Identification of MIF's Proinflammatory Site: A Novel Target for Drug Discovery in Autoimmune and Inflammatory Diseases

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MIF is a potent pro-inflammatory cytokine implicated in the pathogenesis of numerous autoimmune and inflammatory diseases. X-ray crystallographic studies have shown that MIF possesses a pocket at the interface between adjacent subunits. We reasoned that molecules targeting this site could inhibit MIF actions. ISO-1 was specifically designed to fit into the pocket of MIF, an interaction confirmed by the crystal structure of the MIF complexed with ISO-1(*1*). Administration of ISO-1 increases survival during sepsis (*2*) and improves outcome in type 1 diabetes (*3*). ISO-1 is the first small molecule inhibitor of MIF with therapeutic implications and indicates the potential of the MIF pocket for therapeutic interventions in human diseases. We will present our recent studies that generated new potent inhibitors with an IC50 of 100 nM, a 200-fold increase in potentcy over ISO-1(*4, 5*).

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New Delivery Challenges: Nanocarriers for the Delivery of siRNA

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A diverse range of viral and non-viral strategies has been developed for a gene delivery such as plasmid DNA (pDNA) and oligonucleotide. Recently, the development has been extended to a newly discovered molecule, small interfering RNA (siRNA). siRNA suffers from the limitations in being used therapeutically such as low stability in blood poor cellular uptake. The use of cationic and biodegradable polymeric particles has been widely utilised for this delivery. This presentation mainly focuses on the development and investigation of cationic polymers mainly chitosan as well as its derivatives' particles; chitosan nanoparticles as siRNA carriers. Chitosans possess a high density of protonated amine groups which allow them to form non-covalent intra-polyelectrolyte complexes with negatively charged polyanions. These complexes provide protection of nucleic acids from nuclease degradation thereby resulting in high delivery efficiency. Therefore chitosan is a suitable candidate for an effective gene delivery system for siRNA. Several methods have been reported for the preparation of chitosan based nanoparticles. These methods include ionic cross-linking, chemical cross-linking and the use of emulsification to produce cationic PLGA nanoparticles.

During ours as well as other groups' work, certain process and formulation parameters have been extensively studied with the regards of physical and biological properties of the siRNA/chitosan systems to obtain an optimal delivery vehicle. Physical properties particularly particle size, surface charge as well as particle morphology have found to be influenced by homogenisation or stirring rate, molecular mass and concentration of polymers as well as other processes involved in obtaining final products (e.g. centrifugation, freeze-drying).

In vitro evaluations in cultured cells have revealed that the chitosan nanoparticles simply prepared by simple ionic gelation have shown to be more competent in transfecting mammalian cells as siRNA carriers compared to other types of chitosan-based nanoparticles investigated in our studies, either chitosan-siRNA complexes or PLGA-chitosan nanoparticles. Although type and molecular mass of chitosan are main determinants of particle size and surface charge; the two important factors influencing the capability of particulate systems to transfect cells, these parameters have not shown any obvious correlation with the level of the targeted gene silencing by siRNA.

In conclusion, the ability of polycationic polymer chitosan, as a carrier for siRNA is highly dependent on the method of preparation and their physicochemical characteristics of each of the polymeric particles.

Further investigations by our group using chitosan nanoparticles prepared by ionic gelation to deliver MAPK-14 siRNA in macrophage cell line, (J774A.1) cells have demonstrated that the system has the ability to transfect cells and subsequently allow the delivered siRNA to silence the targeted endogenous gene, MAPK p38α with a sustained effect and a relatively low cytotoxicity indicating the potential of this system in the use of lung delivery against lung diseases.

Cytochrome P450 Dependent Drug Metabolism and Outcomes of Drug-Drug Drug-Toxicant Interactions in Diabetes

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Diabetes is the third leading cause of death in many countries after cardiovascular diseases and cancer. Diabetic patients are treated with several drugs, and at the same time they are exposed to a variety of toxic carcinogenic chemicals in the environment. In most of the cases, these drugs and chemicals are oxidatively metabolized by cytochromes P450 (CYPs) dependent enzymes into more water soluble metabolites. More than 5000 *CYP* genes are identified in living organisms and 18 families are described for humans. CYPs in families 1-4 are responsible for about 80-85% of all phase I dependent metabolism of clinically used drugs and thereby determine the response to a given drug dose. Besides detoxification, CYPs often catalyze metabolic activation of pro-carcinogens to their ultimate carcinogenic forms. The variation observed in drug metabolism is mainly due to induction or supression of these enzymes resulting from multiple drug therapies or environmental factors and genetic polymorphisms. Induction or supression of some CYPs have also been observed in diabetic state. Modulation of CYP1A2/1A1, CYP2C, CYP3A and CYP4A1 by some drugs and chemicals results in increased hepatotoxic sensitivity and tissue damage. However, remarkable species differences exist in terms of effects of diabetes on ultimate outcome of hepatotoxicity. Induction of P4502E1 is a well-characterized response in diabetes. Induction of CYP2E1 is observed not only in liver but also in kidney and lung of diabetic subjects. Severe toxicity of chemicals such as benzene, N-nitrosodimethylamine, CCl_4, thioacetamide and drugs such as acetaminophen in diabetes is correlated with induction of CYP2E1 in these tissues. In addition, increasing amounts of reactive oxygen species in diabetic state plays a crucial role in determining the ultimate outcome of organ toxicity initiated by drugs and toxicants.

Profiling and Prediction of Drug Transporter Interactions

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Transport proteins play important roles in the absorption, distribution and elimination of many drugs and there are several examples of clinically significant drug-drug interactions at the transporter level. We performed gene and protein expression studies in human tissues and extensive literature mining in order to identify the most important transport proteins for drug absorption and disposition. We then selected transporters for functional studies, focusing on important but less studied transporters such as the efflux transporter ABCG2. In our first study, 123 registered drugs representing the entire structural space of orally administered drugs were investigated for inhibition of efflux in cells over-expressing wild type ABCG2. 29 new inhibitors of ABCG2 were identified. An easily interpretable computational model with a discriminating power of more than 90 percent for the training set and more than 80 percent for the test set was constructed. Similar results where obtained for other important transport proteins. We believe that our mapping of transporter interactions will contribute to better formulation strategies and better predictions of the performance of new drug candidates earlier in the drug discovery process.

Targeting the Innate Immune System to Fight Infection

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Toll-like receptor (TLR) ligands are potent inducers of innate immunity against invading pathogens, including viral infections. Control of virus replication initially depends on rapid activation of the innate immune responses. Previously, we have demonstrated that ligands for TLR3 and TLR9 induce potent innate antiviral responses against Herpes Simplex Virus (HSV)-2. We have recently found that that TLR ligand-induced production of IFN-β, but not IFN-α, IFN-γ or TNF-α, leads to innate protection against HSV-2. Local delivery of Poly I:C and CpG ODN induced significant production of IFN-β in the genital tract and provided complete protection against intravaginal (IVAG) HSV-2 challenge. There was no correlation between levels of TNF-α in the genital tract and protection against IVAG HSV-2 following TLR ligand delivery. In addition, Poly I:C treated TNF-$\alpha^{-/-}$ or IFN-$\gamma^{-/-}$ mice were protected against subsequent IVAG HSV-2 challenge. To confirm that type I interferon, particularly IFN-β, mediates the TLR ligand-induced innate protection IFN-$\alpha/\beta R^{-/-}$ and IRF-$3^{-/-}$ mice were treated with Poly I:C and then challenged with IVAG HSV-2. There was no protection against HSV-2 infection following Poly I:C treatment in IFN-$\alpha/\beta R^{-/-}$ or IRF-$3^{-/-}$ mice.

We have recently found a new TLR ligand/agonist with strong innate anti-viral and anti-bacterial activities. The antiviral activity of this newly identified TLR ligand is both MyD88 and trif dependent and requires type I interferon signaling.

Our results suggest that TLR ligands can be used to prevent/treat microbial infections. Moreover, the innate antiviral activity of TLR ligands at mucosal surfaces requires IL-15.

Transport of Antisense Across the Blood-Brain Barrier

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Treatment of CNS diseases with antisense has proved problematic because of the assumption that the blood-brain barrier (BBB) is impermeable to these molecules. We have described a transporter located at the BBB that is selective for some oligophosphorothioate antisense analogs (OA). We developed a 42-mer OA directed against amyloid precursor peptide (APP) that decreases brain levels of APP, amyloid beta protein, and measures of oxidative stress and reverses cognitive impairment in a mouse model of Alzheimer's disease (SAMP8) after direct injection into the brain. We found this antisense crossed the BBB at a modest rate by a saturable mechanism to reach brain levels of about 0.25% of the iv injected dose per g of brain. The iv administered antisense reversed cognitive impairments in the SAMP8. Subsequently, we developed antisenses directed at preproenkephalin (PPE), the precursor to the endogenous brain opiate methionine enkephalin (ME). Animals with low levels of ME voluntarily drink more ethanol. We found that antisenses directed against against PPE and given iv crossed the BBB at modest rates to accumulate in brain, reduced brain levels of methionine enkephalin, and increased voluntary ethanol drinking. We have now developed two other antisenses that selectively target transporters located at the BBB. We conclude that the presence at the BBB of saturable transporters for oligophosphorothioate antisense molecules allows both the brain and the BBB to be targeted therapeutically.

Novel Aspects of Estrogen Receptor Signaling for Vascular Disease and Therapy

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Sex hormones are important modulators of vascular function *in vivo*, and the cessation of endogenous estrogen production has been implicated in the onset of cardiovascular disease in women after menopause. Recent large-scale clinical trials have used "equine" estrogens that were derived from horse urine to treat postmenopausal women in order to test the hypothesis whether estrogen therapy can reduce cardiovascular mortality or morbidity (1). These equine estrogens contain more than 30 steroid substances including androgens, some of which have unknown functions. 17beta-estradiol, which is the predominant endogenous estrogen in premenopausal women and is also present in men, is a non-selective agonist of estrogen receptors. Estrogen receptors comprise of different isoforms which display characeteristic distribution within the vascular wall. Both ER alpha and ER beta are known to exist in the nucleus as well as on the cell membrane, where they are involved in genomic as well as in "non-genomic" (*rapid*) signaling. More recently, a novel membrane-bound receptor termed GPR-30 has been identified. Effects of estrogen are markedly different between human arteries and veins (3), which may have important implications for estrogen-associated conditions such as deep-vein thrombosis, a common side effect of hormone therapy. The non-selective estrogen receptor agonist 17beta estradiol, which activates not only ER alpha and beta but also GPR30 (4), has profound vasoactive activities in human epicardial coronary arteries which are mediated by NO-dependent as well as NO-independent mechanisms. We have recently identified novel roles for ER alpha and beta in regulation of epicardial coronary artery tone and found that both receptors display a disticint pattern of responsiveness to receptor-selective and -non selective ligands (5). Most recent data point at novel functions of GPR30, which may provide a novel target for the treatment of disorders associated with menopause. The mechanisms underlying selective estrogen receptor activation will be discussed.

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Mitigating the Reactivity of Acyl Glucuronides in a NEPi Discovery Programme

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This presentation describes the prosecution of a discovery programme to generate orally deliverable neutral endopeptidase inhibitors (NEPi). The work centred around a series of monocarboxylic acid that were demonstrated to achieve required levels of NEP inhibitory potency combined with demonstrated oral delivery potential. The initial candidate UK-414,495 ((2R)-2-[(1-{[(5-ethyl-1,3,4-thiadiazol-2-yl)amino]carbonyl}cyclopentyl) methyl]pentanoic acid) was taken forward to preclinical toxicology studies, where it showed profound toxicity centred around the G.I. tract and the liver. A research programme was mounted to understand the mechanism for this toxicity, with the aim of designing cleaner NEP inhibitors.

In common with many monocarboxylic acid compounds, UK-414,495 is metabolised *via* acyl glucuronidation. Reactive acyl glucuronides have often been implicated in the presentation of toxicity for carboxylic acids with some correlation between potential for toxicity and rate of degradation of the glucuronide[1]. Acyl glucuronides often degrade by O-acyl migration with the potential for trans-acylation of endogenous proteins, leading to toxicity. The greater the rate of glucuronide degradation the higher the likelihood of toxicity.

The acyl glucuronide of UK-447,841 degrades with a half-life of 10 minutes in phosphate buffer pH 7.4. This is one of the most unstable acyl glucuronides known. However, it does not undergo the *O*-acyl migration characteristic of most acyl glucuronides but rapid, eliminative cyclisation to form a cyclic imide. Further work showed that both the acyl glucuronide and the imide reacted rapidly and identically in aqueous solution, pH 7.4, with *N*α- and *N*ε-amino groups of amino acids to form stable amides and with *N*-acetylcysteine and glutathione to form unstable thioesters. The imide also acylated eight lysine *N*ε-amino groups of human serum albumin known to be modified by acyl glucuronides. Thus, it was postulated that the toxicity of UK-414,495 could be attributed to the reactivity of the cyclic imide.

Rapid cyclisation of UK-414,495 glucuronide was attributed to attack on the ester linkage by an unusually nucleophilic glutaramide NH (pKa = 9.76). This suggested a synthetic strategy for preparing analogues that form chemically stable acyl glucuronides. Monocarboxylic acid glutaramides possessing non-aromatic amide substituents exhibited higher NH pK_a and yielded acyl glucuronides with greatly enhanced stability. A potent NEP inhibitor from this second group, UK-447,841, having an estimated pK_a of 16 was selected as a candidate. The decomposition half-life of UK-447,841 in phosphate buffer, pH 7.4 was 51 h. In addition, UK-447,841 was free of the unacceptable toxicity shown by UK-414,495. Whilst not definitively proven, it is hypothesised that the reactivity of the UK-414,495 acyl glucuronide (through the cyclic imide) was responsible for the observed toxicity, which was mitigated by the synthesis of compounds with more stable acyl glucuronides.

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Multifunctional Polymeric Excipients: The Key for Oral Delivery of Peptides and Nucleic Acids?

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The rapid development in biotechnology paved the way for macromolecular drugs such as therapeutic peptides and nucleic acids entering more and more the pharmaceutical arena. So far, however, most of these drugs have to be administered parenterally which is painful, difficult and sometimes even dangerous. The development of oral delivery systems for these therapeutic agents would therefore be highly beneficial. In order to reach a sufficient high oral bioavailability, however, various barriers including the enzymatic barrier being based on peptidases and nucleases and the absorption barrier being represented by the absorption membrane itself have to be overcome. The use of multifunctional polymers exhibiting mucoadhesive, enzyme inhibiting, permeation enhancing, efflux pump inhibiting and targeted drug release properties seems to be a promising strategy to overcome these barriers. Among such multifunctional polymeric excipients various polyacrylates, PEGs and chitosans turned out to be most promising. Moreover, due to the immobilization of thiol groups on these polymers their beneficial properties for oral macromolecular delivery can be further improved. The potential of multifunctional polymers for oral macromolecular drug delivery could meanwhile be demonstrated in various *in vivo* studies showing a tremendously improved oral bioavailability due to the addition of such polymeric excipients.

The Expanding Role of NMR in the Early Steps of Drug Design and Discovery

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In the first steps of lead compounds discovery, a key event is the identification of hits. High throughput screening (HTS) could identify initial hits. If the crystal or NMR structure of target is available, virtual screening can be used to obtain leads. However, the validation of such studies comes only from experimental detection of the interaction.

In liquid state, NMR is able to probe the binding of potent inhibitors of the target even in a mixture of compounds. NMR can give a fast answer and potent knowledge on the feasibility of a new project involving the study of the interaction based on a recently validated target even when only putative ligands are available. The binding of the initial hits of low affinity is detected and the specificity of their interaction validated.

NMR has become an essential tool in the identification of the initial hits and the characterization of the potential lead interaction with the macromolecular targets. Classically, one way to observe such binding is to look at the protein signals. Protein epitope is obtained from powerful NMR methods by means of [^{15}N, ^{1}H]TROSY spectra with ligand titration to follow the chemical shift perturbation. However, mapping of the target residues involved in the interaction between hits and very large macromolecules cannot be investigated easily due to the overlapping and broadening of the protein signals. However, no methods existed to define the binding mode of a ligand if the protein cannot be labeled or is so large that it cannot be assessed by NMR spectroscopy. Moreover, protein receptors expressed in bacteria to obtain ^{13}C, ^{15}N, ^{2}H isotopes labeling should be available in large quantities (mg) and soluble.

What seems to be less informative is to look at the other side of the interaction. Detection of the ligand is proven to be more sensitive due to the fast exchange in the NMR timescale when weak binding is present. Many NMR methods based on differential size have been published. To probe the binding of potent ligands, STD (saturation transfer difference) and WaterLOGSY (water ligand observed *via* gradient spectroscopy) experiments are powerful for hit finding and ligand optimization.

Knowing atoms of ligand contacting the surface of macromolecule and determining the orientation of a ligand with respect to the protein is essential. STD offers the possibility to investigate complexes in solution and derive binding epitopes of the ligands. Examples of the binding epitope resulting from STD NMR experiments are presented. Furthermore, WaterLOGSY methods can be used to map solvent accessibility epitopes.

After a brief illustration of these several NMR methods existing to date and to our best of knowledge, we describe here the application for characterizing the interaction of some phosphorylated synthetic peptides with the human protein β-TrCP (*1, 2*) and the interaction of MLS_B antibiotics with bacterial ribosome.

We applied the TRNOEs (exchange-transferred nuclear Overhauser effects) experiment to know the structural bases allowing the recognition of the ligand by the macromolecule. The constraints of distance were used to obtain the bound structure of compound.

NMR competition experiments have been used to demonstrate the binding of hits to a common site of interaction and to determinate both low- and high-affinity of compounds and to rank order analogs rapidly. Further analysis of STD and WaterLOGSY build-up curves led to estimation of thermodynamic parameters of the binding event.

Starting from the complex structure, optimized ligand can be proposed by the analysis of interactions by NMR. Hence, guidelines for rational synthesis and virtual screening are available from NMR to obtain a lead. Fragment-based screening by NMR has been also successfully applied to the challenging targets such as protein-protein interactions and to generate lead-like compounds.

Therefore, NMR can be used as a screening tool as well as for the rapid validation and characterization of high throughput (HTS) screening hits and elimination of false positives.

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Targeting the Cell Cycle Machinery for Cardiac Regeneration

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Heart muscle cells (cardiomyocytes) lose the ability to divide as the heart matures which has serious consequences for patients who suffer a heart attack (myocardial infarction [MI]) since the damaged adult heart is unable to regenerate new muscle tissue. Instead, scar tissue forms that can lead to heart failure, which is one of the most important causes of morbidity and mortality in the Western world. Understanding the molecular mechanisms responsible for precisely when and why cardiomyocytes lose the ability to divide is crucial if we are to develop new therapies for repairing a heart following an MI. We, and others, have shown that molecules constituting the cell cycle machinery (cyclins, cyclin-dependent kinases (CDKs) and CDK inhibitors) are key regulators for controlling growth in cardiomyocytes. Thus, we recently have demonstrated for the first time that targeted over-expression of the CDC2-cyclin B complex in adult myocytes leads to an increase in the total number of myocardial cells and that inhibition of the G1-S phase transition in myocytes leads to abrogation of hypertrophic growth that can result in heart failure. We also have shown that the CDK inhibitor, $p27^{Kip1}$, and the TGF-β superfamily member, myostatin, independently play pivotal roles in determining the timing of when cardiomyocytes exit the cell cycle and that targeting expression and activity of these molecules in myocytes can significantly affect growth potential of the heart. Thus, p27-null mice have enlarged hearts as a result of an increase in myocyte number whereas increases in myostatin expression causes cells to exit the cell cycle. The identification and controlled delivery of specific cell cycle regulators into adult cardiomyocytes might permit manipulation of their expressions in these cells *in vivo* in an effort to push them back into normal cell division where they could repair a damaged heart.

Stereoselective Synthesis of Squalamine Analogues: Evaluation of Their Antimicrobial Activities

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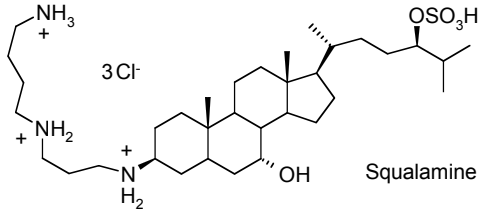
In recent years, among a wide variety of natural low molecular-weight antibiotics, the shark amino-sterol *Squalamine* has recently been the focus of a great deal of attention.

This water-soluble cationic steroid from the dogfish shark *Squalus acanthias* and the close structural metabolite MSI-1436 display potent antimicrobial activities against fungi, protozoa, and both Gram-negative and -positive bacteria. Subsequent studies have shown it to exhibit also a potent antiangiogenic activity and is actually under Phase II clinical trials in chemotherapy (ovarian and prostate cancer). However feasibility of obtaining large quantities of this antibiotic, from natural source or by synthesis, appears challenging. Thus improved preparation of *Squalamine* and analogues are a priority and a new stereoselective efficient methodology will be described.



Squalamine

Moreover, our studies indicate that squalamine possesses promising antimicrobial activities against various multidrug resistant (MDR) strains (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) and these results will be discussed.

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The Discovery of Cholesterol Absorption Inhibitors: from ACAT to Zetia

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The discovery of β-lactams as a novel class of cholesterol absorption inhibitors was a serendipitous finding during the course of a medicinal chemistry effort to identify conformationally restricted inhibitors of ACAT (Acyl CoA: Cholesterol Acyl Transferase). The program, which was driven by an *in vivo* assay of cholesterol absorption as well as metabolism considerations, led to the discovery of ezetimibe (Zetia®), a first-in-class agent to lower serum LDL cholesterol. In addition to our evolving medicinal chemistry program in the area, preclinical and clinical results will be presented showing the potency and efficacy of this agent alone and in combination with statins.

Pharmacogenetics Using an Automated Mass Spectrometry Platform for DNA Analysis

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Traditionally, pharmacogenetics looks at genetic variations in a set of well characterized genes that participate in the activation, deactivation and transport of small molecule therapeutic agents. With the advent of modern DNA analysis it becomes cost effective to analyze a large number of known variations for each patient. This can be very beneficial in choosing among alternative therapies and adjusting dosage.

However, as genomics has matured we have discovered a vast array of additional genetic variations that affect disease etiology and modulate therapeutic possibilities. Besides germ line genetic variations, that are constant in all tissues, we now have access to measurements of tissue-specific effects such as somatic mutations, and gene expression levels and the epigenetic status of genes. This information has great potential to guide therapeutic programs.

In this talk I will show how automated DNA mass spectrometry allows the facile detection of large numbers of somatic mutations in cancers, and also the epigenetic state of promoters in genes important in cancers. I will also illustrate the power of DNA mass spectrometry to sample tissue-specific events non-invasively by detecting rare traces of these events in the general circulation.

Novel Nanomedicine Based Therapeutics

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One of the novel nanomedicine based therapeutics is the use of nanobiotechnology. Nanobiotechnology is the assembling of biological molecules into nanodimension structures. One of the novel approaches is our study on the assembling of biological molecules into soluble single multifunctional nanodimension structures. For examples:

(1) Stroke and myocardial infarction are due to insufficient blood supplies from narrowed or partially obstructed arteries. Oxygen carrying fluids instead of red blood cells can bypass these obstructions, but the reperfusion of oxygen will cause ischemia-reperfusion injuries. We have therefore assembled hemoglobin, catalase and superoxide dismutase together into a single soluble nanodimension structure (PolyHb-CAT-SOD). Being in solution, the nanodimension structure can bypass the obstructed vessels for the hemoglobin to supply oxygen. Oxygen radical produced in the reperfusion can be removed by catalase and superoxide dismutase that are present in close proximity to hemoglobin. Thus, in an experimental stroke model in rats, PolyHb-CAT-SOD did not result in brain edema or increased blood brain barrier, whereas other oxygen carrying solutions including unassembled Hb, CAT and SOD caused these problems. Other research groups have more recently used our approach of PolyHb-CAT-SOD to prevent ischemia-reperfusion injuries in donor hearts and livers in experimental animals.

(2) There is at present no established treatment for melanoma, a fatal skin cancer. We have assembled a soluble nanobiotechnological structure consisting of hemoglobin and tyrosinase (PolyHb-Tyr). In a Murine B16F10 Melanoma mouse model we found that intravenous injection resulted in significant reduction in the growth of the melanoma. Tyrosinase removes tyrosine needed for the growth of melanoma, whereas the hemoglobin component transiently increased oxygen supply to the poorly perfused melanoma to increase the effect of decreased in tyrosine level. Being a single nano-structure, hemoglobin will function only for the same duration as tyrosinase. This will prevent increased oxygen supply when tyrosinase is no longer active.

(3) Another of our approaches is the assembling of hemoglobin and fibrinogen into a soluble nanostructure (PolyHb-Fibr). This solution can carry oxygen and at the same type has a platelet-like function. In experimental animals, the use of PolyHb alone to replace 85% of the total blood volume led to prolonged clotting time. Whereas the use PolyHb-Fibr maintains a normal clotting time when replacing as much as 98% of the total blood volume.

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Can the Block Copolymers Hurdle the Whole Process of DNA Transfer?

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This research is dealing with a molecular medicine which is aiming at introducing defined genes into appropriate cells supplementing dysfunctional genes or introducing new functionalities. In the case of DNA protecting polymers, the various steps for plasmid and DNA transfection are: encapsulation in polyplexes, transcytosis, capture by target cells and cell internalization, intracellular trafficking and finally nuclear import. Block copolymers of the amphiphilic type revealed to be of a surprising efficiency for DNA transfection, especially in the context of muscle dystrophy. Positive polyelectrolyte copolymers can be useful transfecting pulmonary cells, in the context of cystic fibrosis.

This presentation is aiming at placing our work on the block copolymers for DNA transfer in view of the hurdles that the formed polyplexes find on their way to cell nuclei.

1. Influence of the chemical nature of the block copolymers: The synthesis of a copolymer derived from the reference polymer PEO-PPO-PEO, in which the central hydrophobic PPO unit was changed into poly(tetrahydrofuran), has been achieved. The transfection efficiency was found similar, but the toxicity was decreased (1). A totally new triblock was synthesized poly(MeOX-THF-MeOX), in which all three blocks were changed (2). it was concluded that the transfection efficacy was mainly dependent of the amphiphilic nature of the polymer, but that the toxicity was a consequence of the chemical nature. This conclusion is important in that it gives hopes to be able to manage independently efficacy and toxicity. In the case of vectors of the positive polyelectrolyte type like PEI the linear macrostructure is to be preferred versus highly branched macrostructure for the efficacy of transfection independently of the nature of the amine functions (1). The molar mass was an important parameter and was preferably in the 10,000 Da range (3). It was found for linear PEI that a macrostructure with a lower charge density such as in the case of poly(ethylenimine-co-methyl-2-oxazoline) the cytotoxicity was decreased.

2- Nucleic acid encapsulation in polyplexes and circulation in the blood stream: Reducing the size of DNA complexes is a way to aid for instance their passage through nuclear pores. This reduction can be achieved by charge neutralisation. Ionic or non-ionic surfactants were used, and in the same vein amphiphilic polyelectrolytes. Firm adhesion on cell surface through specific molecular recognition can be achieved by increasing polyplex and lipoplexes interactions with epithelial cells to minimize the negative effect of the flow rate and serum, for example, by the addition of tetraglucose residues on PEI (4). This kind of tag can be attached to block copolymers.

3-.Crossing the endothelial barrier, cell targeting and internalization: Endothelium and the endothelial tight junctions constitute fences that prevent the passage of particles of more than 100-200 nm diameter. The problem of the interactions with DNA, particle size and interaction with cell membrane was particularly tackled by our laboratory. It was first demonstrated that the interaction between neutral block copolymer and DNA was repulsive (5) and on model membranes it was shown that the main role of the above triblock POE-PPO-PPO was to permeabilize cell membrane. Ionic channels induced in diphytanoyl-phosphatidylcholine membranes, model of cell membrane, were transiently stable in the open state, while the overall channel-forming structure seemed to remain intact on the membrane for a much longer time. It was concluded that the length of the PPO block controls the insertion.

In this field, strategies mimicking lymphocyte homing or virus infection could be investigated. Short sequences of integrin binding peptides containing the RGD motif such as cyclized RGD-peptides like

GGCRGDMFGCA and AEGEFCRYRGDRRCGDPAK, the non cyclic peptide CYGGRGDTP or tetraglucose moiety are known to facilitate polyplexes capture by endothelial cells and can be bonded to polymer blocks (6,7).

4- Intracellular trafficking: One of the main limiting steps for the transfection is the inefficacy of the delivery of plasmids into the cytosol upon internalisation. Histidinylated cationic polymers have been developed as vector to solve the problem. Plasmids involving original nucleotide sequences have recently been elaborated on the base of the NFκB ability to bind to DNA and to shuttle between the cytosol and the cell nucleus in activated cells (8,9). However, any κB site did not really ensure a high affinity for NFκB. Plasmids bearing an optimised NFκB binding sequence exhibit a remarkable DNA nuclear import and a high level of gene expression in cultured cells and in mice. Alternatively, this kind of nucleic acid sequence can be attached to block copolymers.

5- Nuclear import: In non-dividing cells, the nuclear envelope is the ultimate membrane barrier to gene expression. Efficient nuclear importation has been assessed by adding a nuclear localisation signal (NLS). For instance, the incorporation of some peptides of the SV40 type has been achieved by coupling NLS to polyplex. The NLS can also be linked to the nucleic acids. Also some sugar such as lactose could favour nuclear importation of pDNA.

Conclusion: From a global point of view, the interest of our research is to develop new generations of synthetic vectors useful for gene transfer in the muscle or in the epithelial airways tissues *via* systemic or intravascular administration based mainly on block copolymers, in order to propose treatment of diseases directly impacting muscle such as muscular dystrophy or lungs for cystic fibrosis. Working on the best ever known synthetic vectors, and optimizing their structures according to the above results, therapeutic treatment seems to be feasible with copolymers involving multiple blocks or functions of various dedicated roles, mimicking virus structures.

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Cheminformatics Delivers Super-Antibiotics for Superbugs

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The rise of antibiotic resistance among major human pathogens represents one of the most pressing health issues. The emerging genomic (and other '-omic') platforms generate substantial amounts of data and provide a renewed hope for novel-acting and improved antimicrobials. However, despite all those technological advancements there is still a real shortage of treatment options for resistant infections. We attempt solving this problem using a different approach capitalizing on substantial amounts of already accumulated chemical and biological information. Using the prior-knowledge and powerful machine learning techniques we have created QSAR models of antibiotic activity enabling to advance nonspecific bacteria-killing properties of compounds. The developed structure-activity solutions have been applied to the class of cationic peptides and resulted in the development of novel drug leads, that demonstrated very effective '*in vitro*' and '*in vivo*' killing of multidrug-resistant strains of such major human pathogens as *Pseudomonas aeruginosa, Pseudomonas maltophilia, Staphylococcus aureus* and *Enterobacter cloaca*.

Nature - Source of New Pharmacophores

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Biodiversity is an outward manifestation of chemical diversity. Plants contain a fascinating array of natural products. Since last three decades we have been focusing our efforts to harness the chemical diversity present in the floral diversity. In the process, we have used state-of-the-art technologies, including modern chromatographic techniques, sophisticated and sensitive spectroscopic techniques and a range of biological screening methods.

The field of phytopharmaceutical research and development is now witnessing a major transformation both in terms of concept and in practices. With the advent of super-advanced hyphenated techniques such as LC-NMR, LC-MS/MS, LC-MS/NMR, GC-MS, new spectroscopic methods and high-throughput bioassay techniques, the research in plant-based drug discovery has immensely progressed in recent years. The emerging new field of metabolimics and associated technological advancements also holds great promises for the future of this exciting discipline.

As a result, we have identified various new classes of potential pharmacophores against various diseases. Different clinically important enzymes were targeted such as α-glucosidase, thymidine phoshphorylase, acetylcholinesterase, butyrylcholinesterase, β-glucuronidase, phosphodiesterase, tyrosinase and urease, which led to the discovery of potent and novel pharmacophores. Along with this, a battery of *in-vitro* and *in vivo* bioassays were employed to identify new antibacterial, antifungal, antiparasitic, antioxidant, antiangiogenic and antiglycation agents.

During this presentation, recent trends and future prospects of technological developments in phytopharmaceutical research will be discussed and examples of their utility will be demonstrated by taking the examples of our own research work.

Functional Characterisation of New Immunomodulatory Compounds

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Antibody secreting cells are the effector cells of the humoral immune system. They represent the end stage of the B lymphocyte differentiation program. We have screened a chemical library of 100,000 compounds for their ability to enhance or inhibit the production of antibody in cultures of lipopolysaccharide (LPS)-activated primary mouse B lymphocytes.

The compounds in the Lead Discovery Library (LDL) were selected to provide "lead-like" chemical structures; compounds that have the physiochemical properties and the chemical tractability that is likely to make them suitable candidates for a lead optimisation program. The LDL contains compounds that are less than 400 molecular weight, based on simple medicinal chemistry-friendly scaffolds, and free of reactive chemistries and un-drug like functionality. The compounds in the LDL have therefore been selected to provide structurally simple starting points for the optimisation process. We believe that by screening a library of this nature, we will increase the probability of identifying active compounds, and reduce the incidence of false positives and negatives in the screens.

In this first cell-based screen of the library, approximately 200 compounds have been selected for follow-up study, as they showed strong effects on antibody production, but negligible effects on cell viability. Here, we are applying our unique quantitative cellular and molecular tools and genetic mouse models to map each active compound's activity to specific cellular responses. We expect that some of these compounds will represent novel modulators of B cell differentiation or plasma cell function. Because the culture system used in the screen is one in which several cellular changes occur, the selected compounds have been divisible into functional classes that affect one or other of these cellular responses: isotype switching, ASC/plasma cell differentiation or antibody secretion by plasma cells. Promising preliminary data indicate that compounds showing both activity and selectivity are present in our selected subset. In addition, we have identified a compound with selective toxicity for immature B cell tumour lines. We are currently investigating the molecular genetic and biochemical modes of action of the lead compounds, and will next optimise the most promising lead compounds, to identify derivatives with increased potency.

The newly characterised immunomodulatory compounds we identify here will be valuable research tools, allowing us to probe the biochemical pathways regulating functional ASC production, and to identify the critical components of these pathways. Through the insights gained here, the compounds and their derivatives may be found to have therapeutic potential for the problems of immunodeficiency, autoimmunity, and ASC malignancy, and may be relevant to improved strategies for vaccination.

Interfering with Human Thymidylate Synthase Dimerization: A Strategy for Folate Pathway Down Regulation in Platinum Resistant Ovarian Carcinoma

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Ovarian cancer is the fifth most common cause of death from cancer in women. The standard first-line treatment is a combination of paclitaxel and carboplatin (DDP) or carboplatin alone that rapidly develop resistant mechanisms. The mechanisms behind acquired resistance towards platinum drugs are not clear yet, although it is evident that the process is multifactorial including, enhanced DNA repair. Within this context Thymidylate synthase (TS) represents an effective target, but also in this case commonly used drugs induce early resistance problems. New strategies should be adopted to overcome this problem. TS, involved in the folate pathway, is an obligate dimeric protein showing both catalytic and mRNA regulatory activity. Down regulation of the folate pathway can be achieved through the interference with the protein assembly targeting the protein-protein interface. This event blocks partially the protein dimerization process, alters the catalytic activity and maintains a low level of free and working enzyme that would continue the functional regulation of mRNA. To this aim different unexplored strategies were adopted such as anti-peptide design against the dimer interface**,** and protein labelling for hits and lead finding. The recent achievements in the context of the search for anticancer agents against ovarian carcinoma will be discussed.

This work is part of the LIGHTS project (LIGand to Interfere with Human TS, LSHC-CT-2006-037852) (STREP funded by the FP6 program) www.lights-eu.org.

Cyclotides as a Peptide-Based Combinatorial Template for Drug Design

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The cyclotides [1] are a recently discovered family of plant-derived proteins that have applications in drug design [2] and agriculture [3]. They occur in plants from the *Violaceae* (violet), *Rubiaceae* (coffee) and *Cucurbitaceae* (cucurbit) families and have a diverse range of biological activities, including uterotonic, anti-HIV, antimicrobial, and insecticidal activities, the latter suggesting that their natural function is in plant defence. Individual plants express suites of 10-100 cyclotides at high levels (2g/kg wet plant weight). Cyclotides typically comprise 30 amino acids, contain a head-to-tail cyclised backbone and incorporate three disulfide bonds arranged in a cystine knot topology. In this motif an embedded ring in the structure formed by two disulfide bonds and their connecting backbone segments is penetrated by a third disulfide bond. The combination of this knotted and strongly braced structure with a circular backbone renders the cyclotides impervious to enzymatic breakdown and makes them exceptionally stable. In essence they have the constitution of proteins, but the biopharmaceutical properties of drugs. The cyclotides are the largest of several groups of naturally occurring circular proteins that have been discovered over recent years [4]. This presentation will describe the discovery of cyclotides, their structural characterization, and applications in drug design. Their stability and compact structure makes them an attractive protein framework onto which bioactive peptide epitopes can be grafted to stabilize them. We describe the synthetic routes to cyclotides using solid phase synthesis and illustrate this with applications to drug leads targeting multiple sclerosis and angiogenesis.

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Single-Step *N*-Heterocyclic Annulation Reactions for High Throughput Chemistry

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Single-step derivatization reactions have been extensively used to generate chemical libraries. Primary and secondary amines are especially well suited for this type of modification giving rise to small focused arrays or much larger compound collections at the discretion of the combinatorial chemist. Perhaps the most common of all the amine derivatization reactions is the coupling of an amine with a carboxylic acid or acid chloride to yield an amide. Hundreds of libraries reported in the literature over the past 15 years were created, at least in part, by employing this transformation. Other examples of amine derivatization chemistries include the reaction of an amine with sulfonyl chlorides or isocyanates yielding sulfonamides and ureas. Amines are routinely subjected to reductive amination by reaction with aldehydes or ketones in the presence of a borohydride reagent to furnish collections of higher order amines. In each instance, these high throughput chemistries generate an *acyclic* reaction product. Despite the prevalence of amine derivatization, there are no complementary derivatization reagents or protocols for the single-step conversion of primary amines to *cyclic* reaction products. In an attempt to bridge this gap in methodology, we have developed a conceptually new family of reagents for high throughput annulation. Three reaction manifolds were harnessed for annulation: 1) tandem alkylation - intramolecular acylation, 2) tandem alkylation - intramolecular Michael addition, and 3) intramolecular dialkylation. In general, the reagents rely on a traceless cleavage strategy for product formation and require minimal purification. The first reagent prototype developed was resin-bound 2-bromomethylbenzoate. Its reaction with primary amines afforded an annulation library of isoindolinones. Other annulation reagents since developed provide a broad range of heterocycles - substituted pyrrolidinones, piperidines, benzodiazepinones, quinoxolinediones, morpholinones, piperazines, diazaspirocycles, dihydrobenzoxazepinones, dihydroisoquinolinones - all in a single-step reaction. The details of selected reagent preparation and annulation protocols will be presented. The application of the new annulation reagents in the discovery of biologically active compounds and establishing nascent structure-activity relationships will be exemplified in a medicinal chemistry case history.

The Structural and Biochemical Properties of Non-B Subtype HIV-1 Proteases and Drug Resistance

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All of the basic virology and biochemistry on the human immunodeficiency virus (HIV-1) has been conducted on the B subtype, due to its early discovery in France and the USA. All of the large scale clinical trials of new therapeutic drugs to date have been conducted on the subtype B virus. The genetic variability encountered in HIV-1 poses a major challenge for prevention and diagnostic strategies, therapy response, and vaccine development. There is an extensive and growing literature on sequence data from untreated and treated persons infected with HIV-1 subtype B viruses. This plethora of information has led to increasingly accurate, though complex, interpretations of HIV-1 subtype B drug resistance. Such data are generally not available for non-B subtypes. More information needs to be gathered and analyzed in order to fully understand the important role that differences in protease (PR) sequence of non-B subtypes, including subtype C, play in the interaction of enzyme with the substrates and the inhibitors and in the development of drug resistance. Subtype C virus infects the majority of people worldwide, compared with only 12% of patients infected with subtype B virus. This presentation will describe our studies of a variety of PRs from non-B subtypes, including the effects of mutations in the active site region.

This work is supported by NIH/NIAID grant R37 AI-28571 to BMD.

Discovery of JANUVIA™ (Sitagliptin): A Selective Dipeptidyl Peptidase IV Inhibitor for the Treatment of Type 2 Diabetes

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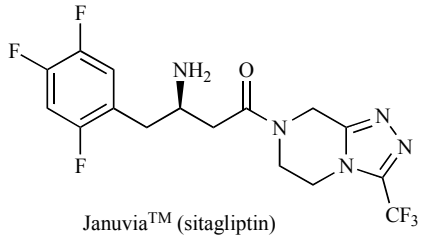
Inhibition of dipeptidyl peptidase IV (DPP-4) has emerged as a new method for the treatment of type 2 diabetes. DPP-4 is an enzyme responsible for the N-terminal deactivation of glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), incretin hormones that induce glucose dependent insulin secretion following ingestion of a meal. GLP-1 also inhibits glucagon release in a glucose dependent manner, slows gastric emptying, reduces appetite, and regulates the growth and differentiation of insulin producing pancreatic β cells. In animal models and humans, DPP-4 inhibitors have been shown to increase circulating levels of GLP-1 and GIP, resulting in improved glucose tolerance. Consequently, multiple DPP-4 inhibitors are reported to be in development for the treatment of type 2 diabetes.

At Merck, the DPP-4 inhibitor program was initiated in 1999 and shortly thereafter the medicinal chemistry team began optimization of two structurally distinct classes of inhibitors derived from either α-amino amides or β-amino amides. Importantly, early research in these laboratories illustrated that the selection of DPP-4 inhibitors for clinical development should take into account selectivity over the closely related enzymes DPP8 and DPP9 which have been associated with toxicity in multiple preclinical species. Optimization of the β-amino amide series led to the discovery of JANUVIA™ (sitagliptin), a highly selective DPP-4 inhibitor that was the first DPP-4 inhibitor approved for the treatment of type 2 diabetes. This presentation will describe the biology, medicinal chemistry, and clinical development pathway that led to the discovery and approval of sitagliptin.



Januvia™ (sitagliptin)

A Strategy for Designing and Screening Inhibitors of α-Synuclein Aggregation and Toxicity: As a Novel Treatment for Parkinson's Disease

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Lesions in the brain known as Lewy bodies and Lewy neurites constitute the main pathological features in the brains of patients with Parkinson's disease (PD) and dementia with Lewy bodies. The main components of the Lewy bodies and Lewy neurites are fibrils of a small protein (14 kDa) named alpha-synuclein. A clear link with PD was established when it was shown that a mutations in the alpha-synuclein gene were found in rare inherited forms of PD. However, the mechanism by which alpha-synuclein deposition is associated with the development of PD is unknown. Several studies indicate that aggregates of alpha-synuclein are toxic to cells, and hence lead to neurodegeneration. Therefore, small molecule(s) capable of inhibiting and/or slowing alpha-synuclein aggregation are an attractive therapeutic approach for preventing the progression of PD and related diseases. The aim of our study was to design and screen for compounds that are capable of inhibiting and/or disrupting the self-aggregation process of alpha-synuclein.

Medications Development for The Treatment of Addictions

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Drug addiction is a major public health problem worldwide which pose a profound burden on morbidity and mortality and drain to the global economy. Approved medications are available for alcohol, smoking cessation, and opiate addiction however these are not widely prescribed. For marijuana, and stimulants, there is no FDA approved medications.

NIDA's division of pharmacotherapies main mission is to find effective and safe medications to treat addictions. To achieve this goal the division which is structured as a virtual pharmaceutical company is vigorously pursuing multiple leads, working with partners in industry and the FDA in coordinating efforts that will hopefully one day will lead to an approved medications for marijuana, and stimulant addiction treatment indication.

Multiple marketed medications and new molecular entities have been tested in preclinical and early clinical development in the last 15 years. Few candidates are emerging as promising leads to pursue in larger confirmatory trials.

Another exciting area for the treatment of addiction is immunotherapy, trials are underway for nicotine and cocaine vaccines, and preclinical safety studies are ongoing for monoclonal antibodies treatment for overdoses.

This presentation will highlight the operation of the division and strategies toward finding targets for the treatment of addiction as well as data from promising medications trials and future compounds that are in the pipeline.

Gene Signatures Predicting Tumor Response to Cytotoxic Drugs

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Patient tumors established subcutaneously in serial passage in nude mice were characterized for their sensitivity towards 12 standard cytotoxic anti-cancer agents. The latter include the alkylating agents cyclophosphamide, ifosfamide, mitomycin C, cisplatin and CCNU, the antimetabolites 5-FU, gemcitabine and methotrexate; the topoisomerase II inhibitors adriamycin and etoposide as, well as the tubulin binders paclitaxel and vindesine. The mean number of tumors treated with any of the various drugs was 54 (range 31-78). The tumor xenografts' gene expression profiles were determined using the Affymetrix HG-U133 plus 2.0 mRNA expression array representing ~38.500 human genes. The hypothesis was that the correlation of drug response to gene expression would identify gene signatures that can predict the drug response of individual tumors to these agents. Predictive gene signatures were found and subsequently verified using the leave-one-out cross-validation (LOOCV) technique.

Tumors were considered as responsive if the drugs effected a tumor volume inhibition to less than 11-41% of the volume of vehicle control tumors (T/C%). The median cut-off over all drugs was a T/C of 25%. Using these criteria, on average one third of the test tumors were sensitive (responders) and two thirds were resistant (non-responders). The bio-informatic analysis yielded predictive gene signatures consisting of 20-129 genes (mean for the 12 drugs: 87 genes). On average, the response rate for predicted responders (83%) was 2.45 fold higher than that for all test tumors (random testing, 34%). This increase of response rates, following signature-guided testing, was consistent for all agents. Conversely, 94% of the predicted non-responders (range: 84-100%) proved to be non-responders in nude mouse studies.

The majority of genes (59%) making up the predictive gene signatures had an unknown function. Known genes were implicated in cell proliferation, apoptosis, DNA repair, cell cycle, metabolism and transcription. The predictive gene signatures presented here for 12 cytotoxic agents have the potential, to substantially increase tumor response rates compared to empirical drug treatment. However, they need to be further validated.

At the present time target directed agents are being studied.

Development and Validation of Emerging "Omic" Biomarkers for New Drugs: An Overview of Lessons Learned from High Technology Materials

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Efficacy and safety are key components of any new drug discovery and development strategy. Biomarkers for early detection of cellular responsiveness and toxicity represent a rapidly expanding area of scientific activity. In particular, the "omic" biomarkers (genomic, proteomic and metabolomic) are being widely applied to address the above issues but correlative validation studies are needed in order to provide interpretive data on whether observed responses are markers of exposure or markers of effect. Gallium and indium are used in clinical medicine for imaging and some therapies while arsenic is currently used in anti-cancer treatments. These elements may hence be considered in the category of clinical drugs on this basis. Micron size particles of gallium arsenide and indium arsenide are of occupational toxicity concern in the semiconductor industry but may be useful as models for combined drug therapies and particle delivery systems. This presentation will provide an overview of studies that examined both proteomic and metabolomic (heme pathway) responses following *in vitro* and short term *in vivo* exposure to these agents in relation to correlative validation studies using ultrastructural morphometric / biochemical endpoints and markers of apoptosis. It will also highlight the ability of proteomic and metabolomic biomarkers to delineate early target cell specific responses of as a function of dose, duration of exposure, gender and chemical agent on an individual or binary compound basis. These studies, based upon experience with toxic chemical agents, should hence provide an operational approach for validating both emerging biomarkers and new drugs at early stages of development. (Supported in part by NIH R01 ES 04979).

Proline-Rich Sequence Recognition: Principles and Molecular Interference

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Low-affinity protein-protein interactions (PPI) between domains of modular proteins and short, solvent-exposed peptide sequences within their binding partners play an essential role in intracellular signaling. An important class of PPIs are proline-rich motifs (PRM) that are speficically recognized by PRM-binding domains (PRD). Aromatic side chains of the PRDs define the binding pockets that often recognize individual proline residues while flanking sequences mediate specificity. Several of these PRM:PRD interactions are associated with cellular malfunction, cancer or infectious diseases. Thus, the design of PRM:PRD inhibitors by using structure-based molecular modelling as well as peptidomimetic approaches and high-throughput screening strategies is of great pharmacological interest. In this talk I will describe the molecular basis of PRM:PRD interactions, highlight their functional role in certain cellular processes and give an overview of recent strategies of inhibitor design.

Pharmacogenomics in Drug-Metabolizing Enzymes: for Personalized Cancer Chemotherapy

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Cancer chemotherapy is characterized by a broad range of efficacy and toxicity among patients. Most anticancer drugs show wide interindividual variability in pharmacokinetics and have narrow therapeutic windows. Since, drug metabolism is often an essential determinant of interindividual variability in pharmacokinetics, pharmacogenomic studies of drug-metabolizing enzymes are expected to rationalize cancer chemotherapy in terms of patient, treatment, and dosage selection.

So far, the candidate gene pharmacogenomic approaches have provided important clues for pharmacogenomic-based personalized chemotherapy with 6-mercaptopurine (6-MP), solely metabolized by thiopurine *S*-methyltransferase (TPMT), and irinotecan, mainly detoxified by UDP-glucuronosyltransferase 1A1 (UGT1A1). Reduced activity of TPMT caused by polymorphisms in the *TPMT* gene and decreased activity of UGT1A1 caused by *UGT1A1*28* are related to severe toxic effects of 6-MP and irinotecan, respectively. In response to these findings, the Food and Drug Administration in the United States has supported clinical pharmacogenetic testing by revising the package inserts for these anticancer drugs.

We looked at the multiple elements of drug metabolic pathways related to the disposition of an anticancer drug S-1 and prioritizing the information obtained. S-1 (Taiho Pharmaceutical Co., Ltd., Tokyo Japan) is an oral anticancer agent combining tegafur (FT), 5-chloro-2,4-dihydroxipyridine (CDHP), and potassium oxonate in a molar ratio of 1:0.4:1. S-1 is currently one of the most widely prescribed agents for the treatment of gastric cancer in Japan. FT is metabolically activated by liver CYP2A6 to form 5-fluorouracil (5-FU). 5-FU thus formed is detoxified by dihydropyrimidine dehydrogenase (DPD). We found that the interindividual variability in the oral clearance of FT was significantly associated with *CYP2A6* genetic polymorphism. However, the area under the concentration-time curve (AUC) for 5-FU in patients treated with S-1 significantly correlated with the AUC for CDHP which was an inhibitor of DPD, but not with *CYP2A6* polymorphisms. Collectively, CDHP exposure, but not *CYP2A6* genotype, is the key rate-limiting step in the S-1 metabolic pathway. Since the CDHP exposure is related to the renal function, creatinine clearance-based dosing of S-1 may be rational.

Pharmacokinetic properties as well as pharmacogenomics should be important for personalized cancer chemotherapy.

The Maillard Reaction for Melanoidins-Sunscreen Formation: New Technical Standards Needed to Evaluate Effectiveness

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The Maillard reaction using sequential topical applications at bedtime of dihydroxyacetone followed by naphthoquinones creates only in the skin's keratin layer over night (6-8 hrs) a keratin-bound melanoidins-sunscreen that lasts two weeks. This reversible chemical reaction does not penetrate into the living epidermis. These melanoidins provide protection in these wavelengths ultraviolet B, UVA, and Soret band for sunburn protection (SPF >15) in normal subjects and all day photoprotection in patients with UVA or Soret band photosensitivities. Standard and current proposed USA FDA regulations for ultraviolet qualitative and quantitative in-vitro and *in vivo* testing methodologies are not applicable for evaluating photoprotection from created melanoidins within the keratin polymers as these melanoidins are destroyed by chemical isolation techniques.

Supramolecular Anticancer Drugs: From Design to Reality

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Several anticancer drugs have been successfully applied for a long time. However, many of them still suffer serious drawbacks in terms of practical use. In this work it is shown that important issues can be addressed by adopting supramolecular approaches. Novel approaches for the production of interesting nanoencapsulated anticancer drugs have been designed and developed. To this end, classical anticancer drugs such as cisplatin and paclitaxel have been employed as model drugs to study these concepts. Cisplatin is a well-known and effective drug for the treatment of different kinds of cancer; however, its use for treatment is restricted by its very poor solubility. We have successfully prepared novel cyclodextrin-encapsulated *trans*-dichloro(dipyridine) platinum(II) drugs for the first time. These compounds feature water-solubility while retaining the anticancer activity of the parent compound. The inclusion complex was found to be significantly more active than the precursor compound and cisplatin alone against colon carcinoma and melanoma cells. Interestingly, the supramolecular complex was found to be soluble in water and its cytotoxic activity against two different cancer cell lines, CT26 colon carcinoma and B16F10 melanoma, was found to be up to 6 times higher than that of the drug cisplatin *in vitro*. Similarly, results on other anticancer drugs, e.g., paclitaxel, are presented and highlighted both as novel approaches and in the context of their applications.

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Synthesis: A Creative Engine for Drug Discovery and Development

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Today a large majority of drugs are small molecules which have been found and sometimes designed to interact with biological targets inducing a biological response of therapeutic interest. The development of the human genome project is thought to lead to an exponential increase of biological targets. A powerful method of exploring these unknown facets of biology is to study the interaction of a small molecule with a biological target and generate informations about its structure and function thereby generating data allowing for more rational drug design.

Such an approach relies upon the ability of the synthetic chemist to generate *collections* of *complex* molecular structures of wide diversity by *short*, *convergent* and *efficient* sequences of reactions. Some projects will aim at perturbing and modulating an area of biological interest. In that case synthesis should be aimed to small molecules occupying a region of the chemical space which is thought to be optimal to "cover" the biological space of interest. Such an approach will be briefly illustrated by showing how new "prostanoid scaffolds" or cyclic hydrazides susceptible to interact with serine-proteases can be generated by short and efficient reaction sequences opening the way for the production of collections of a wide variety of modulators.

Another approach aims at preparing small molecules occupying poorly populated regions of the chemical descriptor space. This should be particularly useful for the discovery of new biological targets of interest for the design of new chemotherapeutical agents. However, it should be kept in mind that the chemical space is so much larger than the biological space that guidelines are needed. Natural products could be a source of inspiration : *some of them* are known to interact with biological systems but *all of them* have been exposed to biological systems in the process of their biosynthesis. Thus these molecules and the synthetic intermediates leading to them occupy a portion of the chemical space which should be close enough to the biological space allowing for interactions and discoveries of new properties. This will be illustrated by the development of an extremely powerful strategy to generate complex "natural product-like" nitrogen-containing heterocycles.

Innovations in the Development of Anti-Platelet Therapies for the Prevention of Thrombosis

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Platelets perform an essential role in haemostosis, the physiological response to injury that prevents excessive blood loss. They also, however, trigger arterial thrombosis which underlies heart attacks and strokes, and are therefore a principal cause of mortality and morbidity. Understanding of how the physiological and pathophysiological functions of platelets are regulated is key to the development of new and effective means to prevent or treat these conditions. Indeed, drugs that affect platelet function have been shown to be efficacious in the prevention of thrombosis, although these are ineffective in a large proportion of patients and are associated with side effects including problem bleeding.

The gold standard anti-platelet drug is aspirin, although substantial progress in our understanding of the control of platelet function at level of basic cell biology, and the use of transgenic mice and *in vivo* models of thrombosis has in recent years catalysed the development of new drugs to combat thrombosis. These include the targeting of platelet-specific receptors or intracellular molecules whose function is associated with thrombosis. In this presentation, current and potential future approaches to anti-platelet therapy will be discussed, with a particular focus previously unexploited target proteins where *in vitro* and *in vivo* data indicate them to be strong candidates, and newly identified molecules and mechanisms in platelets with potential promise. The ultimate goal in the successful development of new anti-platelets therapies is the prevention of thrombosis leaving haemostasis intact.

A Clinical Pharmacogenetic Model to Predict the Efficacy of Methotrexate Monotherapy in Recent-Onset Rheumatoid Arthritis

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Objective. To develop a clinical pharmacogenetic model to predict the efficacy of methotrexate (MTX) in patients with rheumatoid arthritis (RA).

Methods. 205 Newly diagnosed RA-patients with active disease were treated with MTX (initiated at 7.5 mg/week, increased to 15 mg/week after 4 weeks) and folic acid (1 mg/day). If the Disease Activity Score (DAS) was >2.4 at 3 months, MTX was increased to 25 mg/week. Twenty-four baseline variables possibly influencing disease state and drug response were selected. In addition, 17 polymorphisms in 13 genes related to MTX mechanism of action, purine and pyrimidine synthesis were determined. Factors were compared between responders (defined as DAS≤ 2.4 at 6 months) and nonresponders. In case of differences, a stepwise selection procedure identified most significant predictors for response. A clinical score was designed by simplifying regression coefficients of the independent variables. Cutoff levels were chosen based on the clinical score, and positive and negative response rates were calculated.

Results. The model for MTX efficacy consisted of gender, rheumatoid factor and smoking status, DAS and four polymorphisms in *AMPD1*, *ATIC*, *ITPA* and *MTHFD1* genes. The probability of response varied between 0.012 and 0.994. This prediction was transformed into a scoring-system ranging from 0 to 11.5. Scores of ≤3.5 had a true positive response rate of 95%. Scores of ≥6 had a true negative response rate of 86%. Sixty percent of the patients were categorized into responders and nonresponders, whereas a model without genes categorized 32%. Replication of the model in 38 RA patients yielded comparable results.

Conclusion. This study established a model for predicting the efficacy of MTX treatment in patients with RA. The pharmacogentic model may lead to better-tailored initial treatment decisions in RA-patients.

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A New Era of Medicine "Genomic Medicine": Connecting Genes, Drugs and Diseases

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When the Human Genome Project completed the first draft of all human genes, the stage was set for great advances in medicine and biotechnology applications. First, scientists would more quickly identify the genes that foster common diseases and people would start having their genomes analyzed early in life to reveal their risks. Then, armed with that information, we would all adopt lifestyles-maybe even take medicines-tailored to our own needs "personalized genomics or medicine". This enthusiasm was even enhanced by the promises that new combinations of genomic, proteomic and bioinformatics research will provide deeper insights into disease mechanisms, novel markers for diagnostics, new molecular targets for therapeutic intervention and for new drug discovery. The field is being driven forward both by innovative biotechnology companies and by academicians who are introducing the technology required for the parallel identification of individual proteins. This presentation will shed some light on current innovative approaches in functional genomics and proteomics, their biotechnology applications and prospects for developing countries.

A Pharmacological Heart Failure Therapy that Targets Nuclear Pathway in Cardiac Myocytes

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Signals activated by increased hemodynamic overload to the heart finally reach nuclei of cardiac myocytes, change patterns of gene expression and cause their maladaptive hypertrophy. Nuclear acetylation controlled by histone deacetylases and an intrinsic histone acetyltransferase, p300, is a critical event during this process. However, a pharmacological heart failure therapy that targets this nuclear pathway has yet to be established. The acetylated form of GATA4, one of the hypertrophy-responsive transcription factors, and p300/GATA4 complex markedly increased in hypertensive hearts *in vivo*. A natural compound, curcumin, reversed myocyte hypertrophy both *in vitro* and *in vivo* by at least two mechanisms: inhibiting nuclear acetylation and disrupting the p300/GATA4 complex. Furthermore, curcumin treatment almost completely prevented the deterioration of systolic function in two different rat models of heart failure, hypertension and myocardial infarction. Thus, a non-toxic dietary compound, curcumin, will provide a novel therapeutic strategy for heart failure in humans.

Chemical Modified siRNA for the Inhibition of P-Glycoprotein Expression

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Altritol-modified nucleic acids (ANAs) support RNA-like A-form structures when included in oligonucleotide duplexes. Further, the presence of ANA residues can enhance stability to nucleases. Thus Altritol residues seem suitable as candidates for the chemical modification of siRNAs. Here we report that ANA modified siRNAs targeting the MDR1 gene can exhibit improved efficacy as compared to unmodified controls. This was particularly true of ANA modifications at or near the 3' end of the sense or antisense strands, while modification at the 5' end of the antisense strand resulted in complete loss of activity. Multiple ANA modifications within the sense stand were also well tolerated. Duplexes with ANA modifications at appropriate positions in both strands were generally more effective than duplexes with one modified and one unmodified strand. Treatment of drug resistant cells with MDR1 targeted siRNAs resulted in reduction of P-glycoprotein (Pgp) expression, parallel reduction in MDR1 message levels, increased accumulation of the Pgp substrate Rhodamine 123, and reduced resistance to anti-tumor drugs, like daunomycin. Interestingly, the duration of action of some of the ANA modified siRNAs was substantially greater than that of unmodified controls. These observations suggest that Altritol modifications may be helpful in developing siRNAs with enhanced pharmacological effectiveness for the treatment of human cancers.

Novel Design Principles for GPCR Specific Libraries

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The identification of small focused libraries for a given target facilitates the screening and hit validation process. A knowledge-based process (Site-Directed Drug Discovery®) for structure-guided design of small molecule ligands for 7TM receptors has been devised to produce small diverse libraries. The process involves analysis of physicogenetic relationships of binding sites of 7TM receptors and design of pharmacophores by incorporation of target and ligand information. For that purpose, Self Organizing Maps (SOM) have been constructed for GPCR ligands to unravel properties and relationships for different chemotypes and targets. The pharmacophore queries are used for *in silico* screening of compound collections containing up to 10^7 compounds to retrieve small libraries (10^2-10^3 compounds) that are evaluated in information rich *in vitro* screens.

Alkoxyalkyl Acyclic Nucleoside Phosphonates for Smallpox and Othe Viral Diseases

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Acyclic nucleoside phosphonates are an important class of antiviral nucleoside analogs in clinical use for cytomegalovirus (cidofovir), HIV (tenofovir disoproxyl fumarate) and hepatitis B infections (adefovir). Acyclic nucleoside phosphonates have phosphonomethoxyethyl moieties which allow them to bypass the first phosphorylation in the conversion to the active antivirals. However, the intrinsic phosphonate also presents several limitations including poor oral bioavailability and nephrotoxicity caused by accumulation in the proximal tubule of the kidney. Cellular uptake of nucleoside phosphonates into target cells is reduced because of the dual negative charges on the phosphonate moiety and the slow uptake of the compound of this class by fluid phase endocytosis.

We synthesized new type of lipid prodrug of cidofovir which enhances oral absorption and decreases kidney toxicity. Hexadecyloxypropyl-cidofovir is a very active agent with excellent oral activity in lethal poxvirus infections in animals and is in currently in Phase I clinical testing. The strategy is generally applicable to acyclic nucleoside phosphonates. Hexadecyloxypropyl-(*S*)-HPMPA is a antiviral with activity against smallpox, vaccinia, cowpox, cytomegalovirus, BK virus, hepatitis B and drug resistant HIV-1. Similar alkoxyalkyl analogs of tenofovir and phosphonomethoxyethyl-N^6-cyclopropyl-diaminopurine also show improvement of antiviral activity, indicating a potential general role for this approach.

New Bionano Materials for Drug Delivery

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A new method was developed for drug delivery with site specificity. The delivery process involves mechanical force, biological recognition, and chemical interaction. Combination of these three different factors will allow drugs to reach the targets with a minimal side effect. The concept and examples will reported in this lecture.

Functional nanoparticles can be converted to hybrid pro-drugs by chemical synthesis. These bionano materials are used as magic bullets for a gene gun. This approach is on the basis of a chemo-combination strategy; an established example will be presented, in which, cephalosporin 3'phloroglucide esters can be obtained efficiently from cephalosporins and bioactive phloroglucides. Ring opening of the beta-lactam nucleus would occur upon reaction with a bacterial enzyme; then a drug is liberated through a 1,4-elimination. Conjugated products in this family possess a broader spectrum of antibacterial activity than individuals, as reflected by comparison of their biological activities.

Recently, a new photo-dissociation method has also been used in chemo-combination strategy for drug delivery in our laboratory. The lead compounds include a series of new platinum-sulfoxide complexes, which form a class of photo-induced DNA cleavers. Their application to gene therapy requires syntheses of conjugated nanoparticles as biochemical bullets.

In conclusion, exhibition of a dual mode of biological actions by conjugated chemical compounds represents a successful application of the chemo-combination strategy. Its use allows scientists to design and develop various new drugs with dual functions and high efficacy. Attachment of these conjugates to nanoparticles generates magic bullets for drug delivery. This approach may lead to an alternative way in the development of new drugs and biologically active materials.

Genomic Instability in Breast Cancer

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The presence of numerical and structural chromosome aberrations is a common characteristic of tumor cells. Accumulation of these aberrations leads to dramatic changes within the genome. These changes have tumor type and stage specific pattern of segmental losses and gains, resulting in gene losses and gene copy number imbalances. Tumor development and progression is driven by sequential acquisition of specific gene alterations, and chromosome aberrations contribute to these processes. Genomic instability in tumors can be classified as chromosome instability (CIN) and microsatellite instability (MIN). MIN is in general less frequent in tumors than CIN, but is rather frequent in some tumors of the digestive tract. This is mainly due to germline mutations in mismatch repair genes associated with the HNPCC cancer syndrome, but can also be due to somatic mutations and epigenetic mechanism. While MIN is rare in breast tumors, CIN is demonstrated in the majority of breast tumors (about 70%) by aneuploidy, deletions, amplifications and rearrangements. Although the reasons for CIN in breast tumors are not well understood, explanations can partly be obtained from deficient control of genes controlling cell proliferation, apoptosis, DNA repair or chromosome segregation. Among these genes are TP53, MYC, AURKA, BRCA1 and BRCA2. TP53 is relatively well studied and is believed to be a guardian of genome integrity. Myc seems to affect tumor pathogenesis in several ways, including increased proliferation and immortalization of the cancer cells and induction of CIN. The STK15 gene encodes the aurora A kinase, which has been shown to bind to centrosomes and is important in controlling their number and the segregation of correct chromosomes to the daughter cells during mitosis. Genomic instability is high in some hereditary breast cancer, particularly in tumors of BRCA1 and BRCA2 mutation carriers, a finding which is in line with the role of the gene products in DNA repair. Some recent developments in drug therapy are based on molecular and genomic findings about breast cancer pathogenesis. Defects in checkpoint control generate CIN and are believed to facilitate tumorigenesis, but additional disabling of checkpoint signaling is a possible anticancer strategy.

The Development of Marine Derived Anticancer Agents in the Era of Targeted Therapies

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Nature has been instrumental in the acquisition of anticancer compounds. The terrestrial ecosystem led to the discovery of anthracyclines, taxanes, vinca alkaloids and irinotecan. All of them are employed in both curative and palliative approaches in cancer patients. Our marine ecosystem covers 70% of the earth's surface but represents 85% of the biosphere. Moreover multicellular life started in the sea during the Cambrian period, so it is rational to look to the seas as a highly productive source of innovative therapeutics.

Such a hypothesis was reinforced in the past by the discovery, development and regulatory approval of 1-beta-arabinofuranosylcytosine (ARA-C; a chemical analogue among a family of C-nucleotide analogues identified in the Caribbean sponge *T. crypta*),) that still remains the backbone of curative therapy for acute myeloid leukaemia. Emerging evidence underlines the need to implement multitargeted pharmacological approaches to increase the likelihood of tumour control by attacking the process of uncontrolled cell proliferation. In this regard, innovative marine-derived anticancer entities might cooperate to reach this important objective.

Our marine anticancer program seeks new chemical entities, harbouring new modes of action and with activity against tumours resistant to conventional therapies. Our lead compound ET-743/Trabectedin (Yondelis®) is a tetrahydroisoquinoline discovered in the tunicate *E. turbinata*, now produced by semi-synthesis. This innovative compound induces apoptosis *via* selective binding to G-rich sequences within the DNA minor groove and acts upon transcription. Yondelis has received a positive recommendation from the EMEA as therapy for patients with advanced pre-treated sarcoma (STS). This is the first regulatory approval in this setting for the past 25 years: A comparative study in advanced pre-treated STS has confirmed a major impact of the proposed dose (1.5 mg/m2) and schedule (24 hours iv infusion) thus validating the previous phase II results. The median survival, 13.8 months, attained with the infusion regimen is better than that achievable with first line therapy; the progression-free survival at 6 months, 37%, is superior to the 14% proposed as cut-off for active agents; and 50% of cases treated with the infusion schedule have shown tumour shrinkage. The safety profile continues to sustain its feasibility for chronic administration with lack of hair loss, mucositis, diarrhoea and complicated neutropenia. There is now major interest to examine its potential in specific sarcoma sub-types. New data demonstrate a dramatic impact in patients with advanced pre-treated mixoid liposarcoma. A 90% rate of long-lasting, median progression-free survival of 14 months, objective remissions and tumour control has recently been reported. Mixoid liposarcoma relates to a reciprocal 12:16 translocation that leads to a chimeric FUS-CHOP DNA-binding protein that acts as a transcription factor. Early data indicate the capability of Yondelis to down-regulate the activity of genes that are FUS-dependent. A new project is seeking to characterize the clinical potential of Yondelis in other translocation-related sarcomas. Additionally, phase II results demonstrate a 70% rate of long-lasting tumour control in advanced relapsed ovarian cancer, and a phase III registration study comparing liposomal doxorubicin + Yondelis *vs* Yondelis has been completed. Moreover the pharmacogenomic program has confirmed a molecular signature, based on non-functional homologous recombinant DNA repair and effective nucleotide excision repair that clusters both sensitivity and resistance to Yondelis. Such correlations are unique in that they are opposite to the ones applicable to plating salts, anthracyclines and taxanes.

The transtumoral impact of this specific DNA repair profiling is being evaluated, in prostate and in breast cancer, including a patient's enrichment clinical study focusing on triple negative and BRCA mutant patients. Substantial clinical evidence demonstrates an appropriate safety profile with lack of cumulative toxicities thus allowing therapy in a chronic fashion. Yondelis as the standard of care in pre-treated sarcoma represents a major step forward in contemporary anticancer intervention.

Ptidepsin (Aplidine® /APLD) is a cyclic peptide now available by synthesis discovered in the Mediterranean tunicate *A. albicans*. APLD induces antiproliferative effects and acute apoptosis by interacting with multiple targets including blockage of the secretion of VEGF. Clinical data have demonstrated activity in pre-treated malignancies such as multiple myeloma, renal cancer, melanoma and neuroblastoma. Combination studies are ongoing as a foundation for potential phase III registration studies.

PM02734 is a peptidic-based synthetic entity that belongs to the Kahalalide F family. It has unique pharmacodynamic effects as an ErbB3 down-regulator and is under phase I development. The available evidence suggests that this provides a rational argument to explore its therapeutic potential in combination with other ErbB TK inhibitors as well as with monoclonal antibodies that bind to erbB surface receptors.

In conclusion the marine ecosystem is a rational and productive tool to discover new chemical entities harbouring unique mode of action.Such approach copes with the era of molecular/targeted medicine.

The Role of Drug Metabolism and Drug Transport in Idiosyncratic Adverse Drug Reactions

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Characterization of the biotransformation pathways of lead pharmacophores is an integral part of the drug discovery process not only in optimizing ADME properties but also in eliminating potential safety concerns associated with the lead matter. Optimization of ADME properties of new leads often constitutes a significant obstacle in drug discovery. In several instances, biotransformation studies in early discovery have been used to identify metabolic soft spots that render high metabolic instability. Availability of such information has aided in the rational design of compounds with increased resistance to metabolism and overall improvements in oral bio-availability and -activity. Furthermore, there are circumstances in the drug discovery process wherein the presence of toxicophores in otherwise attractive lead matter creates uncertainty around the potential of the drug candidate to cause adverse drug reactions in the clinic. Metabolite identification studies have proved particularly invaluable in this arena. For instance, characterization of stable conjugates derived from bioactivation of pharmacophores provides indirect information on the structure of the electrophilic species, thereby providing insight into the bioactivation mechanism and hence a rationale on which to base subsequent chemical intervention strategies. A team effort on the part of the drug metabolism scientist(s) and medicinal chemists is paramount to the success of such structure-metabolism relationship analyses. Literature and in-house examples, which highlight all of the attributes described above, will be presented.

Glial glutamate and GABA Transporters as Neuroprotective Targets

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Gamma aminobutyric acid (GABA) and l-glutamate (Glu) dominate the balance between inhibition and excitation under normal and pathological conditions in the brain. Although operationally independent, the biochemically integrated GABA*ergic* and Glu*ergic* neurotransmissions do interplay at cellular and sub-cellular levels. However, direct coupling between functionally antagonistic neurotransmitters has remained undisclosed. Here, we show that substrate activation of Glu transporters increases ambient GABA level both *in vitro* and *in vivo*. This action appears to be Ca2+-independent acting through reversal of glial GABA transport. The release of GABA is independent of Glu or GABA receptor-mediated mechanisms. This GABA release can be eliminated by preventing Glu uptake, but is only partially affected by inhibition of Glu decarboxylase. Our results demonstrate that activation of Glu transporters results in GABA release through reversal of GABA transporters. Our findings indicate a new mechanism by which coordinated activation of Glu and GABA transporters determine the ratio of the ambient concentrations of major excitatory and inhibitory neurotransmitters. This transporter- mediated interplay represents a direct link between inhibitory and excitatory signaling, which may function as a negative feedback mechanism to avoid hyperexcitability. Under physiological conditions, Glu-induced GABA release may contribute to tonic inhibition, whereas it can provide a new therapeutic strategy to combat intense excitation in diseases such as epilepsy and ischemia.

Future Development of Oncolytic Virus Therapy

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The new era of oncolytic virus therapy has come; the approach is changing from early basic research to a large number clinical trial. Some of them have already yielded approval of new drugs from November 2005 in China. Oncolytic virus therapy is not a mere dream now under the situation that plenty of suffering patients are waiting for relief from cancer. Clinical trials using oncolytic virus have been performed on over 700 patients world wide, and have revealed tumour size stability and tumour marker decline. Over 10 patients went through a clinical trial regarding oncolytic virus therapy in Japan, and all the clinical trial using oncolytic virus have been conducted by our facility. A total of 12 of patients went through clinical trials using direct injection of the mutated herpes oncolytic virus, HF10, in our hospital. We performed clinical trial for 6 breast cancer patients and 3 pancreatic cancer patients (3 patients in the Dept. of Otolaryngology) Pathological finding showed 30-100% cancer cell death in the clinical trial against breast cancer. No virus shadding from blood and drain tube (by PCR and pfu), no flu-like symptom, and no significant change in blood exam data has emerged so far.

We will show the pathological findings of HF10 from clinical trials including immune stain CD4 CD8, and combination therapy of HSV oncolytic virus with chemotherapy drugs. Representative drug, 5FU, enhances cell-cycle into S-phase, and also reduces viral replication *in vitro*. In the same way, Gemcitabine inhibits the ribonucleotide reductase activity of cells, and also reduces viral replication *in vitro*. Combination of drugs and oncolytic virus has been reported to improve the survival rate and tumour reduction effect *in vivo*.

Targeting Key Transcription Factors in Various Experimental Cardiovascular Pathologies

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Vascular injury initiates a cascade of phenotype-altering molecular events. These involve certain transcription factors, although their regulatory functions in the injured blood vessel wall are poorly understood. We have used both gene- and anti-gene-therapeutic strategies to control a wide variety of cardiovascular pathologic settings. As an example of the former, we demonstrate that the enforced expression of the injury-inducible GLI-Krüppel zinc finger protein YY1 inhibits neointima formation in human, rabbit and rat blood vessels. YY1 inhibits $p21^{WAF1/Cip1}$ transcription, prevents assembly of a $p21^{WAF1/Cip1}$-cdk4-cyclin D1 complex, and blocks downstream $pRb^{Ser249/Thr252}$ phosphorylation and expression of PCNA and TK-1. Conversely, suppression of endogenous YY1 elevates levels of $p21^{WAF1/Cip1}$, PCNA, $pRb^{Ser249/Thr252}$ and TK-1, and increases intimal thickening. YY1 binds Sp1 and prevents its occupancy of a distinct element in the $p21^{WAF1/Cip1}$ promoter without YY1 itself binding the promoter. Additionally, YY1 induces ubiquitination and proteasome-dependent degradation of p53, decreasing p53 immunoreactivity in the artery wall. These findings define a new role for YY1 as both an inducer of p53 instability in smooth muscle cells (SMCs), and an indirect repressor of $p21^{WAF1/Cip1}$ transcription, $p21^{WAF1/Cip1}$-cdk4-cyclin D1 assembly and intimal thickening[1]. Conversely, transcription factors positively regulating the expression of genes whose products stimulate vascular cell growth and inflammation have become attractive targets for interventional strategies. DNAzymes are RNA-cleaving phosphodiester-linked DNA-based enzymes that seek out and cleave their target mRNA in a gene-specific fashion[2,3]. Our studies have revealed that DNAzymes targeting the immediate early gene product, early growth response-1 (Egr-1) inhibit intimal thickening in rat carotid arteries following balloon angioplasty[4], and in-stent restenosis in pigs after coronary stenting[5]. Similarly, DNAzymes targeting a second IEG product c-Jun, a prototypic member of the basic region-leucine zipper family of nuclear proteins, inhibit inducible c-Jun expression in SMCs, block SMC proliferation and attenuate intimal thickening in injured rat and rabbit carotid arteries[6,7]. As well as inhibiting SMC growth, DNAzymes inhibit endothelial cell growth, new blood vessel growth (angiogenesis), vascular permeability, monocyte endothelial cell adhesion *in vitro*, key inflammatory processes such as rolling, adhesion and extravasation, and cardiac ischemia reperfusion injury[8-11]. These findings demonstrate the therapeutic potential of DNA-based strategies that overexpress or underexpress key transcription factors in experimental cardiovascular pathologic settings.

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Histone Deacetylase Inhibitors, Neuroprotection, and CNS Disorders

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HDAC inhibitors (HDACIs) are able to reactivate silenced genes, and these compounds have been shown to be of some value in the treatment of cancer [CTCL], and possibly even neurological disorders as well as certain parasitic diseases such as malaria. In our efforts to identify HDACIs that may show an improved therapeutic profile, we have sought to identify compounds that may show enhanced levels of HDAC isoform selectivity, as it is believed that some of the undesirable side effects of these agents may relate to their overall lack of enzyme selectivity. We have thus been investigating the design, synthesis, and testing of compounds containing various CAP residues that may interact differentially with the surface areas of these enzymes outside their catalytic gorge regions, as well as to more broadly assess the effect of variations in the zinc binding groups (ZBGs). In this presentation I shall summarize our current efforts in this exciting field of research, and present results pertaining to the use of these compounds in stroke, pancreatic cancer, and malaria.

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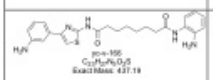
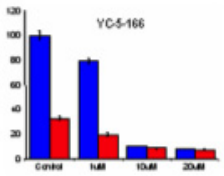
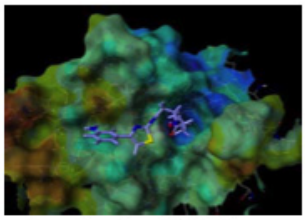
Compound	CLogP (kowwin)	Class I (IC_{50}, μM)				Class II (IC_{50}, μM)	
		Hdac 1	Hdac 2	Hdac 3	Hdac 8	Hdac 10	Hdac 6
	3.50	3.19	0.22	0.0299	>30	3.3	11.17



Effect of an HDAC3 Inhibitor in an Oxidative Stress Model of Cortical Cell Injury.



X-ray Structure of YC-88 in Complex with HDAC8

Structural and Biochemical Studies of Homologous Recombination Toward Drug Design and Discovery

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Double-strand DNA breaks (DSBs) are potential inducers of chromosome aberrations and tumorigenesis. Such DNA lesions are accurately repaired by the homologous recombinational repair (HRR) pathway without base substitutions, deletions, and insertions. Defect in homologous-recombination causes infertility in mammals, indicating that homologous recombination is also crucial during meiosis. To understand the molecular mechanism of the HRR pathway, we have purified human proteins involved in homologous recombination, and have studied their structures and functions by X-ray crystallographic and biochemical analyses. In addition, we identified several novel factors, which may be involved in the HRR pathway, by proteomics and two-hybrid methods, and their structural and functional studies have been performed. These human homologous-recombination factors are potential targets for drug design and screening. Currently, we are performing *in vitro* screening for inhibitors and activators of homologous-recombination proteins. Our recent results will be discussed.

Protein Misfolding and Fibrillogenesis in Neurodegenerative Diseases: From Mechanistic Studies to Therapeutic Strategies

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The process of protein fibrillization, also known as amyloid formatoin, is implicated in the pathogenesis of most, if not all, age-associated neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, Amyotrophic Lateral Sclerosis (ALS), and Huntington's disease. However, the mechanism(s) by which it triggers neuronal death is unknown. Research efforts in our laboratory focus on defining the biochemical mechanisms of amyloid fibril formation of two amyloidogenic proteins, α -synuclein and amyloid-β (Aβ), and how they contribute to each of their respective diseases, Parkinson's and Alzheimer's diseases. To achieve these goals, we rely on a multifaceted approaches that employ tools from chemistry, biophysics, structural biology, proteomics, and molecular and cell biology.

Reductionist *in vitro* studies from our group and others suggest that prefibrillar intermediate referred to as protofibril may be the primary toxic species that is responsible for triggering a cascade of events that culminate in neurodegeneration and cell death in Alzheimer's, Parkinson's disease and related disorders. Although its pathogenic target has not been identified, the properties of the protofibril suggest that neurons could be killed by unregulated membrane permeabilization, possibly by a type of protofibril referred to here as the "amyloid pore". In addition to mechanistic studies, we are employing a combination of chemical and classical genetic approaches to identify small molecule inhibitors and unique unnatural variants as molecular probes and tools for elucidating the molecular and structural basis of protein aggregation and toxicity. Our studies provide new mechanistic insight into the relationship between protein aggregation and neurodegeneration and reveal new therapeutic targets for treating and/or preventing these devastating diseases.

The Effects of Genes Related to the Monoaminergic Pathway on the Action of Antidepressants

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Most pharmacogenetic investigations into antidepressants have been conducted on candidate genes from the monoaminergic pathway, since the action of antidepressants is attributed to the regulation of monoaminergic transmission. This study investigaed pharmacogenetic studies into the effects of genes related to the monoaminergic pathway on the action of antidepressants.

Serotonin transporter, serotonin receptor 2A, G-protein β3 subunit and brain derived neurotrophic factor were selected as candidate genes. It is well known that low BDNF level is associated with MDD and the expression of BDNF is the downstream target of a variety of antidepressants. Therefore we studied that the association between the BDNF level as well as genetic polymorphism and depression susceptibility or antidepressant response.

Severity of depression was evaluated with the 21-item Hamilton Depression Rating (HAM-D-21) scale. Brain-derived neurotrophic factor were assayed with enzyme-linked immunosorbent assay methods, Genetic polymorphisms were examined using a polymerase chain reaction (PCR) method.

Serum BDNF level was significantly lower in MDD than normal control and frequency of the Val66 was higher in patients group compared with control. In mirtazapine treated group, responses at the 2nd and 4th weeks were significantly better for the s/s genotype of the 5-HTTLPR polymorphism than for l-allele carriers. In NET, C carrier patients had a significant faster and better treatment response than C non carrier patients.

Our results suggest that first, the BDNF Val66Met polymorphism play role in a possible susceptibility candidate for with MDD. Second, the HTT and NET genetic polymorphisms were related to the therapeutic response to mirtazapine in MDD patients.

Reporter Mice and Drug Discovery and Development

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In vivo reporter gene and imaging technologies have the potential to contribute to the drug discovery pipeline in several areas. They provide systems that enable the study of the biochemical activity of a target in disease, and in response to a drug, to be monitored over periods of time, and offer more accurate methods of measuring pharmacodynamics and toxicity. Although reporter-gene technology is in its infancy, with further refinement reporter animals could become a valuable tool in the early stages of target and lead identification and preclinical drug development.

In the past few years our group has developed reporter mice for the study of the activity of intracellular activity (1,3). The use of these animal models in association with *in vivo* imaging technologies, demonstrated the power of reporter mouse technology in particular when applied to pharmacological profiling of drugs active on intracellular receptors. In fact, for the first time, the introduction of a surrogate marker offers the possibility directly titrate the action of a drug on its target in space and time avoiding extrapolations based on drug distribution parameters. We also showed the advantages of using reporter mice over the methods currently in use for preclinical drug development which include: *i*.) global view of the tissues affected by the treatment that enables a rapid identification of unexpected, potentially undesired, effects; *ii*) unequivocal assessment of dosage and timing necessary to elicit the pharmacological response; *iii*) longitudinal studies in single individuals during repeated drug treatment that unravels sites of drug accumulation and activity, or the dynamics of the response of the target to the treatment (e.g. with receptor desensitization or down/up-regulation).

In is expected that further improvements of transgene architecture will lead to models that combine pharmacokinetic, pharmacodynamic and toxicological studies in a single step, which should provide a tremendous saving in time and, paradoxically, the number of animals to be sacrificed in the development of novel pharmacologically active molecules.

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Structure-Based Discovery of Aliskiren - A Direct Renin Inhibitor for Hypertension Therapy

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Hypertension is a major risk factor for cardiovascular diseases, affecting more than 25% of adults worldwide. The high percentage of patients with insufficiently controlled blood pressure levels suggest a continual need for improved antihypertensive drug therapies. Direct renin inhibition is a promising new type of high blood pressure treatment that is now emerging with the recent launch of aliskiren, the first in a novel class of orally effective, highly specific nonpeptide inhibitors of human renin of the aspartyl protease family. Targeting the renin system, a key physiological regulator of blood pressure and fluid homeostasis through the actions of the vasoconstrictor peptide angiotensin II, at its first and rate-limiting point of activation has long been recognised as most attractive for therapeutic intervention, and has therefore triggered vast efforts in research over more than two decades worldwide in the quest for developing orally potent renin inhibitors.

The successful topological, structure-based peptide-mimetic inhibitor design concept based on a transition-state mimetic approach leading to the discovery of aliskiren will be highlighted in the presentation. Both computational modeling and protein x-ray crystallography played key roles during the evolution of the design approach during optimization, starting from weakly active initial hits and leading to several distinct small molecule lead series. Pre-clinical pharmacokinetics and pharmacology in various animal models, as well as data from advanced clinical studies with aliskiren will be discussed.

HIT MetASTASIS: Unbalancing Met Activity and its Downstream Survival Signals for New Anticancer Therapies

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Development of novel therapies that are effective in curing cancer is the major challenge for researchers and clinicians. Combining knowledge on cancer biology and chemistry has led to the identification of chemical compounds able to block key oncogenes altered in specific types of tumours. Met is the receptor tyrosine kinase (RTK) that binds hepatocyte growth factor (HGF) and modulates distinct biological events during mouse embryogenesis, depending on the cellular context. The Met oncogene also play a major role during malignant transformation of neoplastic cells, as Met over-expressing cells have growth advantage, are invasive and show decreased sensitivity to chemotherapeutic agents. Mutations and amplification of the *met* gene have been found in different types of cancers and Met is a prognostic marker for a variety of tumours. Studying the nature of Met signals in oncogenesis and establishing therapeutic approaches to efficiently target Met-triggered neoplasia represent two major aims of our laboratory. In particular, we are:

1. investigating how and to which extent survival signals contribute to the oncogenic potential of altered Met;
2. searching for novel Met inhibitors and validating them using biological and biochemical assays as well as engineered animal models.

During embryogenesis, the HGF-Met system is required for hepatocyte survival in developing livers. We recently identified two novel mechanisms composed by well known proto-oncogenes, which are activated by Met to promote cell survival. In particular, we found that Met prevents Fas-triggered degradation of the anti-apoptotic signal Flip acting on the PI3K-Akt pathway. Moreover, we demonstrated that Met acts on PI3K to promote translation and nuclear translocation of Mdm2, which is known to antagonise p53 activity and cell death. The PI3K effectors are selectively used to control Mdm2 activity: **a**) Akt promotes Mdm2 nuclear translocation; **b**) Akt-mTOR pathway is required for Mdm2 translation. Preliminary results indicate that these pathways may contribute to increased survival properties of neoplastic cells with aberrant Met. As mTOR and Mdm2 are often found aberrant in neoplastic cells and currently considered as possible anticancer therapies, we are testing whether their inhibition can un-favour cell survival in Met-triggered neoplasia.

To increase effectiveness in treatment of Met-triggered cancer, we have undertaken a multi-disciplinary approach, which combines cell biology, chemistry, computer-assisted molecular modelling, mouse genetics, and non-invasive monitoring of primary tumours and metastasis, to develop chemical compounds that specifically inhibit Met. Based on computer modelling studies, we have identified new Met inhibitors with unique chemical and physical properties. We are currently validating them for their ability to block Met-triggered biological responses and activation of downstream targets. These new Met inhibitors will be further evaluated using transgenic mice over-expressing Met in a temporally and spatially regulated manner, which have been recently generated in our lab. Met-overexpressing cells will also express the *Luciferase* gene to allow non-invasive monitoring of primary tumours and metastasis *in vivo*. Moreover, they will be of invaluable help for testing anti-Met drug compounds and the effectiveness of combined treatment to un-favour cell survival in Met-triggered cancer.

Antibiotics from Natural Products: Discovery of Tygacil®

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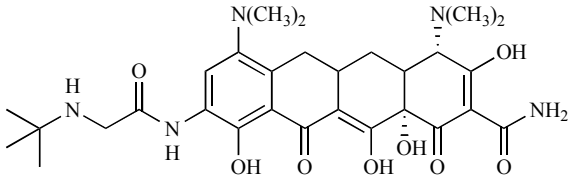
Natural products have commanded a significant role as a discovery platform for novel anti-bacterial and anti-fungal agents. Chemical diversity, particularly of polyketides and glycopeptides, have imparted unique anti-bacterial activities against multiple organisms and in some cases served as chemical biology tools to unravel a molecular target or a specific mechanism of action.

Tygacil® (Tigecycline, GAR-936) is the first member of the glycylcycline class of antibiotics to be approved by the US Food and Drug Administration (FDA). Tigecycline is active against many Gram-positive. and -negative organisms, including methicillin-resistant *Staphylococcus aureus (*MRSA*)*, vancomycin-intermediate and -resistant *Enterococci* (VRE) and extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* (ESBLs). It is also active against many anaerobic bacteria, as well as atypical pathogens. Studies have demonstrated that it binds to the bacterial 30S ribosomal subunit in a novel way and as such results in inhibiting entry of amino-acyl tRNA molecules. The drug overcomes the two major resistance mechanisms associated with the tetracycline class: drug-specific efflux pump acquisition and ribosomal protection.



Tygacil® (Tigecycline, GAR-936)

Research at Wyeth on new antibiotics has also led to semisynthetic mannopeptimycin, muraymycins and pulvinic acids antibiotics with excellent antimicrobial activities. This presentation will describe the medicinal chemistry, biology, microbiology, highlights of the clinical development steps that lead to the approval of Tygacil® and the advances made to date on several classes of natural products derived antibiotics.

Screening for Drugs with Herbicidal, Algicidal and Antiparasitic Properties: a Novel Paradigm to Discover New Antimalarial Compounds

Nadia Saïdani[1,2], Cyrille Botte[2,3], Anne-Laure Bonneau[4], Mickael Deligny, Bernard Rousseau, Jean-François Dubremetz[2], Henri Vial[2], Roman Lopez[4] and *Eric Maréchal*[1]

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Apicomplexans are obligate intracellular parasites infecting virtually all animals from molluscs to mammals. Amongst them, the malarial parasite Plasmodium sp, represents one of the most important threats for humankind, with ~300-500 million clinical cases and ~1.5-3 million deaths each year, most of them African children. More benign, but widely spread is *Toxoplasma gondii*, a parasite causing congenital neurological birth defects and being the most reported opportunistic infection associated with immunosuppressive conditions, including AIDS. A decade ago, *Plasmodium* and *Toxoplasma* were shown to harbour a non-photosynthetic plastid called the apicoplast, which is considered as a relic of an algal chloroplast. Interestingly, some apicomplexan nuclear gene products are imported into the apicoplast and involved in typical plant biosynthetic pathways such as fatty acid, isoprenoid or haem biosyntheses. Plant chloroplasts posses a unique lipid composition: galactolipids represent up to 80% of the chloroplast membrane lipids, whereas in animals, phospholipids represent the major membrane compound. Synthesis of monogalactosyldiacylglycerol (or MGDG) is catalyzed by a specific galactosyltransferase (named MGDG synthase). MGDG can be converted into DGDG by addition of a second galactose, a lipid which is redistributed to non-plastidial biomembranes and is therefore vital for the complete cellular development in plants. We have detected MGDG-like and DGDG-like lipids in lipid extracts of *P. falciparum* and *T. gondii*. A high throughput screening of ~24000 molecules (CEREP® chemolibrary) allowed the identification of inhibitors of Arabidopsis MGDG synthase, with no apparent toxicity in the first trials on human cells (fibroblasts and erythroblasts) and mice. We confirmed the herbicidal properties on Arabidopsis and other plants, and characterized algicidal effects on Chlamydomonas reinhardtii, and antiparasitic properties against *Plasmodium falciparum*, *Babesia divergens*, *Toxoplasma gondii* and *Neospora caninum*. A structure-based diversification of the molecules lead allowed the production of >200 derivatives with improved herbicidal and antiparasitic properties. Novel drug candidates designed based on a new paradigm, i.e. herbicides with antiparasitic properties, will be presented. Related research articles : [1] Birkholtz L.M., Bastien O., Wells G., Grando D., Joubert F., Kasam V., Zimmermann M., Ortet P., Jacq N., Saidani N., Roy S., Hofmann-Apitius M., Breton V., Louw A.I. & Maréchal E. (2006) Malaria J. 5(1):110; [2] Bisanz C., Bastien O., Grando D., Jouhet J., Maréchal E. & CesbronDelauw M.F. (2006) *Toxoplasma gondii* acyl-lipid metabolism: *de novo* synthesis from apicoplast generated fatty acids versus scavenging of host cell precursors. Biochem. J. 394:197-205 ; [3] Botté C., Jeanneau C., Snajdrova L., Bastien O., Imberty A., Breton C. & Maréchal E. (2005). Molecular modelling and site directed mutagenesis of plant chloroplast MGDG synthase reveal critical residues for activity. J. Biol. Chem. 280(41):3469134701; [4] Jouhet J., Maréchal E., Baldan B., Bligny R., Joyard J. & Block M.A. (2004) Phosphate deprivation induces transfer of DGDG galactolipid from chloroplast to mitochondria. J. Cell Biol. 167(5):863-74 [6] Nishiyama Y., Hardré-Lienard H., Miras S, Miege C., Block MA, Revah F,

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Advanced Lead Development - Just How Complex Does if Need to be?

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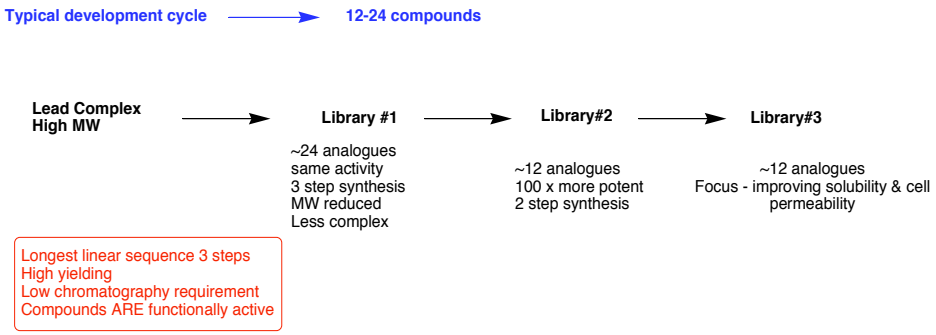
There is little doubt that current medicinal chemists have an amazing arsenal of techniques and technologies at their disposal. With this the case, why then are we not seeing an increase in the number of new drugs entering the clinic? Is it the case that we are working harder and not smarter?

Our group focuses on the implementation of rapid design-synthesis-biological evaluation cycles. Focus is on the speed of each cycle, and development of multiple generations of compounds.



Our rationale is simple - we require access to new molecules and hence the route is immaterial, but we also must minimise chromatography, build in 'space' for further development by pharma, include scope for improved bioavailability, solubility and other PK / ADME considerations. This is best achieved by simple, robust reliable chemistries, 'off the shelf reagents' and focused libraries targeting compounds anticipated to display improved activity, but crucially a subset that is anticipated to be inactive (based on our initial hypothesis).

In medicinal chemistry a negative result can be more important than a small improvement in activity.

Sigma Receptor Ligands as Pharmacotherapy for Psychostimulant Abuse

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Cocaine and methamphetamine have no currently available treatments for their abuse or toxicities. Several attempts have been made over the years to attenuate the effects of these psychostimulants with very limited success. Agents targeting the sites of action of cocaine, such as the dopamine transporter, have simply substituted for cocaine. Treatments for methamphetamine use have been limited to supportive therapies. Recently, it has been noted that sigma receptor ligands can attenuate and block the toxic and the stimulant/rewarding effects of these drugs of abuse. Thus, the sigma receptor is a valid target for medication development for the treatment of cocaine and methamphetamine toxicities and addiction. Sigma receptors exist as two distinct subtypes, sigma-1 and sigma-2. To date, only the sigma-1 receptor has been cloned. Sigma-1 receptors have been demonstrated to be involved in the toxic and addictive effects of cocaine and methamphetamine and are a logical target for the development of novel therapeutics. Although the involvement of sigma-2 receptors is not well-established, their involvement cannot be completely ruled out. This knowledge has been hampered by the availability of selective sigma-2 agents. However, existing data is highly suggestive of their involvement in the stimulant and toxic actions of cocaine and methamphetamine. To this end, we have developed a library of selective sigma-1 agents, high affinity and selective mixed affinity sigma-1/sigma-2 ligands, and highly selective sigma-2 ligands. Data will be presented demonstrating several ligands from this class are able to attenuate the toxic and stimulant effects of cocaine and methamphetamine in mice. Moreover, we have developed predictive pharmacophore models for each subtype that have aided in our design. A single agent, CM-156, a 2(*3H*)-benzothiazolone derivative has demonstrated excellent efficacy in models of cocaine and methamphetamine toxicity and locomotor activities. Furthermore, we have evidence to explain a possible involvement of sigma-2 receptors in the actions of psychostimulants through our highly selective sigma-2 ligand, SN79. Thus, it appears that both sigma-1 and sigma-2 receptors are legitimate targets for psychostimulant medications development. This work is supported by NIDA (DA013978, DA011979, DA023205) and NCRR (P20 RR021929).

Sphingosine Kinases Emerging Therapeutic Targets in Inflammation and Allergy

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During the last few years, it has become clear that sphingolipids are sources of important signalling molecules. Particularly, the sphingolipid metabolites, ceramide and S1P, have emerged as a new class of potent bioactive molecules, implicated in a variety of cellular processes such as cell differentiation, apoptosis, and proliferation. Sphingomyelin (SM) is the major membrane sphingolipid and is the precursor for the bioactive products. Ceramide is formed from SM by the action of sphingomyelinases (SMase), however, ceramide can be very rapidly hydrolysed, by ceramidases to yield sphingosine, and sphingosine can be phosphorylated by sphingosine kinase (SphK) to yield S1P. In immune cells, the sphingolipid metabolism is tightly related to the main stages of immune cell development, differentiation, activation, and proliferation**,** transduced into physiological responses such as survival, calcium mobilization, cytoskeletal reorganization and chemotaxis.

Interest in S1P focused recently on two distinct cellular actions of this lipid: its function as an extracellular ligand activating specific G protein-coupled receptors, and its role as an intracellular second messenger. Several findings reinforced the notion of S1P as an important intracellular second messenger. Activation of various plasma membrane receptors, such as the platelet-derived growth factor receptor, as well as the fMLP receptor, was found to rapidly increase intracellular S1P production through the stimulation of Sphingosine kinase (SphK). Our own work has showed a key role for S1P and SphK1 in FcγRI, FcεRI, C5a, and TNFα triggered proinflammatory responses in monocytes, mast cells, neutrophils and macrophages.

So far two mammalian sphingosine kinases have been cloned (SphK1 and SphK2). Interestingly, when we first cloned SphK, we found the gene to map to chromosome 17q25, laying within an approximate 50cM region, on 17q25, which contains genes implicated in several autoimmune and inflammatory diseases, such as multiple sclerosis, psoriasis and epidermodysplasia verruciformis, strongly suggesting that SphK1 is a possible inflammatory disease susceptibility candidate. More recent work has shown that SphK1 plays important roles in the responses triggered by several immune-effector cells, including neutrophils, monocytes, macrophages and mast cells.

Recently, much attention is to a new class of inhibitors that act by counteracting the functions of the lysophospholid sphingosine-1-phosphate (S1P). S1P is emerging as a potent stimulator of several immune cells and is critical for lymphocyte migration. The sphingosine analogue, FTY720 (fingolimod; Novartis), a high affinity agonist of sphingosine-1-phosphate type-1 receptor (S1P-1), acts primarily by sequestering lymphocytes within peripheral lymphoid organs rendering them incapable of migrating to the sites of inflammation. Phase I, II and III, clinical trials comparing the efficacy of FTY720 containing regimens to conventional immunosuppressive regimens in *de novo* renal transplant patients, has been conducted. Moreover, clinical trials are also on-going in patients with relapsing-remitting multiple scleroses showing obvious benefit for patients receiving FTY720. Interestingly, FTY720 needs to be phosphorylated by SphKs in order to be active.

Very recently, we showed that a general sphingosine kinase inhibitor reduces the inflammation triggered by the anaphylatoxin C5a *in vivo*, and a more recent report by Olivera *et al*, utilising both SphK1 and SphK2 Knock-out mice, showed a role for both isoforms in mast cell-driven hypersensitivity responses *in vivo*. Due to the lack of specific SphK inhibitors many questions remain to be answered. However, one thing is quite clear, that is the involvement of SphK and its product S1P in inflammatory responses, thus suggesting SphKs as novel therapeutic targets for autoimmunity, allergy and inflammation in general.

The Use of TTP Translational Technology to Discover Novel Treatments for Alzheimer's Disease, Cardiovascular Diseases & Diabetes

Adnan Mjalli

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Despite major advances in chemistry, molecular biology, and high throughput screening, drug discovery remains costly and time-consuming with high failure rates in the discovery and development stage. The path leading to the successful design of these drugs involves significant scientific challenges and substantial financial burdens. Despite the considerable preexisting sources of lead molecules there are still many biological targets that have been unsatisfactorily approached with small molecule ligands. To address this, we have introduced a process, which we term "TTP Translational Technology®", encompassing the following three tools: a) TTPredict®; a quick and efficient formulation of reasonable, multiple biomolecular target hypotheses derived from sequence data and/or structural biology, b) TTPSpace®; a formulation of reasonable ligand hypothesis for the targets in the form of novel, highly progressible molecules both for lead finding and lead optimization consisting of greater than 65,000 unique and proprietary TTProbes® and greater than 400,000 novel compounds in the form of TTP Integrated Libraries® and c) TTPScreen®; the verification of the target-ligand hypotheses through biological assays and handling, mining and manipulation of biological and chemical data. In particular, our TTProbes® and TTPredict® are key modules which when taken together are powerful, iteratively - applied tools for going from amino acid sequence, to lead molecule, to preclinical candidate, in *months rather than years*. At TransTech Pharma we have utilized our TTP Translational Technology® in the discovery of preclinical and clinical drug candidates against multiple molecular target classes such as kinases, GPCRs, phosphatases, proteases, and protein-protein interactions including Ig supergene family members. Our programs are directed at multiple therapeutic areas including CNS diseases, cardiovascular diseases, inflammatory diseases, oncology, infectious diseases, and metabolic disorders including diabetes and obesity. All are areas where there are significant unmet medical needs.

Examples of the successful application of TTP Translational Technology® include TTP889, an orally bioavailable small molecule that inhibits the intrinsic pathway coagulation factor IX and/or IXa (FIX/IXa). This compound is being developed for the treatment of thromboembolic disorders. Because TTP889 is a selective partial inhibitor of intrinsic coagulation pathway factor IX/IXa, it should have limited effects on the extrinsic pathway and thus may provide anticoagulant activity with less risk of bleeding. Based on preclinical data and Phase 1 safety and pharmacokinetic results, the once-daily dose of 300 mg TTP889 was selected for the first Phase 2 study of TTP889 (Study TTP889-201, Factor IX Inhibition in Thrombosis Prevention: The FIXIT Trial) to test the hypothesis that TTP889 has antithrombotic effects in humans. TTP Translational Technology® was used to discovery and develop TTP889 from biological target to clinical candidate in three years.

Another example is seen in the TTP glucosekinase (GK) program. GK is the principal gatekeeper enzyme responsible for modulating glucose metabolism through catalyzing the rate limiting step of glucose phosphorylation which is essential for glucose uptake in cells. The enzyme is primarily located in the liver and in pancreatic β-cells. In liver, activation of GK will simultaneously up regulate glucose utilization and storage and down regulate gluconeogenesis. In the β-cells, GK determines glucose utilization and thereby insulin secretion. Using TTP Translational Technology® several series of compounds that activate GK have been identified. Two compounds TTP355 and TTP399 have entered clinical development in less than three years from initial discovery.

A third example of the successful application of TTP Translational Technology® is TTP488; a small molecule orally active RAGE antagonist. RAGE is a membrane protein, member of the Ig supergene family having multiple ligands including AGEs, A-beta, S100b, and HMGB1 (amphoterin). Expression of RAGE and its ligands is increased in pathologic states. Protein-protein interaction targets are particularly recalcitrant to traditional drug discovery methods. Our drug discovery engine produced TTP488 in three years from inception of the program. TTP488 has been licensed to Pfizer for worldwide development.

This presentation will focus on the application of TTP Translational Technology® in the three drug discovery case studies outlined above.

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This presentation will focus on the application of TTP Translational Technology® in the three drug discovery case studies outlined above.

From Vitual Screening to Experimental Data: A Foursome to Inhibit Protein-Protein Interaction Involving Nef from HIV-1

Stéphane Betzi[*], *Audrey Restouin*[†,‡,§], *Sandrine Opi*[†,‡,§], *Stefan T. Arold*[¶], *Isabelle Parrot*[‖], *Françoise Guerlesquin*[*], *Xavier Morelli*[*], and *Yves Collette*[†,‡]

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Despite the initial believe that Protein-Protein Inhibition (PPI's) was refractory to small molecule intervention, improved knowledge of complex molecular binding surfaces has recently stimulated renewed interest for PPI's, especially following identification of 'hot spots' and first inhibitory compounds.

New approaches and development of Computer Aided Drug Design (CADD) have help to circumvent such problems. However, the majority of the scoring functions available to researchers in virtual screenings are not especially relevant to PPI's. We will define why consensus scoring can be an alternative and we will propose GFScore, a non linear ranked-by number consensus scoring function as a solution to this problem. Emphasis will be placed on the actual challenges that still remain, particularly the problem of the treatment of the receptor flexibility and of the water molecules at the interface of the protein protein complexes.

We have applied GFscore to a drug design project that targets a SH3-binding surface of the Human Immunodeficiency Virus Type I Nef protein. We have used virtual screening on the one hand (High-throughput docking and application of a pharmacophoric filter) and search for analogy, on the other hand combined to experimental screening. This new methodology that we have called 2P2I, has permit us to identified drug-like compounds that were further confirmed to bind Nef in the micromolar range (Isothermal Titration Calorimetry), to target the Nef SH3 binding surface (NMR experiments) and to efficiently compete for functional Nef-SH3 interactions (cell-based assay, GST-pull down).

Our results identify the first set of drug-like compounds that functionally target the HIV-1 Nef SH3-binding surface and provide the basis for a powerful discovery process that should help to speed up 2P2I strategies and open avenues for new class of antiviral molecules (Betzi *et al.*, in press).

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Drug Discovery for Allergic Diseases; Tranilast, Suplatast and Feature Drugs

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Allergy has now become amongst the commonest disease in the world and the patients are increasing. There have been significant improvements in our understanding of allergic disease and its management. However there are still many unclear points in the precise mechanism of allergic diseases and therapy. The aim of this presentation is to introduce our studies of drug discovery and development for allergic inflammation in recent decades.

We developed two anti-allergic drugs, tranilast and suplatast which are being used in clinical treatment for allergic diseases in Japan. Tranilast is an oral active mast cell stabilizer. Immunological activation of mast cell is an important trigger in the cascade of inflammatory events leading to the manifestation of allergic diseases. Cromoglycate is a well known mast cell stabilizer, but it is active only by inhalation. Therefore, we searched oral active compound and finely selected Tranilast from almost 300 applicant compounds. Suplatast, Th2 cytokine inhibitor, is also discovered by us. Th2 cytokines are key molecule for the onset of allergic diseases. Suplatast inhibits the production of Th2 cytokines, IL-4, 5 and 13 and resulted in the inhibition of eosinophilic inflammation. Suplatast is now being used as an anti-allergic drug and planned to apply for the treatment of interstitial cystitis. The data regarding the basic research about the efficacy on cystitis will be introduced in this presentation.

Some allergic symptoms including airway hyperresponsiveness (AHR), airway wall remodeling, nasal congestion and severe itching are not yet well controlled by the drugs. Therefore, we are trying to find a new drug for curing above symptoms especially AHR. The trial to discover a new therapeutic agent for AHR will also introduced in this presentation.

Towards Novel Gene Targeted Drugs. Progress in dsDNA Recognition and Cellular Delivery of Peptide Nucleic Acid (PNA)

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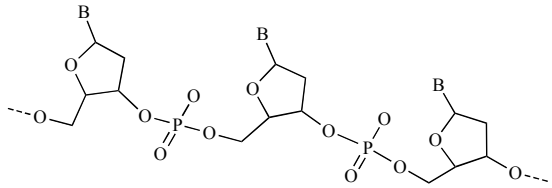
Sequence selective gene targeting is of major interest for drug discovery as well as for basic research and may be accomplished at the mRNA (RNA interference) as well as at the (double strand) DNA level, with the aim of modulating gene expression. Depending on the reagent and the genetic target, the result may be inhibition of translation or transcription, redirection of splicing, activation of transcription, or gene repair, processes that can all be induced by using specifically designed gene targeted oligomers composed of peptide nucleic acid (PNA), a pseudopeptide DNA mimic. However, many challenges still remain before the goal of effective PNA derived medical drugs can be realized. Recent results relating to chemical solutions (that were partly aided by combinatorial approaches) to two of the major challenges: improved bioavailability of PNA oligomers in cell culture *ex vivo* as well as in animal models *in vivo* and effective DNA recognition at physiological conditions will be discussed.



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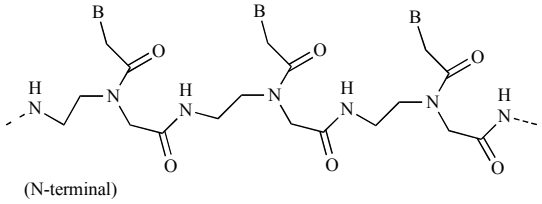

(N-terminal)

PNA

Global Networks for Drug Discovery for Infectious Tropical Diseases

Solomon Nwaka

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Discovering lead compounds with the potential to become usable drugs is a crucial step to ensuring a sustainable pipeline for innovative products. Yet the discovery of drug leads and candidates to sustain the development pipeline for various infectious tropical diseases has not received much attention till recently. The past few years has witnessed the establishment of public private partnerships focusing on drug development for malaria, tuberculosis, and a few neglected diseases. However, most neglected diseases still lack dedicated product development and existing ones require new chemical entities to fill their development pipelines. The talk will summarize current strategies for drug discovery for infectious tropical diseases, and highlight the promise of an innovative drug discovery platform established by WHO/TDR, which involves networks and partnerships between academia and industry in developed and developing countries. The networks include the compound evaluation, medicinal chemistry, PK/metabolism and the drug target portfolio networks, as well as the Helminth Drug Initiative. The achievement of these networks/initiatives and the need for scale up will be discussed.

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The Substance P System as a Target in the Development of Peptidomimetics Acting as Potential Pain Relievers and as Drugs for Treatment of Opioid Addiction

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The tachykinin family of neuropeptides includes three important neuropeptides known as *neurokinin A*, *neurokinin B* and *substance P*. They have binding preference for the so-called neurokinin receptors, where substance P (SP) exhibits highest affinity for the NK-1 receptor. Both N- and C-terminal fragments of SP are shown to retain biological activity. The C-terminal portion of SP is known to interact with the NK-1 receptor and fragments containing this sequence can mimic many of the agonistic effects mediated through this receptor. A biologically potent peptide released from SP is its N-terminal fragment SP(1-7). This peptide is formed by enzymatic conversion of SP (1). It is widely distributed within the central nervous system (CNS). Studies have shown that SP(1-7), as well as the enzyme activity responsible for its release, are significantly elevated in brain areas related to reward and in the spinal cord during morphine withdrawal (2,3). Moreover SP(1-7) is shown to counteract the expression of opiate tolerance and withdrawal (4). This is in contrast to what is seen for the SP receptor (NK-1) agonists, which increases the reaction to opioid withdrawal. Another important area, where SP(1-7) is shown to modulate the SP response is related to processing of pain signals (5). SP is known to facilitate nociception by acting on NK-1 receptors at the spinal level, while SP(1-7) in contrast to SP exhibits anti-nociceptive activity. An additional example of the ability of SP(1-7) to oppose the effect of SP on peripheral nerves comes from studies of the influence of SP(1-7) on the inflammatory response of SP. Whereas, SP is shown to potentiate the outcome of peripheral inflammation in a rat blister model the heptapeptide was found to attenuate the the SP-induced response (6). Furthermore, the SP(1-7) fragment has also been shown to attenuate or modulate SP-induced effects on aversive behavior, on blood pressure and several other behaviors (5). Recent research in our group suggests that the SP(1-7) fragment does not mediate its effects through any of the known tachykinin receptors and not *via* activation of any opioid receptor. The SP (1-7) fragment rather appears to produce its effects through specific sites for this heptapeptide, which are not recognized by its parent peptide. We identified and characterized specific sites for SP(1-7) in the rat spinal cord (7). The affinity of SP(1-7) for these sites highly exceeds those of the other N-terminal fragments of SP. Moreover, in the presence of the GTP analogue Gpp(NH)p at increasing concentrations a dose-dependent inhibition of the SP(1-7) binding was observed. This observation indicated that the SP(1-7) binding in the spinal cord was due to G-protein coupled receptors specific for the heptapeptide. In order to explore the possibility to use the current knowledge on SP(1-7) for clinical purposes we now synthesize both peptide and non-peptide analogues of SP(1-7) and study these compounds with regard to their anti-nociceptive and anti-inflammatory properties. We also study signal mechanisms by which these analogues induce their effects by agonist actions on the specific sites for the SP heptapeptide. In this presentation some SP(1-7) analogues with higher potency than the native peptide will be described. The far aim with the study is to prepare candidate drugs for further development of pain-relievers and anti-inflammatory agents to be used in the clinic (8).

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Design, Application, and Chemical Biology of Tumor-Targeting Drug Conjugates

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Our laboratory launched a research program on the discovery and development of new taxane-based anticancer agents possessing tumor-targeting ability and efficacy against various cancer types, especially multi-drug resistant tumors. These new tumor-targeting anticancer agents (TTACs) are conjugates of the second-generation taxoid anticancer agents with tumor-targeting molecules through mechanism-based cleavable linkers. TTACs are specifically delivered to tumors, internalized into tumor cells, and the potent taxoid anticancer agents are released from the linker into the cytoplasm. We have successfully used monoclonal antibodies (for EGFR) and omega-3 polyunsaturated fatty acids, in particular DHA, as tumor-targeting molecules for drug conjugates, which exhibited excellent efficacy against human tumor xenografts in mouse models. We have also been exploring the use of biotin, folate, and aptamers as tumor-targeting molecules. In order to monitor and elucidate the mechanism of tumor-targeting, internalization and drug release, several fluorescent and fluorogenic probes were developed and we have successfully monitored the receptor-mediated endocytosis and drug release by means of confocal fluorescence microscopy. The use of functionalized single-wall carbon nanotubes (SWCNT) as a template for multiple warhead drug conjugates has been studied and exciting preliminary results are obtained. This lecture will summarize our approaches to efficacious tumor-targeting drug delivery using unique cleavable linkers and second-generation taxoids as the warheads.

Cancer Antineovascular Therapy by Use of Angiogenic Vessel-Targeted Liposomal Drugs

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In the DDS field, several liposomal drugs have been developed for cancer treatment, although the first generation of nanomedicines aimed to reduce side effects and to deliver the encapsulated drugs to the tumor site by passive targeting. Active targeting to cancer cells have been attempted as next generation of DDS drugs. Since administered nanomedicines into bloodstream initially interact with endothelium, endothelial cells are good candidate for the active targeting object. Furthermore, since angiogenic endothelial cells have growing characteristics like as cancer cells, these cells would be damaged by anticancer drugs. Base on these, we have developed a novel modality of cancer treatment by use of DDS technology, in which anticancer drugs are effectively delivered to the angiogenic endothelial cells of a solid tumor. Anti-neovascular therapy (ANET) may cause indirect lethal damage of tumor cells through the damage of newly formed blood vessels with reduced side effects. Moreover, this therapy overcomes drug-resistance tumors. For this purpose, we firstly isolated peptides specific for tumor angiogenic vasculature using a phage-displayed peptide library. Thus obtained pentapeptide APRPG was then used for the modification of liposomes after PEGylation. APRPG-PEG-modified liposomes encapsulating adriamycin, or other anticancer drugs, caused strong tumor growth suppression through possible damaging of angiogenic endothelial cells. The accumulation of APRPG-PEG-liposomes was not so much different from that of control PEG-liposomes. However, intratumoral distribution of APRPG-PEG-liposomes was quite different from the control liposomes: The former colocalized with endothelial cells, and the latter accumulated the surrounding of the blood vessels when the intratumoral distribution of fluorescence-labeled liposomes was examined by confocal laser scanning microscopy. By the way, pancreatic cancer is intractable, and shows poor development of angiogenic vessels suggesting a large number of tumor cells are controlled by a small number of vessel cells. Therefore, we applied ANET to pancreatic cancer model prepared by orthotopical implantation of human pancreatic tumor line cells, and observed the superior growth suppression of the tumor by the treatment of angiogenic vessel-targeted liposomal adriamycin. The present study provides a novel modality of cancer treatment by use of nanomedicines.

Translational Approach to Study Anti-Cancer Drugs Using Mouse Model of Breast Cancer

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Breast cancer is one of the leading causes of death from cancer in the United States. Aurora-A gene is mapped to chromosome 20q13.2-q13.3, a region frequently amplified in 94% of human primary breast cancer. The encoded protein, Aurora-A kinase, is a serine/threonine kinase, containing transforming capacities. Thus, Aurora-A is of considerable interest as a potential drug target. VX-680 and VE-465 are suggested to target Aurora-A developed by Vertex Pharmaceuticals and Merck. Selective inactivation of a specific kinase is indeed a desirable pharmaceutical profile and could be the best strategy to achieve maximal clinical efficacy of an anti-cancer agents, such as Gleevec (also known as imanitib made by Novartis) for chronic myelogenous leukemia targeting Bcr-Abl oncogenic protein kinase. Interestingly, breast cancer development in our MMTV-Aurora-A mice was accelerated on p53-mutant background. Furthermore, we observed that Akt pathway is activated in these mice, and that levels of PTEN is decreased in Aurora-A overexpressing cells, suggesting PTEN-Akt pathway is critical in Aurora-A breast cancer.

Our central hypothesis is that derailed regulation of Aurora-A kinase activity induces mammalian carcinogenesis. This hypothesis is supported by our collaborative efforts, which have been published recently. Firstly, we identified BRCA1 breast cancer tumor suppressor protein as a substrate of Aurora-A; BRCA1 phosphorylation of Ser308 by Aurora-A is essential for mitotic entry of the cell cycle in physiological conditions. Secondly, we have direct evidence that Aurora-A overexpression causes breast cancer by taking advantage of MMTV-Aurora-A transgenic mice model. On the basis of these observations, we will explore how Aurora-A initiates ductal carcinoma in situ (DCIS) and whether Aurora-A inhibitors can inhibit breast cancer development using our mice model system. Molecular basis of translational approach to investigate how small compounds can be utilized in mouse model of human cancer will be discussed.

From Enzyme Mechanism Studies to the Generation of Lead Compounds Against Malaria

Emil F. Pai

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Orotidine 5'-monophosphate decarboxylase (ODCase) catalyzes the last step of *de novo* pyrimidine synthesis. The enzyme decarboxylates orotidine monophosphate (OMP) producing uridine monophosphate. Most decarboxylases use either pyridoxalphosphate, metal ions or delocalization into aromatic systems to stabilize the charge developing in the transition state. ODCase, however, does not employ any cofactors or metal ions and the orotidine base of its substrate OMP is unable to delocalize the charge of the carbanion created upon the release of CO_2. Nevertheless, the enzyme accelerates the decarboxylation reaction by 17 orders of magnitude over the corresponding reaction in water of neutral pH making ODCase one of the two most proficient enzymes known.

Combining structural biology and medicinal chemistry approaches, we set out to investigate the enigmatic reaction mechanism of this enzyme. In the course of our experiments, we discovered a new substrate as well as several suicide inhibitors. As the pyrimidine synthesis pathway had been identified as a promising target for drugs against *Plasmodia* (due to the absence of any salvage pathways in these pathogens) we tested several of the newly synthesized compounds for their effects on the malaria pathogen. Cell assays and *in vivo* mice models were promising and pharmacokinetic studies are underway.

The Use of SUR1/Kir6.2-Selective Potassium Channel Openers in the Prevention or Treatment of Endocrine and Metabolic Disorders Associated with Hyperinsulinemia: Recent Developments and Future Prospects

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Clinical studies conducted with diazoxide, a well-known ATP-sensitive potassium channel opener (K_{ATP} channel opener), have demonstrated the therapeutic interest of this drug in pathologic situations such as type 1 and type 2 diabetes and obesity, either by inducing beta cell rest (prevention of type 1 diabetes), or by rectifying hyperinsulinemia, glucose intolerance and insulin resistance (treatment of type 2 diabetes and obesity). The therapeutic benefits of the drug are linked to its channel opening properties on pancreatic (SUR1/Kir6.2 subtype) K_{ATP} channels and to the resulting inhibition of the insulin releasing process. However, the use of this drug in human medicine has not been proposed in these indications due to appearance of marked unwanted side effects (hypotension, oedema, tachycardia, hypertrichosis, ...) as a result of a lack of tissue selectivity and interaction with other K_{ATP} channel subtypes (i.e. SUR2B/Kir6.1 or Kir6.2). These observations brings us to consider the development in drug design and discovery of new diazoxide analogues expressing high selectivity for the SUR1/Kir6.2 K_{ATP} channel subtype in an innovative approach of the treatment of diabetes and endocrine/metabolic disorders associated with hyperinsulinemia.

Diazoxide analogues for which the chlorobenzenic moiety of the benzothiadiazine dioxide scaffold has been replaced by a pyridinic nucleus (pyridothiadiazine dioxide isosteres) were synthesized and evaluated as potassium channel openers. Several members of the 3-alkylamino-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide series, such as BPDZ 44, were found to markedly and selectively activate pancreatic K_{ATP} channels *versus* smooth muscle K_{ATP} channels. Further chemical and pharmacological exploration identified short branched 3-alkylamino-substituted 7-halo-4*H*-1,2,4-benzothiadiazine 1,1-dioxides, such as BPDZ 73, as very potent and SUR1-selective K_{ATP} channel openers. The latter compound was used as the starting point of a lead optimization program that our department of medicinal chemistry conducted in collaboration with Novo Nordisk in order to discover new therapeutic agents in the treatment of diabetes. This work resulted in the development of several series of benzothiadiazine dioxides as well as of their isosteres, i.e. thienothiadiazine dioxides, expressing potent pancreatic beta cell K_{ATP} channel opening activity. Among thienothiadiazine dioxides, one representative, NN414, revealed marked activity associated with an interesting tissue selectivity, and entered the clinic. Unfortunately, the drug was recently stopped in phase 2 clinical trials due to possible hepatotoxicity.

Current developments of our department of medicinal chemistry in the field of SUR1-selective K_{ATP} channel openers consist in synthesizing benzopyran derivatives (cromakalim analogues). Recent biological results indicated that benzopyran derivatives bearing the appropriate substituents can also express the desired pharmacological profile and are able to markedly inhibit insulin release.

Strategies for the Selection of Drug Candidates for Development: An Overview

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The drug development process is scientifically complex and full of risks, and is therefore, very time consuming and expensive. Recent data indicate that the discovery and development of a new drug costs around 1 billion dollars and takes 10-12 years for the drug to reach the marketplace. In addition, 90% of all drugs in clinical development fail to make to the market. Efforts are being made to reduce attrition of drug candidates during the various stages of drug discovery and development, and to bring safer drugs to the market. The major reasons for the attrition are sub-optimal drug metabolism and pharmacokinetic (DMPK) profile, poor clinical efficacy, toxicity and adverse drug reactions in humans. Given the inherent inefficiency of the development, it is essential to optimize/minimize such factors early in drug discovery process. This has led to greater integration of DMPK functions into early stages of drug discovery process and in addition to potency and selectivity; drug candidates are selected on the basis of DMPK properties, e.g. low clearance, good oral bioavailability, optimum half-life, and an acceptable metabolism profile in preclinical species and humans. Currently, a wide variety of *in vitro* and *in vivo* screens are in place to obtain valuable information about ADME parameters for lead candidates. The *in vitro* studies include (a) metabolic stability in liver microsomes, hepatocytes or with recombinant cytochrome P450 enzymes, (b) metabolite formation in liver microsomes, hepatocytes or with recombinant cytochrome P450 enzymes, (c) absorption/transport studies in Caco-2 cells or cell lines over expressing various transporters, (d) cytochrome P450 inhibition, (e) cytochrome P450 induction, and (f) plasma protein binding. The *in vivo* studies include (a) pharmacokinetic studies *via* various routes of administration (oral, intravenous, subcutaneous, etc), (b) tissue distribution (e.g., brain penetration) and (c) metabolite identification in various biological fluids (plasma, bile, urine, etc.). This presentation will summarize the *in vivo*/*in vitro* techniques used for rapid determination of the DMPK profiles and role of these studies in the selection of the drug candidates for further development. Knowledge of metabolic profiles of these candidates in an early stage of drug discovery is essential to select compounds with favorable pharmacokinetic credentials and to aid medicinal chemists for rational drug design.

Chemistry and Biology of Glycopeptide Antibiotics

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The glycopeptides (vancomycin, teicoplanin) are the antibiotics of choice for serious infections due to Gram-positive pathogens. The search for derivatives active against multidrug-resistant bacteria resulted in the discovery of hydrophobic derivatives of glycopeptides, active against Methicillin-resistant *Staphylococcus aureus* (MRSA), Glycopeptide intermediately susceptible enterococci (GSE,) and Glycopeptide resistant enterococci (GRE) in a manner different from that of the parent antibiotics. The introduction of both polar and hydrophobic substituents in the same molecule has enabled to reduce the negative pharmacological consequences of the introduction of a hydrophobic substituent into a glycopeptide.

In cooperation with *Rega Institute for Medical Research, Leuven, Belgium* (Erik De Clercq, Jan Balzarini*),* antibiotic derivatives were evaluated against the cytopathicity of HIV-1 and HIV-2 and against the cell-transforming effect of Moloney murine sarcoma virus (MSV). The introduction of a hydrophobic substituent is beneficial for antibacterial and antiviral types of activity. The deglycosylation of glycopeptide derivatives does not influence anti-HIV activity, whereas for antibacterial activity the presence of sugars represents a critical determinant. Glycopeptide aglycon derivatives were found to be selectively active against HIV-1, HIV-2 and MSV, whereas they lost antibacterial properties. Inhibition of viral entry is the molecular event responsible for the anti-HIV activity of this type of compounds

Brain-Selective Estrogen Therapy

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Estrogens are potent neuroprotective compounds. However, treatment of maladies affecting the central nervous system (CNS) with estrogens is hampered by undesirable side-effects such as peripheral feminization, stimulation of estrogen-sensitive malignancies and risk of venous thrombosis. Differential expression of distinct estrogen-receptor (ER) subtypes has been implicated in the pleiotropic and tissue-specific effects of estrogens, and a plethora of non-genomic actions has been brought into connection with estrogens' neuroprotective action. After a brief overview of strategies to target ERs in the brain by selective ER-modulators (SERMs), this presentation focuses on prodrug design from naturally occurring estrogens to confine the action of the steroid to the CNS and, thereby, reduce or even eliminate systemic side-effects of hormone treatment. The prodrug approach may be considered an alternative strategy that is not burdened by the excessive costs, enormous challenges and high risks associated with an extensive screening and discovery program to find "neuro-SERMs" with an optimal activity profile. Specifically, estrogen-derived *para*-quinols will be presented as brain-selective prodrugs with details on chemistry, physicochemical and biological properties, as well as efficacy against a variety of neurological maladies such as postmenopausal hot flushes, cognitive impairment, depression and stroke.

GPR30 as a Novel Biomarker and Drug Target

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Estrogen is a critical hormone in the development, normal physiology and pathophysiology of numerous human tissues. Although the functions of estrogen have traditionally been ascribed to the nuclear receptor family of soluble transcription factors, recent studies demonstrate that the G protein-coupled receptor, GPR30, binds estrogen and mediates rapid cellular responses to this steroid. Signaling initiated by GPR30 is mediated at least in part by transactivation of epidermal growth factor receptors at the cell surface. Effector functions appear to be regulated by a combination of this transactivation and direct signaling events triggered by GPR30. With our recent description of a GPR30-specific agonist, studies are now able to begin to dissect the functions of GPR30 vs. classical estrogen receptors in human physiology and disease. Our continuing drug discovery efforts have identified novel ER- and GPR30-selective compounds that show great promise as lead drug candidates. Recent work shows that GPR30 is capable of mediating gene expression and cellular proliferation. In addition, GPR30 is overexpressed in both breast and endometrial cancers. In the latter, GPR30 overexpression is inversely correlated with survival, suggesting GPR30 to be an important prognostic biomarker and novel target for drug development.

Nanoparticles as Delivery Systems for Subunit Vaccines - A Pharmaceutical Approach

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With current gene and protein technology it is now possible to identify specific regions of some whole organisms or cells which are likely to be recognized by the immune system, and to reproduce them synthetically as subunit vaccines. These so called epitopes are very safe because they are non-living but they also tend to be only poorly immune stimulating. To improve the immunogenicity of a poorly immunogenic antigen, our approach is to use nanoparticles as delivery systems. Nanoparticulate delivery systems are thought to enhance the immune response by more closely mimicking a virus or microorganism due to the possibility of multimeric antigen presentation and their large size compared to subunit antigens.

Our group has developed and characterised the following colloidal delivery systems:

- functionalised liposomes (mannosylated or including adjuvants such as Quil A)
- immune stimulating complexes (ISCOMs)
- cationic ISCOMs (termed Pluscoms)
- ISCOM implants
- polymeric nanoparticles on the basis of microemulsions
- in situ gelling chitosan solutions containing chitosan nanoparticles
- cubosomes

In this presentation, we will give an overview about the various nanoparticulate delivery systems our group has developed for the delivery of subunit vaccines. We will describe new results in this field, both on physico-chemical characterisation and immunological activity of these systems.

Development of New Therapeutic Approaches in Allergy and Lung Disease: The Antisense Strategies

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Bronchial asthma is a chronic inflammatory disease with increasing prevalence and incidence worldwide. Several hallmarks characterize the complex pathogenesis of the disease including recurrent and reversible bronchoobstruction with airway inflammation, mucus hypersecretion and development of airway hyperresponsiveness[1]. The chronic nature of the disease results over time in a state of airway remodeling. The dysregulation of the immune system is defined by a Th1/Th2 cell imbalance resulting in overproduction of many pro-allergenic and pro-inflammatory cytokines such as IL-4, IL-5, IL-9 and IL-13[2,3]. The transcription factor GATA-3 promotes the development of naïve T cells into Th2 cells and directly induces production of Th2 cytokines by transactivation of the promoters for IL-5 and IL-13[4,5]. Moreover, GATA-3, even expressed at low levels, inhibits the expression of t-bet, which is required for Th1 cell development and subsequent production of IFN-γ[6]. More recent data indicate that the post-embryonic expression of GATA-3 is not restricted to T cells, but is detectable also in eosinophils, basophils, mast cells and epithelial cells[7,8]. Since all of these cell types play an important role in the pathogenesis of bronchial asthma and other allergic diseases, GATA-3 represents an interesting novel target for anti-allergic therapeutic strategies[9].

DNAzymes represent a novel class of antisense molecules which are not yet established in the treatment of any human disease. In terms of stability, specificity, and bioavailability they have several important properties which make them attractive tools for drug development. 10-23 DNAzymes represent a group of RNA-cleaving DNA molecules and consist of a catalytic and two substrate recognition domains[10,11]. We have developed a catalytic DNA molecule targeting GATA-3 mRNA. This GATA-3-specific 10-23 DNAzyme, termed gd21, was tested in a murine model of experimental allergic asthma. Due to secondary and tertiary structures of the target mRNA not all theoretical target sites are actually accessible for DNAzyme cleavage. Therefore, the cleavage activity of seventy GATA-3 DNAzymes (designated as gd1 - gd70) were initially tested using a multiplex cleavage assay adopted from Cairns *et al.*[12]. Kinetic analysis was performed with active molecules to select the DNAzyme with the highest substrate cleavage activity by incubating individual DNAzymes with GATA-3 mRNA for different times and subsequent analysis of the cleavage products. DNAzyme gd21 was identified to possess the highest activity, as it was able to cleave approximately 50% of the substrate within 5 minutes and about 90% within one hour. The gd21 cleavage reaction showed a dose-dependent pattern. Therefore, gd21 was used throughout all subsequent experiments.

Intranasal administration of gd21 in a model of acute experimental asthma effectively prevented airway inflammation and mucus production together with development of airway hyperresponsiveness (AHR) to methacholine. In a model of secondary allergic response, gd21 markedly reduced airway inflammation and normalized AHR. The strong anti-inflammatory and airway function normalizing effect was also present in a model of chronic allergic asthma. In its anti-inflammatory and anti-asthmatic capacity gd21 was an even more effective approach than other antisense strategies, such as antisense DNA (asDNA) and small interfering RNA (siRNA). In contrast to the other antisense strategies gd21 showed no off-target effects, neither *in vitro* nor *in vivo*. Furthermore, *in vitro* experiments indicate that pulmonary surfactant has properties of a natural transfacting reagent and may support cellular uptake of gd21 in the airways. These results suggest that local application of the GATA-3 specific DNAzyme is a promising novel approach for the treatment of allergic bronchial asthma.

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Targeted Topical Drug Delivery

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Understanding chemical structure - vehicle formulation - skin transport relationships for the absorption of drugs and toxins through the skin is important in the further development of dermatological products and transdermal delivery systems, and in risk prevention strategies for toxicological dermal exposure. Our interest has been in developing these relationships through a mechanistic understanding of how chemicals and nanoparticles interact with and pass through the skin and how this transport is modified by the formulation/device in which the chemical is applied.

In our view, these transport relationships are best defined in terms of a fractional maximum flux, derived by expressing transport is expressed in terms of a solute's maximal chemical potential. We have found that solute size and melting point is the major determinant of such transport and that transport is also affected by binding of solutes within the various layers of the skin. The stratum corneum acts as a reservoir for many solutes and is characterised by both 'fast-release' and 'slow-release' binding sites. Rehydration of the skin after a previous exposure can cause the solute to be released leading to a pharmacological effect long after application. Transport in the skin is also affected by epidermal metabolism, epidermal transporters, dermal blood flow and lymphatic clearance. Evaluation of the kinetics of transport in the skin after topical exposure to thin, finite doses of products is more complicated than the traditional infinite dose forms used to enable an easy kinetic analysis. However, the latter formulations can lead to misleading conclusions in relation to, for instance, the role of viscosity in determining skin transport and should be used with caution. The transport of nanoparticles into the skin is currently controversial with published available which both supports and rejects transport into the viable epidermis. Uptake of nanoparticles into the appendages has been shown but the depth of penetration within appendages is dependent on the nature of the nanoparticle and the state of the appendages.

Formulations and choice of chemical structures are not always the best methods to overcome the immense stratum corneum barrier to skin transport of solutes. Microneedles, iontophoresis, sono-phoresis and micro-abrasion are alternative strategies we have found to be effective in facilitating targeted solute delivery through the skin. Such technologies yield different chemical structure - vehicle formulation - skin transport relationships than what has been reported with traditional topical passive delivery. Further, such approaches may be used to not only deliver solutes into the skin but also to monitor solute concentrations in the body *via* the skin. Whilst major advances have been made in understanding the transport of solutes and naonparticles *via* the skin, the dynamics of the interaction of solutes with epidermal and dermal structures *in vivo* is less well developed. Multiphoton microscopy and related imaging techniques are now emerging as new tools addressing this area.

PI3Kγ and PI3Kδ Partners in Crime in Inflammation? Rheumatoid Arthritis and Beyond

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Dysregulated signal transduction in lymphocyte dependent and independent immunity is known to be associated with the development of various autoimmune and inflammatory diseases. Consequently, targeting intracellular signalling of the pro-inflammatory cytokine network heralds hope for the next generation of anti-inflammatory drugs. Phosphoinositide 3-kinases (PI3Ks) generate lipid-based second messengers that control an array of intracellular signalling pathways that are known to have important roles in leukocytes.[1] According to recent progress in the development of selective PI3K inhibitors[2], and the beneficial effects of these inhibitors in models of acute and chronic inflammatory disorders, we discuss the therapeutic potential of blocking PI3K isoforms for the treatment of rheumatoid arthritis and other immune-mediated diseases. For both of these isoforms we have developed, with the help of X-ray crystallography, potent and selective inhibitors. Our development candidates are orally available and show beneficial effect in several murine models of inflammatory diseases such as RA (e.g. collagen induced arthritis, CIA)[3, 4].

Targeting the Holliday Junction: A New Target for Cancer Research?

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Nucleic acids are a major target for antitumour agents, with platinating agents and alkylating agents such as cisplatin and melphalan, topoisomerase inhibitors such as amsacrine and doxorubicin and DNA cleaving agents such as bleomycin being amongst the most utilised clinical agents. However, the advent of molecular therapeutics means that such cytotoxic agents are no longer sought, with more specific agents with lower side effects, exemplified by imatinib mesylate, being the major goal of cancer research around the world. Although this has been taken to mean that targeting nucleic acids is no longer feasible, this mistaken idea is being challenged by various approaches. Targeting nucleic acids *via* siRNA or gene-selective small molecules still holds promise, with the latter compounds also under development as artificial transcription factors. More promising is the targeting of higher order DNA structures, particularly quadruplex structures that may form within duplex DNA strands or on the telomeric ends of chromosomes. This has already seen the movement of one compound into preclinical development and prompted us to investigate alternative higher order DNA structures that may hold therapeutic promise.

The Holliday junction is a four-strand DNA junction involved in homologous recombination. It has been studied extensively in combination with processing enzymes but a native structure only became available in 1999. In 2007, we published the structure of the first small molecule to bind non-covalently to the Holliday junction, which revealed a novel mode of binding, involving adenine-displacement and formation of a pseudo-base pair with thymine, with the ligand binding across the junction. At the same time, we also disclosed a second relatively novel mode of DNA binding involving duplex crosslinking. In this presentation, we will describe the further developments in medicinal chemistry and Holliday junction binding and our first steps towards investigation of the biological activity of these compounds.

Discovery of MK-0354: A Niacin Receptor Partial Agonist with Acute Antilipolytic Activity but Little Flushing Effect in Preclinical Species and in Humans

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Niacin (nicotinic acid) is a water-soluble vitamin that at high doses in humans favourably modulates essentially all serum lipid and lipoprotein parameters. As a result, niacin has been used for the prevention and treatment of cardiovascular disease for many years. The recent resurgence of interest in this area has focused on niacin's ability to increase high density lipoprotein-cholesterol (HDL-C) to a greater extent than other currently available drugs. The use of niacin as a therapeutic however, is limited by a number of associated side-effects, most notably a highly uncomfortable cutaneous flushing response which can limit patient compliance. The development of novel agonists of the niacin receptor that have the beneficial lipid effects but with fewer side-effects would clearly be of value.

Recent mechanistic investigations have shown that niacin may exert its free fatty acid (FFA) lowering effect through activation of a G-protein coupled receptor (GPCR) localized on adipocytes. Subsequently, two class 1 (rhodopsin-like) G_i-coupled orphan GPCRs (GPR109a and GPR109b) that share 95% identity and which are both expressed in human adipocytes and macrophages were identified as possible molecular targets for niacin. The activation of one or both of these receptors results in a decrease in intracellular cAMP. This in turn leads to inhibition of lipolysis, *via* negative modulation of intracellular lipase activity, thereby decreasing plasma free fatty acid levels. It has been postulated that this pathway may also ultimately be responsible for the changes in lipid profiles. Nonetheless, although several activators of GPR109a can raise HDL-C in humans, more compelling evidence is still lacking.

In this presentation, our discovery of the receptors GPR109a and GPR109b will be briefly outlined. The identification of agonist ligands for GPR109a *via* both high throughput screening and classical SAR approaches across multiple series will be described. The *in vitro* and *in vivo* assay systems that enabled us to identify compounds with interesting properties for further investigation will be highlighted, leading to the discovery of MK-0354, which behaves as a partial agonist of the niacin receptor in recombinant cell-based assays. *In vivo*, MK-0354 inhibits lipolysis with comparable efficacy to niacin in acute models and shows a markedly improved therapeutic window between plasma FFA reduction and cutaneous flushing in multiple species. In Phase I studies in healthy volunteers, MK-0354 was well tolerated, induced a robust reduction of plasma FFA comparable to an extended-release niacin formulation, and showed only a modest flushing effect at high doses.

How to Learn Lessons from Failures: Discovery and Development of Novel Neuronal L-type Calcium Channel Blockers

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Modern target-based drug discovery involves (1) target identification, (2) high throughput screening of random, focused or drug libraries, (3) 'hit-to-lead' optimization, and (4) *in vivo* efficacy and toxicology studies. Alternatively, the 'old-fashioned' efficacy-based drug discovery approach implemented (1) design and synthesis of the pharmacologically relevant compounds, and (2) *in vivo* studies for anticipated efficacy and safety.

We have developed a strategy which merges 'modern' target- and 'old-fashioned' efficacy-based approaches on the early stages of drug discovery. Our strategy involves (1) design and synthesis of novel analogs of the compounds with known pharmacological effects, (2) *in vivo* 'proof-of-principle' for efficacy and safety in well-established animal models, (3) *in vitro* screening and detection of the *in vitro* site of action for the compounds with an appropriate *in vivo* profile, (4) optimization of the series for enhanced binding at the identified *in vitro* target, (5) building up an expanded therapeutic potential for the series.

The strategy is exemplified by discovery of the MPP-021 Series, novel and selective neuronal L-type calcium channel blockers, and their development for the treatment of traumatic shock and pain and as cognitive enhancers and neuroprotectants.

Predicting Enzyme Induction and Drug-Drug Interactions

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Induction of drug metabolizing enzymes, such as the cytochromes P450 (CYP) is known to cause drug-drug interactions due to increased elimination of co-administered drugs. This increased elimination of co-administered drugs may lead to significant reduction or complete loss of efficacy. For example, coadministration of the herbal medicine St. John's Wort (antidepressant and CYP3A4 inducer) with indinavir (HIV protease inhibitor and CYP3A4 substrate) results in a loss of antiviral activity (57% decrease of indinavir AUC). Due to the significance of such drug interactions, many pharmaceutical companies employ screening and characterization models which predict CYP enzyme induction to avoid or attenuate the potential for drug interactions with new drug products.

The most common mechanism of CYP induction is transcriptional gene activation. The activation is mediated by nuclear receptors, such as AhR (aromatic hydrocarbon receptor), CAR (constitutive androstane receptor), and PXR (pregnane X receptor or SXR-steroid X receptor), that function as transcription factors. Early high throughput drug candidate screening models utilize these nuclear hormone receptors in ligand binding or cell-based transactivation/reporter assays. In addition, immortalized hepatocyte cell lines can be used to assess enzyme induction of various drug metabolizing enzymes. Cultured primary human hepatocytes, the best established *in vitro* assay for predicting enzyme induction and most accepted by regulatory agencies, is the predominant assay to evaluate a wide variety of drug metabolizing enzymes. Unfortunately, *in vivo* animal models such as rodents and dogs do not respond to enzyme inducers in the same manner as humans and therefore are inappropriate to predict human enzyme induction or drug interactions. However, transgenic animal models and the cynomolgus monkey have been shown to mimic human enzyme induction and may be appropriate *in vivo* animal models for predicting human drug interactions.

The presentation will describe each of the predictive *in vitro* and *in vivo* models, as well as interpretation of results and correlations to actual drug interactions in patients.

High Throughput Functional Genomics Strategy to Discover New Therapeutic Targets for Complex Diseases

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Complex diseases, such as coronary artery diseases (CAD) and obesity, have multifactorial and polygenic etiology. Thus, high throughput functional genomics strategy is needed to discover new therapeutic targets for their treatment. In order to characterize specific high fat satiety signals, we have used the serial analysis of gene expression (SAGE) method to identify the genes specifically regulated by high fat (HF) compared to low fat (LF) meal. The SAGE strategy consists to isolate short expressed sequence tags specific for each transcript and to ligated them in order to form long concatemers that can be sequenced to measure the expression level of each transcript, including novel genes. We have identified several new candidates which are secreted by intestine specifically after HF meal. We have cloned their full length cDNA and expressed their proteins in *E. coli* in order to inject them in mice and to test their effects on CAD risk factors. Thus, this study has identified new therapeutic targets for the prevention and treatment of obesity and related diseases.

Perspectives of Novel ATP- and Non-ATP-Competitive Kinase Inhibitors: Targeting Polo-like Kinase 1 for Anticancer Therapy

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Anti-mitotic/tubulin-targeted agents, such as taxanes and the vica alkaloids, have proven to be effective treatment options for many cancer types. The clinical success of these agents suggests that other agents targeting mitotic signaling pathways may likewise increase patient survival and lead to cures. Polo-like kinase 1 (Plk1), a human serine/threonine kinase, which was originally identified and cloned by our group, has been established as one of the most promising drug targets for anticancer therapy. Plk1 is a key regulator responsible for many aspects of mitosis. Our previous data demonstrate that Plk1 is overexpressed in many types of human cancers and serves as poor prognostic marker for tumor patients. Moreover, the aberrant expression of Plk1 promotes aggressive proliferation of tumor cells by inhibiting tumor suppressors and overriding cellular checkpoints, indicating its tight involvement in carcinogenesis and its potential as a therapeutic target. Indeed, many approaches have been pursued to downregulate Plk1. More recently, several ATP-competitive inhibitors, targeting the kinase domain of Plk1, have been investigated and are currently in clinical trials. Yet, like other ATP-competitive inhibitors, most of them suppress several member of the super family of protein kinases.

In order to obtain specific inhibitors of Plk1, we have focused on the unique polo-box binding domain (PBD) of Plk1. Based on a high-throughput fluorescence polarization assay we have used the PBD of Plk1 for the specific screening of small molecule-inhibitors. The identified cell-permeable inhibitors selected from the diverse chemical libraries block efficiently the function of the polo-box domain of Plk1. The treatment with these compounds induces mitotic arrest and activates apoptotic pathways, leading to a strong inhibition of the proliferation in various human tumor cells. Furthermore, treated tumor cells exhibit phenotypes characteristic for the downregulation/depletion of Plk1, implying that the inhibitors are specifically aimed at Plk1.

Taken together, our data demonstrate that targeting specific members of the class of mitotic kinases is a promising strategy to fight cancer. The novel potent inhibitors of Plk1 represent a new aspect for anticancer intervention. Perspectives of ATP- and non-ATP-competitive inhibitors will be discussed.

An Efficient, General Synthesis of ^{11}C- and ^{18}F-incorporated PET Tracers Toward Revolutionizing Drug Development and Diagnosis

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Positron emission tomography (PET) is a particularly powerful noninvasive method for the investigation of *in vivo* biochemistry, especially in the human organ. The need for the development of new PET tracers has grown with the increase in its use for diagnosis in medicine and for drug development processes in an early stage. In this context, we have developed 15*R*-TIC, a stable prostaglandin analogue, which selectively binds with a prostacyclin receptor (IP_2) in the central nervous system. The tolyl group in 15*R*-TIC was intended as a trigger component to introduce a ^{11}C radio-nuclide for PET molecular imaging. The introduction of such a short-lived ^{11}C nuclide ($t_{1/2}$ = 20 min) into its organic framework required the development of rapid *C*-methylation. This problem was solved by a rapid coupling (65 °C, 5 min) of [^{11}C]methyl iodide and an arytributylstannane (excess) in the presence of a tri-*o*-tolylphosphine-bound coordinatively unsaturated Pd(0) complex, a Cu(I) salt, and K_2CO_3 in DMF. This one-pot protocol was slightly improved to be a two-pot procedure, giving 15*R*-[^{11}C]TIC methyl ester (2.5 GBq) with high reproducibility.[1] PET studies of monkey and human were successfully executed by intravenous injection to visualize IP_2 distributed in the brains. By comparing with PD/PK studies of 15*R*-TIC [^{11}C]methyl ester, which was labeled at the ester moiety, we found that 15*R*-[^{11}C]TIC methyl ester penetrates the blood-brain-barrier and then undergoes enzymatic hydrolysis of the ester in the brain to produce 15*R*-[^{11}C]TIC, which binds to IP_2. The MCAO PET studies using a monkey model demonstrated the efficacy of 15*R*-TIC methyl ester (iv injection) for such an ischemia model as judged by the $^{15}O_2$ consumption and the uptake of [^{18}F]FDG.[2]

We have been expanding the rapid methylation to alkyne, alkene,[3] and alkane in order to establish four kinds of methylations. Such methylations would realize ^{11}C-labeling of almost any organic compounds, allowing the PD/PK studies in both animals and human to promote evidence-based medicine. In addition, we elaborated the rapid *C*-methylations and *C*-fluoromethylation by methyl iodide and fluoromethyl iodide, respectively, in combination with an organoborane.[4] Thus an adequate PET tracer library would be constructed by complemental use of stannanes and boranes. The novel *C*-[^{11}C]methylations have peculiar benefits: (1) The metabolic stability of a C-$\underline{^{11}CH_3}$ group is much higher than N-$\underline{^{11}CH_3}$, O-$\underline{^{11}CH_3}$, and S-$\underline{^{11}CH_3}$ routinely used so far, and therefore, the PET image obtained by such a metabolically stable tracer is highly credible; (2) The methyl group is a minimum-sized carbon substituent and non-polar to give only a little change for the original function of a compound. Accordingly, the molecular designing of a PET tracer could be executed within a predictable realm. Moreover, the methods would be applied to other carbon isotope units such as $CH_2{}^{18}F$, $^{13}CH_3$, $^{14}CH_3$, CD_3, and $CH_2{}^{19}F$, allowing the studies of AMS (Accelerator Mass Spectrometry) and MRI. Particularly, PET and AMS using ^{11}C and ^{14}C, respectively, are two wheels to promote a human microdose study.

As such, we have been synthesizing novel PET tracers based on the combination of the design of disease-associated specific molecular probes with the ^{11}C and ^{18}F-labeling by the rapid carbon-carbon bond forming reactions to introduce a microdose concept at an early stage of the drug development to explore a drug candidate tolerant of current clinical studies. The construction of a library of novel PET tracers is also important subject to prepare disease-associated markers for diagnosis.

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Prevention of Cardiovascular Diseases by Regulation of Endothelial cell-Neutrophil-Cytokine Interaction

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The reactivity of endothelial cells (ECs) to proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) is critically important for the pathogenesis of cardiovascular diseases. Cytokine-stimulated ECs exhibited increased synthesis of platelet-activating factor (PAF), increased production of granulocyte-macrophage colony-stimulating factor (GM-CSF) and reactive oxygen species, and increased expression of PAF receptor and ICAM-1. The adhesive interaction between cytokine-activated ECs and human neutrophils resulted in massive release of superoxide from neutrophils, which was mediated by the collaboration of GM-CSF and PAF synthesized from ECs. The activity of cytokine-stimulated ECs to stimulate superoxide release in neutrophils and to produce PAF declined markedly in parallel as ECs matured during cultivation under a confluent condition, suggesting that EC functions are altered during cell maturation and the reduced neutrophil-activating activity may be, in large part, ascribed to down-regulation of PAF synthesis. Mitogen-activated protein kinases (extracellular signal-regulated kinase [ERK] and p38) and phosphatidylinositol 3-kinasse (PI3K) were found to play a critical role in cytokine-mediated activation of neutrophil functions such as superoxide release, migration and adherence. Some calcium channel blockers (CCBs; amlodipine, nicardipine, azelnidipine, etc.) suppressed cytokine-induced functional activation in ECs, neutrophils and monocytes. Activation of the ERK and PI3K/Akt pathways induced by TNF-α, but not by GM-CSF, was selectively affected by some CCBs. Suppression of inflammatory reaction by some CCBs and angiotensin II type I receptor blockers (ARBs) may provide a favorable effect on prevention of vascular diseases in hypertensive patients treated with these specific blockers. We suggest that appropriate regulation of EC-neutrophil-cytokine interaction by such a drug as certain CCBs and ARBs may be a strategy and potent inhibitors of cytokine activation for prevention of cardiovascular diseases.

Discovery and Development of Orally Bioavailable 5-HT6 Receptor Antagonists for Cognition Enhancement in Treating Alzheimer's Disease. Fingerprinting Approach to Clinical Candidates. Parallel Optimization in "Constellation" of CNS Targets

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5-HT6 receptors are expressed in brain regions associated with learning and memory. Specific blockade of their function increases central cholinergic and glutamatergic neurotransmission and enhances cognitive processes.

We report the development of a novel, bioavailable and safe small molecules with favorable selectivity profile over competitive development candidates - modulators of 5-HT6 receptors.

Several development stage molecules such as sulfonyl-containing 5-HT6 ligands as well as atypical antipsychotic drugs with cognition modulating properties with variable selectivity profile on 5HT receptors were selected as a starting point and reference compounds in the discovery process.

They also provided grounds for "fingerprinting" of a desirable molecular, animal pharmacological profile and design of development studies.

Cognition disorders, arguably due to their inherent intricacy, have not benefited from single structure-based design. Reflecting on that character we have selected a "constellation" of key biotargets for specific modulation including 5-HT6, 5-HT2C, AChE, calcium L-channel, NMDA, SERT and few other GPCR and ion channel targets as desirable ones for developing antagonist towards. While hERG, 5-HT2B and a few other "black holes" were identified as undesirable considering their safety profile. Subsequently we attempted exploratory research including rational design, synthesis, and SAR studies with tri- and bicyclic series and substituents patterns modulating potency/selectivity as well as PK and safety trends within the "fingerprint".

We will demonstrate a successful parallel optimization and selection of a lead candidate and backup compounds with desired molecular pharmacology fingerprint as well as significant improvement of efficacy, PK/PD and toxicity profiles over competitive compounds. Administration of these molecules significantly reversed chemically-induced cognition deficit in multiple species behavioral models.

This study provides further support for the potential therapeutic utility of 5-HT6 receptor antagonists with improved profile over disorders characterized by cognitive deficits such as Alzheimer's and schizophrenia.

Vaccine Delivery: Synthesis and Investigation of a Highly Pure, Multi-Epitopic Lipopeptide Vaccine Candidate

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The lipid core peptide (LCP)-system, a peptide vaccine delivery system incorporating a lipidic adjuvant, carrier, and peptide epitopes into a single molecular entity, has been demonstrated to adjuvant peptide epitopes without the need for additional adjuvants. We have also developed a method for the synthesis and immunological evaluation of highly pure, LCP-system analogues. The developed system was used to synthesize a multi-epitopic, prophylactic lipopeptide group A streptococcus (GAS) vaccine using native chemical ligation, with immunological evaluation performed in B10.BR mice.

Peptide building blocks, incorporating antigens from GAS strains common to the Aboriginal populations of northern Australia, were synthesized using stepwise solid-phase peptide synthesis. 2-Amino-D,L-dodecanoic acids were incorporated into the system to act as an inbuilt adjuvant. Thioester peptides were synthesized using a trityl-associated mercaptopropionic acid leucine linker. The multi-epitopic lipopeptide GAS vaccines were synthesized in high purity and good overall yield. The synthesized vaccines were assessed in B10.BR mice. Subcutaneous administration was performed at the tailbase (30mcg day 0, and 3mcg boosts on days 21, 28, 35, 42, and 49) in phosphate buffered saline. Systemic antigen-specific IgG antibodies were elicited in response each of the incorporated peptide antigens without the need for additional adjuvants.[1, 2]

We have also developed synthetic self-adjuvanting GAS vaccine candidates composed of a universal helper T-cell epitope, several GAS B-cell epitopes, and an immunostimulatory lipid moiety designed to mimic dipalmitoyl-*S*-glyceryl-cysteine (Pam_2Cys). Systemic antigen-specific serum IgG antibodies were detected following subcutaneous immunization of BALB/c ($H\text{-}2^d$) mice with each construct without the need for any additional adjuvants. We have investigated the structure activity relationships of different constructs by varying the points of attachment of the peptide antigens and the lipid adjuvant. Changes to the vaccine construct structure were found to greatly effect the level of systemic antigen-specific IgG antibodies elicited.

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Differential Production of Connective Tissue-Type and Mucosal Mast Cells from Human Embryonic Stem Cells for Anti-Allergy Drug Screening

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Mast cells (MC) function as effector cells in allergy and atopic disease. Therefore, anti-allergy drugs have been established to diminish the mast cell function. However, since the acquisition of an abundance of human MC (hMC) is difficult because of no culture method producing massive hMC, most anti-allergy drugs targeted animal MC. Thus, efficient discovery of effective anti-allergy drugs needs to establish the culture system of massive hMC.

In human, two types of MC have been characterized; connective tissue-type and mucosal MC (CTMC and MMC). CTMC contain tryptase, chymase, MC carboxypeptidase and cathepsin G in their secretory granules, are predominantly located in normal skin and in intestinal submucosa, and involve in atopic dermatitis. MMC contain tryptase in their secretory granules, but lack the other proteases, are the main type of MC in normal alveolar wall and in small intestinal mucosa, and involve in allergic rhinitis or bronchial asthma. Although MC can be generated from human adult CD34+ hematopoietic progenitor cells *in vitro*, these MC are mainly MMC. So far, there lacks an evidence for the direct derivation of CTMC from adult hematopoietic progenitors.

Embryonic stem cells (ESC) derived from the inner cell mass of preimplantation embryos can be maintained as undifferentiated cells in culture without apparent limits. They also endow a capacity to differentiate into all cell types of tissue including blood progenies. Then, human ESC (hESC) are considered as a potential cell source for hMC.

We recently achieved successful production of hESC-derived CD34+ hematopoietic progenitors, using co-culture with mouse fetal liver stromal cells for 1-2 weeks. In suspension culture favoring MC differentiation (15% FBS, SCF+IL-6+Flt3-ligand) within 3weeks, hESC-derived progenitors generated mature MC that shared a chymase / tryptase double positive phenotype and strongly expressed c-Kit, similar to human skin derived CTMC. On the other hand, hESC-derived multipotential hematopoietic progenitors obtained in clonal culture developed into MC for a longer time (over 5 weeks) and only expressed tryptase, with no or few chymase, similar to human CD34+ cell-derived MMC.

Since the current culture system of hESC can produce differentially a large number of CTMC and MMC, our study may highlight a new understanding for MC development and finally benefit clinical trials for these MC-related disorders.

Synthesis of Fosmidomycin Analogues: Towards Potent Antimalarials

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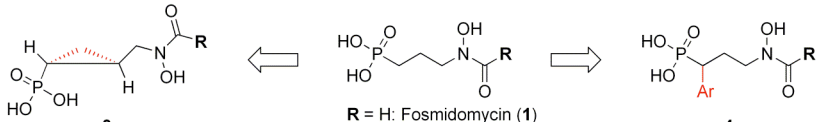
In combination with clindamycin, fosmidomycin (**1**) was recently shown to be efficacious in the treatment of uncomplicated *P. falciparum* malaria in a small cohort of paediatric patients. The mechanism of action of this structurally simple antibiotic, originally isolated from *Streptomyces lavendulae*, involves the blockade of the non-mevalonate pathway for the biosynthesis of isoprenoids, which is absent in humans. Fosmidomycin was found to be a potent inhibitor of 1-deoxy-D-xylulose 5-phosphate reductoisomerase (Dxr) of *P. falciparum*. FR900098 (**2**), the acetyl congener of fosmidomycin, was shown to be approximately twice as active against *P. falciparum in vitro*, as well as in a *P. vinckei* mouse model.

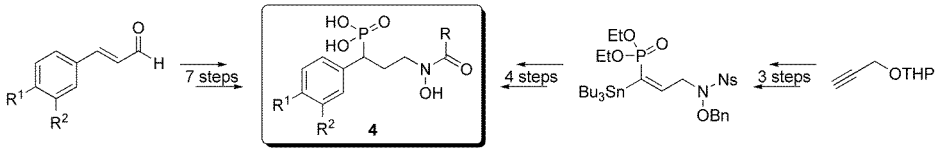
In order to study the structure-activity relationships, modifications of the retrohydroxamate and the phosphonate moieties have been reported but largely failed to improve fosmidomycin's antimalarial properties. Remarkably, modifications addressing the three carbon spacer that connects both functionalities are relatively scarce.

Here we present a series of fosmidomycin/FR900098 analogues characterized by structural variations of this three-carbon spacer.



First we synthesized a series of cyclopropyl analogues (**3**) of fosmidomycin featuring restricted conformational mobility. From this series the (1*R*,2*S*)-isomer with an *N*-hydroxy-*N*-acetamide group emerged as the best Dxr inhibitor and proved as potent as fosmidomycin in inhibiting *P. falciparum* growth. This result demonstrates Dxr's preference to accommodate analogues with a *trans*-orientation of both substituents.

Next we focussed on the synthesis of α-aryl substituted analogues (**4**). First we followed a *de novo* synthetic approach starting from substituted cinnamaldehyde. Although this route proceeded in high overall yield and was amenable to scale-up, it only permitted to synthesize a limited number of examples. Therefore, a more divergent synthetic method was developed featuring a palladium-catalyzed Stille coupling to introduce the aryl group at a later stage in the synthesis.



Compared with fosmidomycin, several analogues displayed enhanced activity towards different *P. falciparum* strains. An analogue with a 3,4-dichlorophenyl substitution in the α-position of fosmidomycin emerged as the most potent analogue of this series. It is approximately twelve times more potent in inhibiting the growth of *P. falciparum* than the lead fosmidomycin.

Assessing the Infection Risks of New Anti-Inflammatory Therapies

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Tuberculosis is a rare but serious complication of anti-TNF therapy. Despite sharing a common therapeutic target, the TNF antibody infliximab poses as much as a 10 fold greater monthly risk of reactivation of latent TB infection compared to the soluble TNF receptor etanercept. We examined the cellular and molecular basis of this difference using a whole blood culture model. Infliximab inhibited TB-induced production of IFNγ (required for protection) and increased production of TGFß (associated with TB risk). In contrast, etanercept inhibited TGFß production and did not affect IFNγ, even when tested at a supra-therapeutic concentration. These cytokine effects were accompanied by differential effects on the ability to control intracellular TB growth. To better understand this phenomenon, effects on global gene expression were examined by Affymetrix microarray. 381 TB-induced genes were identified. Of these, 113 were deactivated by infliximab, 109 by adalimumab, but only 11 by etanercept ($P < .001$). The 11 etanercept-inhibited genes were entirely contained within the set of 79 genes also inhibited by infliximab and adalimumab. Examination of host-bacterial interactions in whole blood culture may prove to be a valuable tool in assessing the infection risks posed by new anti-inflammatory therapies.

Developing Novel Anti-inflammatory Therapy for Asthma/COPD

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This study is aimed at developing a new class of anti-inflammatory drug that blocks intrapulmonary activation of complement cascade for the treatment of asthma and COPD. Activated complement components are potent pro-inflammatory mediators. Significant elevated level of intrapulmonary C5 activity correlated with the development of airway inflammation and airway hyper-responsiveness (AHR) in both human patients and rodent models. We had demonstrated that anti-C5 monoclonal antibody (mAb) can be successfully delivered by nebulization with common Jet-Air nebulizers. The Intra-pulmonary delivery of anti-C5 mAb inhibits complement activity in airways without significant systemic effect. This treatment ameliorates established severe airway inflammation and prevents the development of AHR in rodent models. We had also demonstrated that intra-pulmonary C5 inhibition utilize unique sets of mechanisms of action, significant different from corticosteroid therapy or other existing therapies. We will discuss our approaches of moving forward this very important drug candidate.

The Long March Towards Effective and Selective Drug Treatment of Cancer

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The anti-cancer section of the ICDDD will be devoted to a wide-ranging look at various modalities and approaches to cancer treatment and drug discovery. This historical introduction will highlight aspects of progress in the search for selective anti-cancer therapies, pointing up just a few areas of special interest to serve as background for the invited and session lectures as well as topics addressed in diverse posters contributed by scientists from around the world. The lecture will recount, among other things, the strange circumstances that led to the birth of cancer chemotherapy towards the end of the second World War. Then there will be a brief look at significant lessons that have been learned from experience with established agents like the DNA-intercalating agents and antibiotics. Finally the promise of new agents acting upon hypoxic cancer cells commonly occurring in the midst of solid tumour masses will be described, along with encouraging signs that this approach may spearhead a new paradigm for drug design.

Challenging R&D Paradigms by Use of Microdose and Microtracer Studies During Non-Clinical Development and Beyond

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Industry's bid to search for novel drug substances stable to CYP-dependent clearance, with significant gains in safety, efficacy or both, now challenges some of the well-established *in vitro* metabolic screening and discovery tools used to support extrapolation to the *in vivo* situation. Furthermore, predicting human drug exposure from animal studies is often hampered by marked species-differences in drug disposition. The net effect of removing or substantially reducing the role of CYP-dependent clearance is an apparent increase in the role of more esoteric non-CYP-dependent and non-enzymatic routes of clearance for which *in vitro-in vivo* extrapolation (IVIVE) techniques are yet to be fully developed. The uncertainty of reliably predicting human drug exposure from IVIVE, the role of non-CYP-dependent drug-metabolising enzymes and the species differences in ADME all highlight an increasingly important requirement to find alternative 'tools' to better select new drug candidates for development.

Human microdosing studies provide an important adjunct to IVIVE and offer an opportunity to change the paradigm of drug research and development to improve our ability to select drug candidates with appropriate ADME profiles prior to full development. Similarly, microtracer studies offer opportunities to acquire pieces of important ADME information much earlier in development with paralleled opportunities to use reduced or better designed studies to answer key questions arising during early clinical development.

This paper will briefly outline Servier's strategy toward utilising microdosing and microtracer studies to help support decisions taken during pre-development and development programmes.

Intracellular Calcium Release Channels - Promising Targets for the Treatment of Heart Failure and Arrhythmias

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Heart failure (HF) is one of the most common causes of mortality in the world. Contractile failure and cardiac arrhythmias are main determinants of sudden death in patients with HF. Alterations in intracellular calcium handling play a prominent role in the pathogenesis of abnormal contractility and arrhythmias in HF. Recent findings indicate that the HF is associated with defective regulation of intracellular calcium release channels (ryanodine receptors, RyR2), associated with incomplete closure of these channels during diastole. Leakage of calcium through RyR2 channels leads to lower calcium levels in the sarcoplasmic reticulum calcium stores, resulting in depressed contractility. Moreover, abnormal release of calcium may trigger lethal cardiac arrhythmias.

We have discovered that a novel class of drugs such as the 1,4-benzothiazepine JTV519 can prevent diastolic leakage of RyR2 calcium release channels. JTV519 normalizes the RyR2 subunit composition by enhancing the binding affinity of the channel-inhibitory protein calstabin2. In animal models of HF and cardiac arrhythmias, JTV519 improves cardiac contractility and prevents lethal cardiac arrhythmias. At present, JTV519 derivatives are undergoing preclinical testing and are expected to advance into phase I/II clinical trials within the next year. Thus, normalizing intracellular calcium release channels constitutes a promising new therapeutic modality for heart failure.

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Serotonin/Noradrenaline Reuptake Inhibitors: A Case History in the Challenges of CNS Drug Discovery

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Stress urinary incontinence (SUI) is characterized by the involuntary loss of urine due to a sudden increase in intra-abdominal pressure (eg from coughing, sneezing or exercise). Inhibition of serotonin and noradrenaline reuptake (SNRI) has been shown to be an attractive dual pharmacology mechanism for the treatment of SUI.

This talk will highlight the SNRI medicinal chemistry program at Pfizer, and exemplifies the challenges involved in tackling drug targets which require BBB penetration for efficacy. The interplay of potency, selectivity, ion channel activity, BBB penetration and pharmacokinetics will be discussed.

Optimization of these properties led to the identification of the SNRI clinical development candidate PF-184298. The detailed *in vitro*, *in vivo* and human pharmacokinetic profile of PF-184298 will be presented.

Triazine Derivatives as Protein A Mimetics for the Treatment of Autoimmune Disease

Boulos Zacharie

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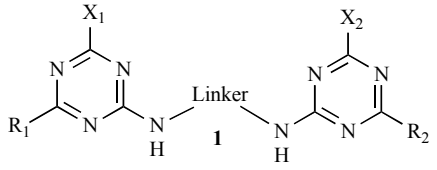
Introduction: Many autoimmune disorders (rheumatoid arthritis, systemic lupus erythematosus, idiopathic thrombocytopenia purpura (ITP), glomerulonephritis or vasculitis) are related to the presence of pathogenic antibodies and/or immune complexes in the system. Cypress Bioscience developed a protein A column to remove these autoantibodies and immune complexes during an apheresis procedure. The Prosorba® column was approved by the US FDA in 1987 for ITP and in 1999 for rheumatoid arthritis. The immune system does not suppress and is a therapeutic option for treatment-refractory patients.[1]

Overview: Protein A (MW = 42,000) is found on the surface of the bacteria *Staphylococcus aureus*. This protein binds with high affinity to the tail (Fc) portion of human and mouse antibodies. Protein A has potential therapeutic utility, but its toxicity and cost limit its therapeutic use. There is a definite need for a non-toxic small molecule mimetic of protein A which can be administrated as a drug.

I will present the structure-activity relationship (SAR) of a series of triazine dimers of general structure 1 that were developed as protein A mimetics. The triazine framework has been shown to provide a very effective scaffold for the construction of protein binding ligands.[2] Three thousands compounds were synthesized and selected examples will be discussed. Some of these compounds were equipotent to protein A in a competitive IgG binding ELISA assay. These compounds also demonstrate interesting *in vivo* activity in inflammation disease models. We have now developed more soluble analogues that show good oral activity.



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Conclusion: A first in class series of low molecular weight synthetic molecules is described that mimic the ability of protein A to bind to human IgG antibody. The SAR studies demonstrate the importance of the presence of at least one 1,3-phenylenediamine substituent. The hydrophobicity of these 1,3,5-triazine dimers is important for binding to the IgG tail portion. These compounds show potent *in vivo* activity in standard inflammation models by intravenous, oral or topical routes. A second generation series of compounds has also been developed and will be presented in due course. These compounds offer a unique approach for the treatment of autoimmune diseases by virtue of their novel biochemical target.

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Structure-based Investigation of Wnt Signaling Inhibitors

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Through structure-based virtual ligand screening and NMR spectroscopy, several drug-like compounds have been identified and those compounds have been shown to be able to disrupt the protein-protein interaction events at different points of the Wnt signaling pathway. Wnt signaling plays a critical role in embryonic development and in the regulation of cell growth. Inappropriate activation of Wnt signaling has been implicated in cancers and other human diseases. Therefore, the inhibitors we obtained will be helpful in formulating rational approaches to the development of novel pharmaceutical agents that can interfere with specific Wnt signal events that contribute to human diseases. In the presentation, we will focus on two proteins in the Wnt pathways.

The first target is PDZ domain of Dishevelled (Dvl). Dvl transduces Wnt signals from the receptor Frizzled (Fz) to downstream components in canonical and non-canonical Wnt signaling pathways. The Dvl PDZ domain is thought to play an essential role in both pathways, and we recently demonstrated that the Dvl PDZ domain binds directly to Fz receptors. Since we identified the first inhibitor of the Dvl PDZ domain (Biochemistry, 44:15395-15503, 2005), using NMR-assisted virtual ligand screening and design, we now have identified/created more than 60 small-molecule inhibitors of the Dvl PDZ domain and many of them have submicromolar binding affinities. Furthermore, we also showed that some of the small-molecules are able to penetrate the cell membrane and have inhibitory effects to the Wnt signaling pathways in different cell biology and animal model studies.

The second target is LRP5/6. LRP5/6 was originally found as a candidate gene of insulin dependent diabetes mellitus; together with frizzled receptor, it works as a co-receptor of Wnt molecules in the canonical Wnt signaling pathway. The function of LRP5/6 in the Wnt signaling pathway can also be regulated by DKK molecules which function as Wnt antagonists. In our studies, we identified molecules that could activate the Wnt pathway by blocking the interaction between LRP5/6 and DKK as well as compounds that could inhibit the Wnt pathway by disrupting the binding between LRP5/6 and Wnt. In addition, *in vivo* tests showed that the most potent Dkk antagonistic compounds could stimulated bone formation locally and systemically while the most potent Wnt inhibitors stopped/slowed the growth of different cancer cells.