Update on Clinical Trials in Adenocarcinoma Patients Immunised with Mannan-MUC1

Seven different clinical trials (Phase I, II, III) have been completed using mannan-MUC1 in adenocarcinoma patients. Dose, route of administration, MUC1 fusion protein, peptides, peptide mimics, peptide mutants and different cytokines were examined, and will be discussed. The current ongoing trial using autologous dendritic cells cultured and primed with mannan-MUC1 *ex vivo* before re-injection will also be discussed.

No 20

New Cytotoxic Drugs: Evaluation Process of a New Class of Cu(II) Complexes

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In the process of evaluation for new anticancer drugs we have tested a new series of Cu(II) complexes. The reaction of $[(dien)Cu(ONO_2)](NO_3)$ with 2-amino-5-methylthiazole (2A5MT), 2-amino-2-thiazoline (2A-2Tzn), imidazole (im), N,N´-thiocarbonyldiimidazole (Tcdim), 2-aminothiazole (2AT) and 2-ethylimidazole (2Etim), gave a new series of mixed-ligands compounds of the general formula $[(dien)Cu(B)ONO_2)](NO_3)$; [dien: diethylenetriamine, B: 2A5MT, 2A-2Tzn, im, Tcdim, 2AT and 2Etim].

An approach algorithm was employed: First the candidate drugs are tested for their antiproliferative activities against a panel of human tumor cell lines. The drugs that showed any promising results were then tested for their cytostatic (DNA synthesis inhibition and cell cycle arrest) or cytotoxic activity (induction of programmed cell death). Finally, mode of action (based on the previous results) was estimated by examining gene expression, signal transduction pathways and combination with other drugs.

We tested all complexes for antiproliferative (cytostatic and cytotoxic) activity against a panel of cell lines (Hela, L929, HT29 and T47D). All $[(dien)Cu(B)ONO_2)](NO_3)$ complexes had an activity against colon cancer cells (HT-29), inducing G_2/M cell cycle arrest, an effect that for most of the complexes could be attributed to $p34^{cdc2}$ inhibition by tyrosine-phosphorylation and/or to induction of (cyclin-dependent kinase inhibitor) $p21^{WAF1}$. Other cell lines were resistant to the majority of the complexes, except $[(dien)Cu(2A5MT)ONO_2)](NO_3)$, that had showed the highest anti-proliferative activity against HT29 cells also. The predilection for colon cancer cells and the relatively low toxicity against normal (L929) cells justified further investigation of this group of compounds. A new series of modified Cu(II) complexes are under development and the preliminary results showed a potent anticancer activity against colon cancer cells.

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No 21

Genome, Drugs and Bioethics

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The human genome mappings is a major step in the continuing exploration on how our bodies work, how we have evolved and related to other species. Furthermore, the knowledge from the human genome leads now to the goal of discovering the function of genes and how they relate to

each other in order to succeed the development of new diagnostics, drugs and vaccines for diseases.

Thus, by using various relative genomics (e.g. SNPs maps) and proteomics techniques, rich information will be added to the archive of tools for studying complex genetic diseases, population migration patterns, and a variety of pharmacogenetics possibilities which lead to the personalized medicine as well as to identification of genes involved in drugs efficacy and / or site effects.

On the other hand people have long taken various substances to improve performance. Such substances are e.g. caffeine, nicotine and amphetamines for alertness and concentration, steroids, erythropoeitin and human growth factor for athletic ability as well as various cosmetics etc. Moreover, new classes of drugs enhancing heritable traits, genetic selection of optimal embryos and more effective genetic engineering and enhancement technologies will grow out of the Human Genome Project. Thus, it is quite possible that most genetic technologies would be used not for currying a (rare) disease by a specific drug or process but for enhancing normal characteristics, such as raising I. Q. creating with this way a genetically – based caste system where poorer people won't stand a chance, excluded from the principle of equal genetic opportunity.

Such problems have led to the concept of geneticitation, which has been introduced to describe the various interlocking and imperceptible mechanisms of interaction between medicine, genetics, society and culture. Thus, as we learn more about our genome and ourselves, we will confront new bioethical issues involving privacy, discrimination, intellectual property, proper education and the sociopolitical dimension as well.

No 22

Immune Responses to Human Tumors and the Development of Peptide and Recombinant Cancer Vaccines

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Tumor cells are recognized as non-self by the immune system, and elicit an immune response, which often leads to their elimination. Initial evidence for the existence of a specific antitumor immune response in humans was provided by the development of T-cell lines/clones with specific cytolytic activity against autologous melanoma tumor cells. Human and animal tumors are infiltrated by mononuclear cells designated tumor infiltrating lymphocytes (TIL) which are comprised primarily of T cells. TIL may represent the immune response of the host to the tumor and their presence has been associated with better prognosis and improved survival. TIL have been shown to be a better source of T cells with antitumor immunity than peripheral blood lymphocytes. We and others have developed and propagated in rIL-2 TCR+ T-cell lines/clones from TIL from patients with malignant melanoma and ovarian carcinoma, exhibiting cytotoxicity restricted to autologous tumor cells and/or cytokine production restricted to autologous tumor cells. These melanoma or ovarian carcinoma T-cell lines and clones recognize autologous tumor cells alone, as well as allogeneic tumors cells that express the same peptide/MHC epitopes. These T cells do not recognize other allogeneic tumor cells, normal cells or K562 cells.

To further access the immunogenicity of tumor cells, we investigated whether fresh (uncultured) TIL from patients with malignant melanoma or ovarian carcinoma contained clonally expanded T cells. Unique T-cell receptors (TCR) serve as fingerprints of each individual T-cell clone. The maximum theoretical number of unique -chain TCR transcripts is approximately 1012. Therefore, the probability of randomly finding multiple identical copies of a single -chain or -chain TCR transcript within an independent sample of T cells is negligible. Specific-antigen driven clonal T-cell responses are identified by the presence of multiple identical TCR transcripts. TIL from patients with melanoma or ovarian carcinoma and certain other tumorscontained substantial proportions (range of 25% to 90%) of identical - and -chain TCR transcripts after nonpalindromic adaptor-PCR (NPA-PCR) amplification, cloning and sequencing. These results demonstrate the presence of oligoclonal populations of T cells in these tumors. It is very likely that these T cells have undergone antigen driven proliferation and clonal expansion in situ in the tumor, in response to as yet unidentified antigens.

Recently, tumor antigens that elicit antigen-specific MHC-restricted T-cell responses have been identified, initially in malignant melanoma.

Peptides derived from these antigens are recognized by T cells in association with HLA. Certain of these antigens are expressed only on tumor cells (tumor -specific antigens). Others are expressed on tumor cells and on normal cells of the same lineage (such as malignant melanoma cells and normal melanocytes), or on embryonic or fetal cells (tumor-associated antigens). Tumor-specific or tumor-associated antigens can be: (i) a newly expressed antigen; (ii) a point mutation, on a gene that is usually expressed on normal cells; (iii) a posttranslational modification; (iv) the result of a chromosomal translocation. Peptides derived from these tumor antigens are recognized by T cells in association with MHC. T cells also recognize in association with MHC, peptides derived from: (i) activated oncogenes; (ii) protooncogenes, which are regularly expressed (same sequence) on normal cells and at much higher density on tumor cells; (iii) oncofetal antigens; and (iv) tumor suppressor genes. These are mutated and overexpressed in certain tumors and therefore cannot control cell growth. Mutated, or wild type peptides from these molecules are recognized by T cells in association with MHC.

Tumor cells are masters in disguise and disete to escape destruction by the immune system. They use several mechanisms for this purpose: (1) tumor cells downregulate the expression of HLA class I and thus of HLA class I/peptide complexes. We have found that HLA class I expression on human ovarian carcinoma tumor cells correlates with T-cell infiltration in vivo and T-cell expansion in vitro in low concentrations of rIL-2. (2) tumor cells or other cells in the tumor environment, such as monocytes, produce immunosuppressive factors, such as TGF- 2 and IL-10. We have found in ovarian carcinoma that inhibition of T cell proliferation by IL-10-producing monocytes could be reversed by adding neutralizing antibodies to both IL-10 receptor and to TGF- 2. (3) Induce anergy in T cells infiltrating tumors. CD3-zeta message and protein are absent or downregulated in TIL from a number of different tumors. CD3-zeta message and protein expression is restored after in vitro treatment with rIL-2.

The molecular cloning of tumor antigens and the identification of peptides derived from these antigens that are recognized by T cells, in association with MHC, permit the design for the first time of tumor vaccines, based on peptides or on recombinant antigens, for the treatment of patients with certain cancers, preferably after surgery and chemotherapy. In particular these vaccines are being/will be used for the treatment of patients with minimal residual disease and for prevention of relapse. These vaccines can be also used for the vaccination of those individuals at high risk to certain cancers, because of genetic predisposition. However, the mechanisms of tumor escape previously discussed will interfere in the development of successful tumor vaccines and steps need to be taken to counter these mechanisms. Clinical trials to evaluate these vaccines and various vaccination approaches will be discussed.

No 23

Theiler's Murine Encephalomyelitis Virus-induced Demyelinating Disease in Mice as a Model for Multiple Sclerosis

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TMEV induces in susceptible strains of mice biphasic disease, namely early acute disease followed by late chronic demyelinating disease. Early acute disease is characterized by replication of the virus in the gray matter of the central nervous system (CNS) predominantly in neurons, but also astrocytes, oligodendrocytes and microglia. After few weeks the virus is completely cleared from the gray matter and animals develop late chronic demyelinating disease. The virus persists at very low level in microglia/ma-crophages, astrocytes and perhaps in oligodendrocytes. Both phases of the disease are associated with extensive infiltration of the CNS by CD4+ and CD8+ T cells, monocytes/ma-crophages, few B cells and plasma cells. Immunosuppressive studies with cyclophosphamide and anti-thymocyte serum and studies investigating infection of mice with TMEV with genetically deleted CD4 +or CD8+ strongly suggest that both CD4+ and CD8+ T cells are essential for clearance of the virus. It has been also suggested that CD4+ and CD8 +; T cells play a role in the development of demyelinating disease. Very little information is available

about antigenic specificity of these T cells, in particular during late chronic demyelinating disease. These T cells may be specific for viral structural protein(s) but additional T cell populations specific for autoantigens, such as proteolipid (PLP) have been also described. Generation of T cells specific for PLP is very likely the result of epitope spreading.

TMEV-induced demyelinating disease is consider to be one of the best experimental animal models of MS. This is based on (i) similar histopathological changes observed in the CNS of TMEV infected mice and in the CNS of patients with MS; (b) Major Histocompatibility Complex (MHC) association of susceptibility to TMEV and to MS; (c) epidemiological evidence suggests viral etiology of MS. TMEV-induced CNS disease in SJL mice has been systematically studied in our laboratory. Most recent findings involve demonstration of the role of iNOS, cytokines and estrogen in this model of demyelination. Recent studies suggest that in both TMEV infection and in MS, the progress of the disease, including relapses and increasing damage to the white matter depends on generation of T cell mediated immune responses to host CNS myelin antigen (s). These immune responses are generated in the process of epitope spreading, either intra or intermolecular (involving predominantly PLP and myelin basic protein {MBP}). Treatment of patients with MBP and/or PLP peptide analogs, with substitution in TCR contact residues or MHC contact residues may be a promising avenue in averting the generation of pathogenic T cells and may help to ameliorate progress of the disease. Although the mechanism of protective effect of these analogs is not fully understood, it has been suggested that they induce regulatory T-cells. Such studies including treatment of TMEV-infected mice with potent analogs of MBP and PLP are in progress in our laboratory in collaboration with Dr. Matsoukas.

No 24

Effect of non-Peptide Thrombin Receptor Antagonists and RGD-Analogues on Angiogenesis in the Chick Chorioallantoic Membrane

M. Papakonstantinou[1], N. Tsopanoglou[1], Y. Sarigiannis[2], J. Matsoukas[2], G. Stavropoulos[2] and M. Maragoudakis[1]

[1]Department of Pharmacology, University of Patras, Greece, [2]Department of Chemistry, University of Patras, Greece

Thrombin, a multifunctional serine protease, apart from its central role in blood coagulation constitutes a powerful agonist for a variety of cellular responses mediated mainly by the PAR receptors. The identification of the thrombin receptors and the demonstration that are members of the super-family of G-protein-coupled receptors opened a new area in the development of agents that may selectively inhibit the effects of thrombin without affecting fibrin formation.

The angiogenic action of thrombin has been shown to be mediated by activation of the thrombin receptor PAR-1 and be independent on its ability to form fibrin. Recently, the thrombin-induced angiogenesis in the chick chorioallantoic membrane was shown to be suppressed by DOL_5, a thrombin receptor non-peptide antagonist designed to be analogue of thrombin receptor activating peptides (TRAP) and carry the pharmacophoric features of Phe and Arg residues present in the active pentapeptide SFLLR. Novel modified compounds of the biologically active DOL_5 were synthesized in an effort to develop more potent thrombin receptor antagonists.

In this report, we studied the effects of F-DOL_5, Ebom and Ebzl, three prospective thrombin receptor antagonists, on angiogenesis in the CAM system. F-DOL_5 suppressed angiogenesis as compared to control and in combination with thrombin reversed its angiogenic effect. Ebom and Ebzl were also shown to suppress thrombin-induced angiogenesis although in a lesser extent. Comparing them to DOL_5, F-DOL_5 presented similar activity whereas EBom and Ebzl were less active. These results suggest that there is a possibility for developing specific inhibitors of the angiogenic action o thrombin without effects on blood coagulation that can be used in the treatment of angiogenic diseases where suppression of angiogenesis is desirable.

In addition, a series of RGD-analogues incorporating or not the salicyl acid moiety were tested in the CAM system. These compounds that combine in the same molecule the active group of aspirin and the extracellular matrix-related RGD

tripeptide were primarily designed to be antagonists of the GPIIb/IIIa platelet receptor that mediates platelet activation. Indeed, these compounds have been shown to exhibit antithrombotic activity since they abolish collagen-induced platelet aggregation. Testing them in the CAM system, we found that they possess considerable anti-angiogenic properties.

No 25

Synthesis of Conformationally Restricted Retinoids of the Acitretin Type

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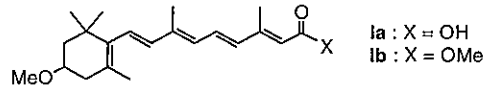
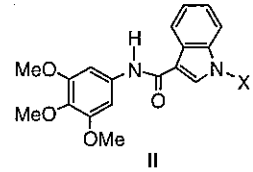
A series of conformationally restricted analogues (IIa-d) of the retinoid Acitretin (Ia) and its methyl ester (Ib) were obtained using amide IIe as key intermediate. The latter was synthesized from the commercially available 3,4,5-trimethoxyaniline and indole-3-carboxylic acid. Attempts to cleave the phenacyl esters IIb and c with PhSNa in order to obtain the corresponding acids IIf and g failed. The main product in both deprotection reactions was identified as the amide IIe, resulting from a PhSNa-mediated amide bond cleavage





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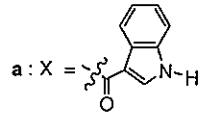

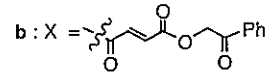

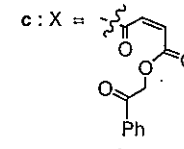

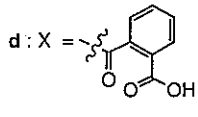

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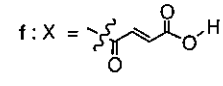

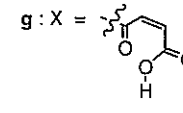

No 26

A Rapid Capillary Electrophoretic Method for the Resolution of Cerebrospinal Fluid Proteins with Qualitative and Quantitative Characteristics that Facilitate the Diagnosis of Multiple Sclerosis

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A specific humoral immune response in multiple sclerosis (MS) is the production into the central nervous system of immunoglobulins which appear as oligoclonal bands (OBs) in agarose isoelectric focusing (IEF) of cerebrospinal fluid (CSF). Among the cases with clinically definite

MS, up to 95% have oligoclonal IgG bands in their CSF. Capillary zone electrophoresis compared to flat bed IEF provides reproducible results, requires shorter analysis time, and allows quantitative determination. It was therefore examined whether CE can be used as an alternative procedure to IEF for MS diagnosis. Separation of CSF and serum proteins is performed using 25 mM borate buffer, pH 10, containing 25 mM SDS at 30 kV and normal polarity. Sandoglobulin ® was used as control immunoglobulin to identify the positioning of γ-globulins in the electropherogram. CE analysis of CSF and serum samples from patients with clinical definite MS (n=3) and controls (n=5), demonstrated (1) peaks in the region of γ-globulins of the CSF samples of patients, which were absent in controls and (2) the absence of the peaks, which were present in CSF samples of the patients, in the serum of the same patient. The relationship of the CE peaks to OBs of IEF is presently examined.

No 27

Use of Nuclear Magnetic Resonance to Study the Conformational Properties of Novel Analogs of RGD

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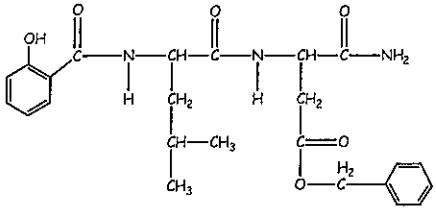
2-HO-C_6H_4-Leu-Asp(OBzl)-NH_2 was designed based on RGD and synthesized in an attempt to find better antithrombotic or antiangiogenetic drugs. Such drugs act through specific interactions with integrins. A conformational analysis of 2-HO-C_6H_4-Leu-Asp(OBzl)-NH_2 was sought as it is a bioactive molecule. In addition molecules possessing a variety of biological activities are under study. These studies aim to explore the stereo-electronic requirements of integrins for drug binding in an attempt to synthesize novel analogs with better pharmacological profile.



2-HO-C_6H_4-Leu-Asp(OBzl)-NH_2

No 28

Angiogenesis and Cancer

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Angiogenesis is a complex biological process, in which many types of cells, a prethora of modulators and the extracellular matrix play a role. Physiological angiogenesis takes place in embryonic development and the endometrium of females during the reproductive cycle.

In adults angiogenesis is evident only in repair processes (inflammatory conditions, wound healing etc.). In many pathological conditions, however, including cancer, angiogenesis is deranged and contribute to the pathology of the disease. Recently angiogenesis is recognized as necessary condition for tumor progression and metastasis and is considered as suitable therapeutic target for developing novel anticancer drugs.

Possible advantages of anti-angiogenic therapy of cancer are: easy access to target (vessels), independent of development drugs resistance, applicable to many types of cancer and very few expected side effects of therapy.

Angiogenesis is activated in cancer by the progressive mutation of cancer cells, which leads to the expression of angiogenic factor causing mu-

tual activation of endothelial and cancer cells (angiogenic switch).

The past few years many angiogenic and anti-angiogenic factors have been discovered. The new techniques of molecular biology made possible the study of these modulators of angiogenesis at the molecular and cellular level, their receptors and the transduction mechanisms involved.

These modulators are involved in the growth, survival and apoptosis of endothelial and cancer cells. Among these factors is thrombin, which has been the subject of our research for the past 15 years. In this presentation the role of thrombin in angiogenesis will be reviewed and the implications of thrombin-induced angiogenesis in tumor progression and metastasis as well as in drug development will be discussed. In addition the current status of anti-angiogenic therapy will be presented.

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No 29

Commercialising Academic Research: The Challenges, the World-wide Pharmaceutical and Biotechnology Industry Developments and the Need for Partnering

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The past few years have seen the pharmaceutical industry undergo more change than ever with consolidation creating fewer and larger companies. Cost/benefit ratio awareness and cost-containment by government reforms and market economics have increased the pressure in the industry for even more effective drug development and commercialisation. At the same time, collaborations have surged to the point that most pharmaceutical companies see each other as potential partners; a remarkably different view compared to the doing business 10-15 years ago.

Following maturity of many of the innovative product classes and companies' expansion in new geographic markets, top-line growth became too modest to excite investors. The industry responded to this financial drive by the easiest route: Consolidation & Alliances. This in turn has created mega-companies that find it difficult to maintain a certain percentage growth because the absolute amounts of *incremental* turnover per year needed to deliver an attractive growth (e.g. 10-15% per year) o the shareholders are billions of dollars, which means that pharmaceutical companies need to develop more new compounds than what they have in their own research. As a result, big, mid-size and also small pharmaceutical and biotech companies depend now to a large extent to licensed projects, i.e. discovered and perhaps also partly developed by institutions and small biotech companies.

Alliances have surged to the point that most pharmaceutical companies see each other as potential partners; a remarkably different view compared to doing business 10-15 years ago. There have been more than 7,000 alliances concluded over 5.5 years. On average, around 20% of the actual revenues of big-pharma companies' derives from products licensed from the "outside" many years ago. This number is projected to almost double, based on the recently licensed compounds now in development. More importantly, while the number of deals has increased by 50% since 1996, the deal values have increased almost 250% as big companies compete for the same assets.

Developing pharmaceutical compounds is a very expensive and risky business. On average, a new compound, or a New Chemical Entity (NCE), takes 12 years from the time it was discovered to reach the market, and cost estimates range from $200-800m, while going through the following stages (based on regulations): *Preclinical Tests* (3 or more years in-vitro and in-vivo studies to show biological activity of the compound against the targeted disease, including safety evaluation); *Phase I* (testing the safety and pharmacokinetics in a small number of healthy volunteers - compounds in phase I have a 15-20% chance of making to the market); *Phase II* (larger number of patients, e.g. 200-300 to test the NCE's efficacy, providing proof of concept -

compounds in phase II have a 25-35% chance of making to the market); *Phase III* (detailed and large phase of clinical development in thousands of patients establishing statistically significant differences in the efficacy and safety of the NCE vs. marketed drugs - compounds in phase III have a 60-70% chance of making to the market). Then all the data is compiled in a Dossier Filing submitted to the regulatory authorities (e.g. FDA and EMEA) for review and approval to market.

The prohibitive cost, specialised skills and lack of infrastructure prevents local pharmaceutical companies from developing novel compounds. So far, Greek pharmaceutical companies are not able to develop novel compounds.

There is therefore a good case for certain Greek researchers and institutions that are interested, to focus on projects that have the potential to lead to *novel* pharmaceutical compounds that can be *partenered* with European and American biotech and pharmaceutical companies who are able to undertake the expensive and risky development and of course, the final marketing of the products on a global basis.

No 30

New Metal-Complexes and their Biological Activity

D.A. Kyriakidis, A.A. Pantazaki. D.P. Kesisoglou, D. Kovala-Demertzi, K.T. Papazisis and A.H. Kortsaris

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The interaction of many inorganic compounds with cellular components such as DNA and proteins, as well as their involvement in many cellular processes, have been extensively studied. Information derived from *in vitro* studies concerning metal binding or interaction with DNA and DNA of living cells appears to form essentially the same adducts. Several anticancer drugs that are capable of DNA intercalation or causes DNA alteration are related to topoisomerase activities. The cytotoxic effects of some agents have been suggested to result from distortion of the DNA double-helix, which induces topoisomerases to break and relax DNA.

In the present study we use the high nuclearity complexes of Ni, from trinuclear to decanuclear fused metallacrowns to study different biological parameters such as interaction with DNA, anticancer and antibacterial activity (1,2). In addition, the biological effect of two classes of newly synthesized complexes of Pt(II) and Pd(II) with 2-acetyl pyridine 4N-ethyl thiosemicarbazone will be used as interference compounds to topoisomerases *in vivo*.

Antibacterial activity was performed against a range of Gram positive and Gram negative bacteria. Minimal Inhibitory Concentration (MIC) was determined using the method of progressive double dilution in liquid media. The antiproliferative activity was carried out using the XTT microculture tetrazolium colorimetric assay through which an assessment of drug-induced cellular growth inhibition is attained. Drug potency is expressed in terms of IC50 values (drug concentration resulting in 50% growth inhibition) calculated by dose-response curves obtained by XTT-assay.

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No 31

A New Polypeptide Carrier of Multiple Antigenic Epitopes for the Development of Diagnostic Assays and for Immunoregulation

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Our concept in the design of the *S*equential *O*ligopeptide *C*arriers, (Lys-Aib-Gly)$_n$ or (Aib-Lys-Aib-Gly)$_n$, (SOC_n), was to construct an artificial support with structural rigidity and regularity, so

that peptide epitopes could be anchored without conformational restrictions and steric hindrances. Conformational analysis of SOC_n pointed out that the carrier adopts a 3_{10}-helical structure, which remains unchanged even after anchoring of the epitopes.

It was also demonstrated that SOC_n-conjugates preserve their original conformation, due to the carrier's helicoid structure. The constructs help the reconstitution of the native epitopes and mimicking of antigens especially when anchoring different types of epitopes.

The SOC_n-conjugates, when used as antigenic substrates displayed significant biological activity, while the developed immunoassays were sensitive, convenient and reproducible in screening antibody specificities. Animal immunizations generated, depending on the bound to SOC_n peptides, either an immune spreading or a limited expansion of the B-cell repertoir.

No 32

Molecular Targets in Cancer Therapy

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Every cell in a multicellular organism receives signals from the effective molecules of the extracellular matrix and neighboring cells. These signals are transmitted, via transmembrane receptors and cascade proteins of the intracellular message system, inside the cell and often to the nucleus regulating almost every physiological function of the cell. Protein tyrosine kinases constitute a family of receptors that regulate major cellular events, such as cell proliferation, differentiation, cell adhesion and apoptosis. Mutant tyrosine kinases and/or their aberrant activity are associated with human cancer and other hyperproliferative diseases. Strategies for inhibition of aberrant tyrosine kinase activity, such as antisense oligonucleotides, antigenic stimulation and small molecular inhibitors have been developed. STI571, a phenylaminopyrimidine derivative, is considered as the pioneer of the small molecular inhibitors available to date. It is a successful tyrosine kinase inhibitor, which is currently approved and used for treatment of chronic myelogenous leukemia and gastrointestinal tumors.

No 33

Hormonal and Local Effects of Activated Renin Angiotensin System (RAS)

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The kidney plays a central and perhaps dominant role in cardiovascular homeostasis. RAS is a set of interacting and mutually supportive hormones secreted from the kidney and adrenals that regulate fluid and electrolyte balance and govern blood pressure. Evidence also exists that tissue of local renin systems in heart, vasculature, adrenals, brain, kidney, and reproductive organs are pathophysiologically involved with organ function and organ damage in the case of hypertension.

The effector molecule of RAS is the octapeptide angiotensin II, which exerts a central dipsogenic effect, is a powerfull, direct and immediate vasoconstrictor and increases sodium reabsorption acting directly on the proximal tubules and indirectly –via aldosterone- on the distal tubules. In addition, angiotensin II promotes erythropoiesis, thus increasing the oxygen-carrying capacity of blood, stimulates plasminogen activator inhibitor-1 (PAI-1), thus inhibiting fibrinolytic systems and acts as a growth factor for a variety of cell types. Thus, the acute RAS activation is protective for the minute-to-minute regulation of blood pressure, but the chronic RAS activation is associated with target organ damage and enhanced cardiovascular risk.

Therefore, it should not be surprising that ACE inhibitors have produced functional and clinical outcome benefits in clinical trials of patients with

congestive heart failure, systolic dysfunction after myocardial infarction, diabetic and non-diabetic nephropathy etc. Because such enzymes, as chymases can substitute for ACE, the ACE inhibitors may not completely block angiotensin II formation. However, they keep enhancing bradykinin accumulation and secondary stimulate vasodilatory prostaglandins and nitric oxide. The newer class of antihypertensive medications, sartanes, act as angiotensin receptor blockers (ARB) and selectively block AT1 receptors that mediate the known effects of angiotensin II. At the same time, sartanes allow high plasma angiotensin II levels, which may stimulate AT2 receptors and mediate vasodilatory and antiproliferative effects. In this regard, ARB's alone or in combination with ACE inhibitors have been also found to favourably affect target organ damage.

No 34

Pharmacogenomics and Biological Complexity

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Pharmacogenomics was initially based and developed on the premise that the pharmacological response is determined directly by one or a few genes acting in a linear and easily predictable way.

We know today that the final phenotype of pharmacological response is not a simple monogenic trait. It is instead determined by the highly complex molecular interaction of several gene products, which are involved in the disposition (absorption, distribution, metabolism and excretion) and the action of the drug. All these proteins interact at several levels of complexity. These data have an impact on the practice and strategic orientation of Pharmacogenomics, as well as the process of new drug development. In order to compress the time for the selection and evaluation of new products two major changes have taken place in the process: Introduction of high throughput screening and employment of integrated approaches including cell-based and model-organism-based systems.

It is expected that analysis of associations of SNPs and haplotypes with the pharmacological response will certainly yield important information on the role of genetic variation. However the final phenotype of drug response will remain much more difficult to define, because it represents the result of complex interactions at various levels of complexity.

No 35

The Molecular Basis of Hypertension: A Solved Mystery or a Controversial Issue?

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Angiotensin II (AII), Asp[1]-Arg[2]-Val[3]-Tyr[4]-Ile[5]-His[6]-Pro[7]-Phe[8], the primary active hormone of the Renin-Angiotensin-System (RAS), is a major vasoconstrictor implicated in the cause of hypertension. Angiotensin I (AI), (Asp[1]-Arg[2]-Val[3]-Tyr[4]-Ile[5]-His[6]-Pro[7]-Phe[8]-His[9]-Leu[10]), is the inactive precursor of the bioactive hormone AII and is converted to AII by the angiotensin – converting enzyme I (ACEI). In the present communication the three dimensional structure of the N- catalytic site of the somatic form of ACEI (sACE) is presented through comparative modeling with the

recently solved X-ray structure of the spermatic form of ACE (*g*ACE) and structural homologies with other metalloproteases. Furthermore, the structural features of AI and AII and several analogues of AII in solution are reported by the use of NMR and molecular dynamics. Structural comparison of the solution structure of AII with the X-ray structure of AII bound to the mAb FAB131 is reported. The molecular basis of recognition of AII from its G-protein coupled receptor AT_1 was investigated through homology modeling studies of the AT_1 based on known X-ray structures and docking calculations using literature derived restraints.

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No 36

Conformation and Bioactivity: Design and Discovery of Novel Antihypertensive Drugs

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Peptidomimitism is applied in the medicinal chemistry in order to synthesize drugs that devoid of the disadvantages of peptides. AT1 antagonists constitute a new generation of drugs for the treatment of hypertension designed and synthesized to mimic the C-terminal segment of Angiotensin II and to block its binding action on AT1 receptor. An effort was made to understand the molecular basis of hypertension by studying the conformational analysis of Ang II and its derivatives as well as the AT1 antagonists belonging to SARTANs. Such studies offer the possibility to reveal the stereoelectronic factors responsible for bioactivity of AT1 antagonists and to design and synthesize analogs possessing novel pharmacophore segments that may improve their pharmacological profile.

No 37

NMR Solution Models of the Angiotensin-I Converting Enzyme Catalytic Sites

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The Angiotensin-I Converting Enzyme (ACE) is a Zinc Metallopeptidase which has been isolated at mid '50s and constitutes one of the major partners of the Renin-Angiotensin System. ACE catalyzes the hydrolysis of the Angiotensin-I (A-I) carboxy-terminal dipeptide His-Leu. A-I is liberated to the blood from the Angiotensinogen due to the catalytic action of the Renin, and after the catalytic action of ACE, A-I is transformed to the vassopressor octapeptide Angiotensin-II (A-II). ACE is encountered in two distinct forms in humans, the *somatic* and the *testis* form. These differ from the structural point of view, mainly in size (1306 and 732 AA for *somatic* and *testis* isoform, respectively) and number of catalytic sites (2 and 1, respectively). As far as the ACE

role in blood pressure is concerned, the inhibition of ACE enzymatic activity against A-I was considered as one of the major challenges, in the field of Chemistry, Medicine and Pharmacology, against hypertensive disease and congestive heart failure. Therapy today and after extensive research for the last 30 years has been achieved through inhibitors based on the pioneering work of Ferreira S.H. (1) and Ondetti M.A. (2), which until recently were using the structures of other Zn Metallopeptidases as templates for drug design. Only recently the X-ray structure of *testis* ACE has been determined (3).

The reconstitution of the polypeptide skeleton which represents the amino acid sequence of the two catalytic sites of ACE, where Zn is bound, has been performed using Fmoc/*t*Bu chemistry on 2-chloro trityl resin and addition of Zn(II) has yielded the catalytic site models. The solution structures of these constructs have been studied using high-resolution multinuclear NMR spectroscopy. Data analysis has led to the following results: (a) identification of the amino acid Zn(II) ligands and (b) the features of the secondary and tertiary structure of these models.

These NMR models of ACE catalytic sites indicate that the peptide chain is folded around the Zn ion in a way that three helical fragments are formed. The N- and C-terminal fragments, of the constructs, where the potential Zn ligands are sited, bear α-helix structure and the overall fold exhibit great similarities with the Zn-containing catalytic sites of other Zn-metallopeptidases which belong, as ACE does, to the superfamily called *gluzincins*.

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No 38

Does Leptin, the Appetite Regulator, Play a role in Autoimmunity?

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Leptin was first described as an adipocyte-secreted hormone that regulates food intake, energy expenditure and metabolism (1-3). It is a non-glycocylated 146 aa long peptide of 16 KD, encoded by the ob gene (1-3). Leptin adopts a helical cytokine structure, mainly similar to IL-2, and it's receptor (OB-R) is a member of the class I cytokine receptor super-family which includes IL-6, IL-11, IL-12, leukemia inhibitory factor (LIF), granulocyte colony stimulating factor (GCSF), ciliary neurotrophic factor (CNF) and oncostatin M (4). OB-R comes in different isoforms which are found primarily in the central nervous system, but also in tissues of the periphery, such as the reproductive, hematopoietic and immune systems (1-3,5). Leptin has been shown to be important for pubertal progression and also to influence hematopoiesis, inflammation and immunity against pathogens, mainly in studies in animals (1-3,5,6).

Both CD4 and CD8 T lymphocytes express leptin receptors and leptin enhances proliferation and activation of human circulating T lymphocytes in a dose-dependent manner (7,8). In addition, leptin has been shown to modulate CD4 T lymphocyte activation towards a Type-1 (pro-inflammatory) phenotype by stimulating the synthesis of IL-2 and IFN-γ (7,8). Recently, a deleterious effect of leptin on autoimmunity has been shown in studies involving animal models of autoimmune diseases. In particular, it was shown that acute starvation, which prevents the increase of serum leptin levels, delayed the onset of experimentally induced autoimmune encephalomyelitis (EAE) in mice and induced a Type 2 (anti-inflammatory) cytokine switch and attenuated clinical symptoms. Furthermore, it was shown that there was a high leptin production in inflammatory infiltrates and neurons during the acute phase of the disease, and that anti-OB-R antibodies inhibited the proliferative response of autoreactive T cells in vitro (9,10).

Leptin's role in mediating Type 1 immunity is strengthened by the fact that leptin-deficient mice have minimal IFN-γ production and a moderate production of IL-4 and are unable to mount a Th1 response (9,10). These experimental findings taken together with the observations (1,2) that in humans women have higher leptin levels than men with the same body mass index (BMI =

[weight (kg)/height (m^2)]), along with an increased susceptibility of women to autoimmune diseases, create a platform for studies on the immunomodulatory role of leptin in human autoimmune diseases and especially multiple sclerosis, the human disease imitated by the EAE model.

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No 39

Cellular Senescence and Carcinogenesis: Antagonism, Synergism and Therapeutic Perspectives

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One of the fundamental differences between tumor versus normal cells in culture is that while the former proliferate indefinitely, i.e. they are *immortal*, the later can divide only for a limited number of times, after which they become *senescent*. Currently, the most widely accepted interpretation for the biological function of cellular senescence is that it serves as a tumor-protection mechanism. Thus, the delineation of the mechanisms underlying the above phenomenon is of primary importance for understanding and combating cancer development.

Senescent cells exhibit specific morphological and functional features, most prominent being their inability to proliferate. This is the result of the overexpression of cyclin-dependent kinase inhibitors and subsequently the activation of the p53 and pRb pathways. Inactivation of these tumor-suppressor proteins by viral oncoproteins, such as SV40 LT antigen, is able to annul cell-cycle checkpoints and drive the cells to immortalization. In addition, senescent cells exhibit a pro-inflammatory phenotype, marked by the overexpression of matrix metalloproteases (MMPs). The constant number of cell duplications that a certain normal cell strain can undergo in vitro has hypothesized the presence of a counting mechanism, i.e. a "mitotic clock". The molecular basis of this "clock" has been shown to be the shortening of telomeres, as a result of the "end-replication problem". Continuous telomere erosion results in irreversible growth arrest in normal cells. In contrast, the majority of cancer cells express telomerase, an enzyme that restores and maintains telomere length, thus leading to immortalization, one of the cornerstones of cancer.

Although cellular senescence is supposed to be an anticancer mechanism, advancing age is the major risk factor for cancer development. A hypothesis has been raised to face this apparent controversy. According to this, the accumulation of senescent cells in the tissues of the elderly, due to their increased MMP secretion, can produce a "permissive" microenvironment, for the development and migration of cancer cells. This hypothesis has recently been supported experimentally. This "antagonistic pleiotropy" has also been shown at the molecular level. The overexpression of the tumor-suppressor p53 in mice increased their resistance to tumor development but, on the other hand, led to premature senescence, indicating the complex relationship between (cellular and organismic) ageing and carcinogenesis.

All these new findings on the mechanisms underlying ageing and tumor development can direct new approaches for diagnosis and treatment. For example, detection of telomerase can be a useful marker in cancer diagnosis, prognosis and monitoring. Furthermore, low molecular weight

telomerase inhibitors, as well as antisense oligonucleotides could be of therapeutic importance. Finally, compounds that can stabilize p53 protein have been shown to inhibit tumor growth in mice.

No 40

HARP Domains with Different Activity on Angiogenesis

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Heparin affin regulatory peptide (HARP) is a heparin-binding molecule, initially purified from perinatal rat brain as a molecule that induces neurite outgrowth. It is also present in non-neuronal tissues, including uterus, heart, cartilage and bone extracts, suggesting that the function of HARP is not restricted to the neuronal system. A growing body of evidence indicates that HARP is involved in the control of cellular proliferation, migration and differentiation. Moreover, there seems to be a strong correlation between HARP expression and tumor growth and angiogenesis, and both protein and mRNA of HARP have been identified in blood vessels and capillaries in mammary gland, prostate, uterus and chicken embryo chorioallantoic membrane (CAM).

HARP has an approximate molecular weight of 18 kDa when analyzed by SDS-PAGE. It consists of 168 amino acids, the first 30 corresponding to a signal peptide. The mature molecule is highly conserved among species (with an overall homology of 98% among human, rat and bovine) and it is rich in basic amino acids, mainly lysines, forming two clusters at the termini of the molecule. Each one of the two lysine-rich termini of HARP form an á-helix. HARP also contains 10 cysteines contributing to the formation of two â-sheet domains at the central region of the molecule.

We have previously shown that human recombinant HARP expressed in bacteria was mitogenic for three endothelial cell types when it was presented to cells as a substrate [8]. We have also shown that HARP is angiogenic *in vivo*, in the chicken embryo CAM assay and *in vitro*, using different types of endothelial cells. The purpose of this study was to identify regions within the molecule of HARP with potential activity on angiogenesis. We have studied the biological activity of naturally derived peptides (generated after treatment of HARP with plasmin *in vitro*), truncated forms of HARP produced by transfected cells and synthetic peptides corresponding to different regions of the molecule. We have studied the effect of the above peptides on the adhesion, migration and differentiation on matrigel of human umbilical vein endothelial cells (HUVEC) and on angiogenesis *in vivo*, in the chicken embryo CAM.

Our results indicate that different regions of the molecule of HARP may induce or inhibit angiogenesis. Further studies are in progress in order to clarify the physiological significance of these data and to investigate the potential use of these peptides as inducers or inhibitors of angiogenesis.

No 41

Biological Markers: Another, Novel Necessary, Approach

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The biological markers, a host of laboratory-measurable parameters, undoubtedly comprise a unique tool at a clinical doctor's disposal. They are used as an aid to timely diagnosis, in the

prognosis of relapses anticipated, assessing the response in a given pharmaceutical regimen and, alas, in predicting the outcome of a gene-specific disease.

During the last few years, there has been a massive increase in the number of novel biological markers, especially in the field of cancer research, where there are many problems yet unsolved. The measurement of many markers in a patient, at a given moment may not merely sustain the doctor's suspense but may also complicate the matter even more. It is fair to say that despite all efforts, no biological marker with unique specificity has been found as yet. On the other hand, the development of novel methodology has helped to improve the accuracy of assessment.

Finally, measuring biological markers without administrative control within the same hospital unit by many different laboratories usually means a lower quality of assessment and a considerable increase in terms of the cost of laboratory expenditure.

No 42

Protein Screening in Blood Sera and Cerebrospinal Fluid in Patients with Multiple Sclerosis and Monoclonal Gammopathy by MECC

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Capillary electrophoresis (CE) is a modern analytical technique, which could have an impact in the clinical laboratory, due to its potential for rapid, automated, high efficiency separation of analytes of diverse size and character with minimal sample requirements. The aim of this experimental work was the development of a micellar electrokinetic capillary chromatography (MECC) method for determination of immunoglobulins in cerebrospinal fluid samples of patients with multiple sclerosis and in serum samples of patients with monoclonal gammopathy.

Multiple Sclerosis (MS) is a long-lasting autoimmune disease of the central nervous system (CNS), which is associated with the destruction of myelin. MS usually appears at the age of 20-40 years. A specific humoral immune response in MS is the production into the central nervous system of immunoglobulins, which appear as oligoclonal bands (OCBs) in agarose isoelectric focusing (IEF) of cerebrospinal fluid (CSF). Among the cases with clinically definite MS, up to 95% have oligoclonal IgG bands in their CSF. These bands, γ-globulins, are thought to result from a restricted antibody response directed against autoantigens or viral antigens. MECC was performed using 25 mM borate buffer, pH 10, containing 25 mM SDS at 20 kV and normal polarity. Sandoglobulin® was used as control immunoglobulin to identify the positioning of γ-globulins in the electropherogram. Analysis of CSF and serum samples from patients with clinical definite MS and healthy individuals demonstrated the presence of two peaks migrating as γ-globulins in the CSF samples of patients. These peaks were absent from controls and the serum of the same patients.

The monoclonal gammopathies are a group of disorders characterized by the presence of a monoclonal protein or paraprotein (IgM, IgG, or IgA) in the serum. Each monoclonal protein is produced by a clone of plasma cells in the bone marrow. Very few patients show evidence of systemic amyloidosis, oteosclerotic myeloma, macroglobulinemia, multiple myeloma, and lymphoma. If these conditions are excluded, then the patient is classified as having a monoclonal gammopathy of undetermined significance (MGUS). Patients with IgG MGUS present with a variable picture of demyelination, axonal, or mixed neuropathies. CE analysis of serum samples from IgG MGUS-positive patients, using the same analytical conditions as for MS analysis showed a peak at 11 min, representing an IgG population and is absent in normal (MS-negative) serum samples, used as controls.