed-ligand Pt(II) compounds of 4ASA and a variety of other N-donor ligands is in progress aiming to improve the solubility and bioactivity of the compounds.

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A molecular overlap tool for investigating potential binding mode similarity in sets of compounds

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The Developmental Therapeutics Program (DTP) currently offers web accessible tools such as COMPARE that use biological similarity comparison algorithms to mine the compound databases provided by DTP. By applying a method that focuses on structural aspects of these compounds, we provide an independent measurement that identifies structural commonalities that may help distinguish particular sets of compounds that are more likely to have the same underlying biochemical mechanisms of action. We present a new tool where sets of compounds can be aligned based on maximizing their molecular volume when hydrogen bond donors and acceptors are superimposed. The algorithm employs an evolutionary programming method that overlays structures based on a substitution matrix of atom types. Examples of the utility of this new application will be presented along with details about its accessibility through our web pages and integration with COMPARE.

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Development of an Mdm2/p53 fluorescence polarization high throughput inhibitor screening assay

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Mdm2 regulates p53 tumor suppressor function by three mechanisms: binding to the transactivation domain of p53, exporting p53 out of the nucleus, and ubiquitinating p53 for degradation. Mdm2 hyperactivity, due to amplification/overexpression of Mdm2 or mutational inactivation of ARF locus, is undesirable because it inhibits the function of wild type p53 and can lead

to the development of a wide variety of cancers (such as sarcoma, ependymomas, soft tissue tumors, glioblastomas, and astrocytomas). Thus, the development of anti-Mdm2 therapies may restore normal p53 function in tumor cells and induce growth suppression and apoptosis. We have developed a fluorescence polarization (FP) assay to identify small molecule inhibitors that block the binding of Mdm2 to a high affinity (~4 nM) p53-derived fluorescent peptide. The change in fluorescence polarization of the ligand upon binding to a 33 Kda Thioredoxin-Mdm2 fusion protein is more than 200 mP and has an assay performance indicator value (z value) greater than 0.5 (indicating excellent response). The assay is performed in a high-throughput, 384-well, format capable of detecting single digit nanomolar inhibitors. Buffers and buffer modifiers, ligand and protein concentrations, as well as assay volumes were optimized. The assay was validated with reference Mdm2 inhibitors by dose-dependent inhibitor titrations to determine Ki values which were independently confirmed by isothermal titration calorimetry (ITC).

Cancer vaccines

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Humoral Immune Responses to MUC1 in Women with BRCA1/BRCA2 Mutations

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MUC1 is being studied as a target antigen for the immunotherapy of cancer. Natural antibodies to MUC1 are present in the circulation of cancer patients and healthy controls. Early breast cancer patients with a natural humoral response to MUC1 have a higher probability of freedom from distant metastases and a better disease-specific survival, suggesting a possible role of MUC1 antibodies in controlling haematogenous tumour dissemination and outgrowth. The objective of the study was to evaluate humoral immune responses to MUC1 in women at hereditary high risk of breast ovarian cancer. IgG and IgM antibodies to MUC1 were measured by ELISA in serum samples obtained from 79 women with a BRCA1 (N = 67) or a BRCA2 (N = 12) mutation, 23 women with BRCA1/BRCA2 mutations in the family but themselves not mutations carriers and 125 age-matched healthy controls. History of breast cancer was present in 24 and in 4 of the BRCA1 and BRCA2 mutation carriers, respectively. MUC1 IgM antibodies levels did not differ significantly between mutation carriers, non-carriers and healthy controls. MUC1 IgG ab levels did not differ significantly between controls and non-carriers. A significant difference in MUC1 IgG ab levels was found between controls and carriers of a BRCA1/2 mutation.

Table 1

Study population (N = 227)	N	MUC1 IgG arb. U/ml Median (range)	Mann-Whitney U test two-tailed P
a. Proven carrier BRCA1or BRCA2	79	0,72 (0,39-9,13)	a/c <0.0001
b. Mutation in family, patient not carrier	23	0,84 (0,49-6,67)	b/c n.s.
c. Controls	125	0,95 (0,43-5,70)	

MUC1 ab levels did not differ significantly between mutation carriers with or without history of breast cancer. In conclusion, natural IgG ab to MUC1 ranked lower in BRCA1/BRCA2 mutation carriers than in healthy controls. Prophylactic immunotherapy with MUC1 substrates may be a strategy to reduce the risk of breast/ovarian cancer in BRCA1/BRCA2 mutation carriers, strengthening tumour immune surveillance. A possible relation between BRCA1/BRCA2 mutations and the immune system remains to be explored

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Prolonged antigen presentation of monocyte-derived dendritic cells loaded with plga-microspheres

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Dendritic cells (DCs) are professional antigen-presenting cells (APCs) that are specialized to capture antigens in peripheral tissues, to migrate into secondary lymphoid organs and to prime T cells. Hence they are increasingly exploited as cellular vaccines in tumor immunotherapy. Currently, in most of the ongoing DC-based vaccination studies DCs are differentiated from monocytes during 5 to 6 day cultivation in the presence of GM-CSF and IL-4, and are externally loaded with tumor-specific antigenic peptides or tumor lysates before being re-injected into the patients. However, peptide-MHC complexes have a rather short half-life, and the single peptides are restricted to their specific MHC allele, and, finally, the composition of tumor lysates contain a bulk of tumor unrelated proteins and are quite undefined. In order to obtain a sustained antigen release which is favor to a prolonged antigen presentation by DCs, in the present study we encapsulated protein antigens or MHC class I- or class II-restricted peptides into biodegradable poly(D,L-lactide-coglycolide). The yielded antigen-containing microspheres (MS) can release their content over a time period of several days to weeks. We observed that human monocyte-derived DCs (MoDCs) generated under serum free conditions were able to efficiently take up MS particles. Using MS loaded with tetanus toxoid (TT) or a synthetic peptide derived from tetanus toxin sequence 947-967 (tt30) as model antigens for MHC class II restricted presentation, we observed that MoDC loaded with MS-tt30 were able to induce a strong T cell response, which is comparable to those induced with soluble tt30. Interestingly, 10 days after MoDC taken up MS-TT, stimulation of TT-specific T cells can still be observed, while MoDCs loaded with soluble TT only induced a very weak proliferating response. Moreover, 5- to 10 fold lower amount of antigen was required for stimulating TT- or tt30-specific T cells with MS-TT. Currently we are evaluating whether the MHC class I-restricted presentation of microencapsulated antigens taken up by MoDCs exerts similar prolonged kinetics. In conclusion, microencapsulation of proteins or antigenic peptides is a potent system to deliver antigen to DCs. This could provide a considerable contribution to improve the DC-based vaccines, in terms of prolonged antigen presentation with a low antigen amount.

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A phase I/II vaccination study of patients with minimal residual prostatic adenocarcinoma after radical prostatectomy using autologous dendritic cells pulsed with recombinant PSA (Eladem tm)

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Dendritic cells are potent antigen presenting cells activating naive T lymphocytes and initiating cellular immune responses. Twenty-four prostatectomized patients, with rising PSA values (1-10 ng/mL) as the only sign of progressive prostate cancer, were vaccinated with Eladem (autologous dendritic cells pulsed with recombinant human PSA protein) in an attempt to trigger an immune reaction against cells expressing PSA. Bone scans and pelvic-abdominal CT scans performed before the inclusion were negative for all patients. To prepare Eladem, 1010 peripheral blood mononuclear cells from a single apheresis were cultured in the presence of GM-CSF and IL-13 for 7 days, purified by elutriation and pulsed with human rPSA. Eladem was administered in 3 cycles of 3 doses over a period of 5 months. Each dose was divided into 1 ID, 4 SC and 1 IV injections. All 24 patients received the planned 9 administrations. The mean cell count injected per administration ranged from 1.5-7.2 $\times$ 10^7 cells. The total number of injected cells ranged from 1.3 $\times$ 10^8 to 6.5 $\times$ 10^8. Mean age of the patients was 66 years (range 53-80), mean time since prostatectomy 3.7 years (range 1.3-9.7) and mean PSA at baseline 3.1 ng/ml (range 1.0-7.6). The cell therapy was generally well tolerated. No SAE was observed and there were no significant laboratory findings. Four patients experienced a total of 5 adverse events considered to be reasonably related to the therapy: macular rash (3) asthenia (1) and halitosis (1) all of mild intensity. No emergent signs of autoimmunity or significant change in autoantibody status were noted. Seven patients withdrew from the study during the Month 6-12 follow-up because of progression or local disease recurrence. Circulating cancer cells (CCC) were detected by RT-PCR with PSA-specific primers in 6 patients at baseline. CCC were no longer detectable in all the 6 patients at Month 6. Eleven patients had a transient post-baseline decrease of PSA on 1 to 3 occasions ranging from 6%-39%. First decrease were observed at Month 1 in 7 patients, Month 3 in 2 patients and at Months 5 and 9 in 1 patient each. Five patients with negative results at baseline became positive for PSA-specific cytotoxic T cells. No significant correlation between PSA, CCC and cytotoxic T cells was observed. In conclusion, Eladem therapy proved to be both feasible and safe in the treatment of prostate cancer patients.