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PLENARY LECTURES, AWARD LECTURES, PRIZE LECTURES, LECTURES

PLENARY LECTURES – **PL.01**

GENOMES, STRUCTURAL BIOLOGY AND DRUG DISCOVERY: NEW CHALLENGES FROM DIFFICULT TARGETS AND NEGLECTED DISEASES

Tom Blundell

Professor Sir Tom Blundell.FRS, FMedSci Director of Research and Professor Emeritus,Department of Biochemistry, University of Cambridge. 80 Tennis Court Road, Cambridge UK, CB2 1GA

The knowledge that is now emerging from genomics of man and pathogens and from biochemical and structural proteomics programs has the potential to accelerate drug discovery. Genome sequences, and most recently non-synonymous single nucleotide polymorphisms and somatic mutations, when taken together with structural and functional information on the gene products, can provide insights into the relationship of human genetic variation and disease. This is also helpful in identifying new targets for drug discovery; it is an exploration of biological space.

On the other hand, high-throughput biophysical and structural analyses can be used to investigate the chemical molecules that proteins might bind; this is an exploration of chemical space. I will argue that this is best achieved by structure-guided and fragment-screening techniques, which inform not only lead discovery but also optimization of candidate drug molecules.

One major challenge for drug discovery arises from the very large surfaces that are characteristic of many of the protein complexes, for example those involved in receptor recognition and signal transduction. This is especially true of complexes that are assembled from preformed globular domains, where it is particularly difficult to bind a small molecule to the large, relatively flat surfaces of such proteins involved in protein interactions. However, recent analyses of multiprotein systems involved in cell regulation and signaling have identified a large number in which one component involves a flexible or unstructured region of the polypeptide chain. An example involves the complex of the human recombinase, Rad51, and the product of the breast cancer associated gene, BRCA2, which offers an interesting site of interaction that is being used to target agents that would be helpful during chemo- or radio-therapy. We suggest that proteins forming interactions with a ligand that comprises a continuous region of flexible peptide may be more tractable targets than where complexes are formed from preformed globular protein structures.

I will describe such developments in academia for diseases of poverty, including recent progress in targeting Mtb targets.

PLENARY LECTURES – **PL.02**

A CHEMICAL APPROACH TO CONTROLLING CELL FATE

Sheng Ding

The Scripps Research Institute

Recent advances in stem cell biology may make possible new approaches for the treatment of a number of diseases. A better understanding of molecular mechanisms that control stem cell fate as well as an improved ability to manipulate them are required. Toward these goals, we have developed and implemented high throughput cell-based phenotypic screens of arrayed chemical and gene libraries to identify and further characterize small molecules and genes that can control stem cell fate in various systems. This talk will provide latest examples of discovery efforts in my lab that have advanced our ability and understanding toward controlling stem cell fate, including self-renewal, survival, differentiation and reprogramming of pluripotent stem cells.

AWARD LECTURES – **AL.01**
NAUTA AWARD FOR PHARMACOCHEMISTRY

ARE PYRIDAZINES PRIVILEGED STRUCTURES?

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The term "privileged structure" originates from Evans and coworkers (1) who stated "what is clear is that certain privileged structures are capable of providing useful ligands for more than one receptor." A typical example of a privileged structure is provided by the benzodiazepines which have led to cholecystokinin (CCK) antagonists, to ocytocin antagonists, to C5a antagonists, to GABA A agonists, to potassium channel blockers, to γ-secretase inhibitors, to PDE (IV) inhibitors and to Src kinase inhibitors (2). Several privileged structures were since identified: diphenylmethanes, spiropiperidines, biphenyltetrazoles, phenylethylamines, benzazepines, 2.2-dimethylbenzopyrans (3). I want today to focus on a new privileged structure candidate, namely the pyridazine scaffold. The use of pyridazines in medicinal chemistry presents a significant amount of advantages. First pyridazines allow the insertion of elements capable of interactions that the corresponding carbocycles do not give. Second they allow a greater number of combinations; it becomes therefore easier to be original. Pyridazines also are rigid analogues of endogenous substances that themselves are often nitrogenous metabolites of amino acids. In decreasing the log P values the pyridazine nucleus increases the water solubility. In addition to all this advantages one has to consider the good ADME profiles and *in vivo* safety of pyridazine drugs. Another interest of pyridazines is their capacity to act as original functional surrogates. Thus aminopyridazines can be used as carboxamide as well as amine surrogates. Finally the many examples of pyridazines used either as a structural element or as a main scaffold, justify largely their status as privileged structures.

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AWARD LECTURES – **AL.02**
THE PROUS INSTITUTE –OVERTON AND MEYER AWARD FOR NEW TECHNOLOGIES IN DRUG DISCOVERY

(R)EVOLUTIONS IN DRUG DISCOVERY THROUGH CONCEPTUAL AND TECHNOLOGICAL INNOVATIONS

Klaus Müller

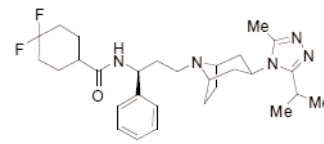
F. Hoffmann-La Roche Ltd, Pharmaceutical Research Science & Technology Relations. CH-4070 Basel, Switzerland

Within only a few decades, Drug Discovery has undergone dramatic changes as a result of mostly parallel developments in many areas of chemistry, biology, biophysics, molecular medicine and pharmacology, as well as informatics. Drug Discovery has become highly multi- and interdisciplinary being pushed by and constantly pushing the development of new technologies and novel concepts. The surge of molecular biology and biotechnology resulted in a paradigm shift from chemical intuition-dominated to molecular bio-mechanism-based approaches. The implementation of structural biology in industrial environments, together with critical developments of heterologous protein expression, purification, and crystallization technologies, and the establishment of a solid theoretical basis to understand molecular recognition, complemented by many novel molecular concepts, provided a firm 3D-structural basis for ever more efficient rational molecular design. Miniaturization, parallelization, and automation technologies put high-throughput compound handling and ultra-HT screening on qualitatively and quantitatively new levels, and resulted in the introduction of HT bio-analytical tools for physicochemical and *in vitro* pharmacological compound properties. The latter produced a dramatic paradigm shift of the Drug Discovery process and the emergence of multi-dimensional lead optimization much beyond potency and selectivity. There is no end to such developments. Much is currently undertaken to address the persistent difficulties in the transition from the early discovery to mid-stage clinical development. With enhanced conceptual and technological possibilities in genomic research, biomarker studies, systems biology and physiology, translational medical approaches are now recognized as a most promising paradigm.

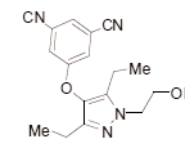
What are key factors enabling innovations in the Drug Discovery process? Innovations are done by talented people. However, their research environment is a critical enabling factor for innovations to occur. As innovations often take place at interfaces between disciplines, interdisciplinary research should be actively fostered. An open and entrepreneurial research atmosphere is key for new ideas to emerge. Recognize your creative talents and with them generate new visions and challenging targets. Encourage your scientists to explore new territory, give them enough support and trust - and be patient. Follow their endeavors with empathic interest without falling into the trap of micro-management. Take a balanced approach towards internal ver-

sus external developments. Do not be afraid of internal developments, which are often more focused, more timely, less costly, and much more adapted to current and future needs; however, be also aware of critical external expertise, complementary technologies, components, or materials, which may be critical to integrate for success at any stages in the design or development processes.

The development and success of the Drug Discovery process at Roche provides ample illustration for these concepts.



maraviroc



lersivirine

AWARD LECTURES – **AL.03**

UCB-EHRLICH AWARD FOR EXCELLENCE IN MEDICINAL CHEMISTRY

NEW HIV THERAPIES: THE DISCOVERY OF MARAVIROC AND LERSIVIRINE

Tony Wood PhD

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The UN estimates that 33 million people were living with HIV infection in 2007, and there remains an urgent need for new and improved medicines to treat the infection. The most successful new therapies will suppress drug-resistant viruses, cause fewer side-effects than existing agents, and be convenient to use in highly active antiretroviral therapy, or HAART.

If viral resistance is to be avoided or circumvented, drugs with new mechanisms of action will be important. The chemokine receptor CCR5 is the major co-receptor for the fusion and entry of macrophage-tropic (M-tropic) HIV-1 into cells. M-tropic strains are prevalent in the early, asymptomatic, stages of HIV infection, and CCR5 antagonists have been an intense area of research within the HIV field.

In addition to new mechanistic classes, non-nucleoside HIV reverse transcriptase inhibitors (NNRTIs) remain a cornerstone of HAART. Here, novel agents with a broad spectrum of activity against the virus with clinically significant drug resistance mutations in the reverse transcriptase (RT) enzyme are highly desirable.

In all instances, several factors are important: reducing dose and pill burden, minimising the risk of side-effects and drug–drug interactions. These will improve tolerability and ease of compliance.

The lecture will describe the invention of the potent CCR5 antagonist maraviroc, starting from high-throughput screening leads, with a particular focus on the tactics used to design a compound with a maximum therapeutic window over QT prolongation. It will also describe the discovery of the new non-cross-resistant NNRTI lersivirine using structure-based drug design. I will compare and contrast these two different approaches, emphasising the common role of simple lead quality criteria such as ligand and lipoidal efficiency in guiding design.

PRIZE LECTURES – **PR.01**

2010 IUPAC-RICHTER PRIZE LECTURE

BEHIND THE DESIGN OF DARUNAVIR FOR HIV/AIDS AND β-SECRETASE INHIBITORS FOR ALZHEIMER'S DISEASE

Arun K. Ghosh

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Aspartic acid proteases are involved in the pathogenesis of a variety of human diseases and a number of aspartic acid proteases have become important drug-design targets. The highly active antiretroviral therapy (HAART) with HIV protease inhibitors in combination with reverse transcriptase inhibitors continues to be the major treatment regimen for HIV infection and AIDS. However, one of the major challenges of HAART-therapy is the emergence of multidrug-resistant HIV-1 variants. In view of this problem, we have conceptualized our structure-based design of protease inhibitors by targeting the protein backbone to combat drug resistance. This 'backbone binding' design strategy led to diverse classes of potent HIV-1 protease inhibitors including Darunavir, an FDA approved treatment for HIV/AIDS patients harboring multidrug-resistant HIV-1 variants. Aspartic acid protease, memapsin 2 (beta-secretase, BACE-1), has become an important drug-design target against Alzheimer's disease (AD). Our structure-based design has led to the development of drug-design templates and tools against this important protein target for the treatment of AD. We have designed a number of potent and selective inhibitors for clinical development. This lecture will focus on design-concepts, general structure-based design strategies and development tools for HIV-1 protease and beta-secretase inhibitors.

PRIZE LECTURES – **PR.02**

EFMC PRIZE FOR A YOUNG MEDICINAL CHEMIST IN INDUSTRY

DISCOVERY, STRUCTURE-ACTIVITY RELATIONSHIPS AND EFFICACY OF NOVEL BK-CHANNEL OPENERS

Dr Antonio Nardi

Senior scientist, Grünenthal, Global Drug Discovery, Aachen, Germany

An account of the scientific research carried out in the large-conductance calcium-activated potassium channels (BK) field will be presented. Focus will be given on the different drug design strategies (scaffold hopping, bioisosteric approaches, etc) and synthetic tactics that led to the development of the currently most potent BK modulators. These modulators demonstrated good *in vivo* efficacy in a number of precliclinal animal models, which will also be discussed during the presentation.

PRIZE LECTURES – **PR.03**

EFMC PRIZE FOR A YOUNG MEDICINAL CHEMIST IN ACADEMIA

HOW CAN COMPUTERS SUPPORT DRUG DISCOVERY? *IN SILICO* APPROACHES FOR COMPOUND PROFILING, PREDICTING TARGETS, AND FOR PREDICTING LIGAND ACTIVITY AGAINST PARTICULAR PROTEIN MUTANTS

Andreas Bender, PhD

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Today, a vast number of associations (hundreds of thousands to millions) between small molecule structures and their respective target affinities are known. Those associations are analyzed here for the prediction of *on-target* as well as *off-target* effects of novel compounds, and to analyze factors contributing to the promiscuity of new structures – and, thus, knowledge from the past can be used to guide the discovery of bioactive structures in the future.

In particular, we describe the development of *in silico* models to predict protein targets of compounds, such as those contained in common safety profiling panels[1], to prioritize targets to be screened in safety evaluations of novel structures. For the analysis of compound promiscuity, a usually undesired feature of active ingredients, we present computational models which firstly provide estimates for compound promiscuity, and secondly give insight into features frequently associated with promiscuous compounds.[2] Finally, we will discuss ideas how phenotypic profiling of compounds can be integrated with *in silico* techniques to derive a comprehensive assessment of the biomodulatory capabilities of a compound.[3] Applications will in particular be presented on the prediction of compound activity against particular mutants of HIV Reverse Transcriptase as well as against subtypes of the Adenosine class of G-Protein Coupled Receptors (GPCRs), using so-called proteochemometric modeling approaches. The models were prospectively validated using >400 new data points with model predictivity approaching assay reproducibility, which is an encouraging results of this novel technique.

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LECTURES – **L.01**

ACTIVITY BASED PROBES OF PROTEASES: APPLICATIONS TO FUNCTIONAL PROTEOMICS AND MOLECULAR IMAGING

Matthew Bogyo, Galia Blum, Laura Edgington, Fangfang Yin, Jiyoun Lee, and Michael McConnell

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Protease are regulated at the level of their activity in both normal and disease conditions. Thus it is difficult to monitor the dynamic nature of this regulation in the context of a live cell or whole organism. Our laboratory has developed a series of activity based probes that specifically bind protease targets through an enzyme catalyzed chemical reaction. These reagents freely penetrate cells and can be used to enrich complex proteomic samples for monitoring of global patterns of protease activity as well as to directly image protease activity in live cells and whole animals. We have applied these probes to study the role of specific proteases in the process of angiogenesis and metastasis in mouse models of cancer as well as during the process of inflammation in mouse models of atherosclerosis and asthma. In addition, we have developed probes that bind caspases associated with the process of apoptosis. These reagents allow the direct non-invasive imaging of apoptois in vivo. We are currently developing these tools to further study the cell biology of tumor response to chemotherapy. Advances in development and applications of these imaging probes will be presented.

LECTURES – **L.02**

REACTIVE NATURAL PRODUCTS PROBING PROTEIN COMPLEX FUNCTION

Hans-Dieter Arndt

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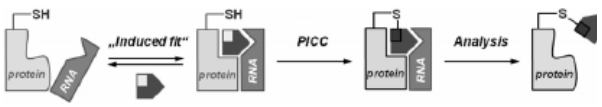
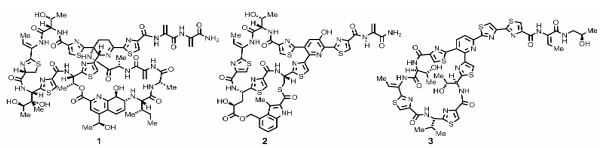
One of the major challenges in chemical biology is the characterization of ligands able to specifically bind to and modulate larger surfaces of biomacromolecules.[1] Recently, we reported a affinity-based strategy for the covalent capture of ligands on oligonucleotide-protein targets („**p**roximity **i**nduced **c**ovalent **c**apture", **PICC**), which utilizes engineered Cys-mutants at the protein surface.[2] These investigations were geared at the thiopeptide class of antibiotics,[3] which inhibit the GTPase associated region (GAR) of 70S ribosomes with nM to pM affinities by locking a bipartite RNA-protein interface.[4,5]



A detailed analysis of extended PICC-experiments with a panel of intrinsically reactive thiopeptide natural products (e.g. **1**-**3**) and semisynthetic derivatives on their reconstituted RNA/protein target and on full size 70S ribosomes will be presented. Successful mapping of the ligand position is demonstrated at amino-acid resolution, and integrated MD-simulation studies revealed characteristic features of the highly complex bipartite binding interface. The results will be discussed in light of recent structural investigations,[6] studies of resistance mutants *in vitro* and in bacteria,[5] and off-target effects of thiopeptide ligands.[7]



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LECTURES – **L.03**

TRANSNUCLEAR MICE MADE BY SOMATIC CELL NUCLEAR TRANSFER FROM B AND T LYMPHOCYTES

Hidde L. Ploegh

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The generation of mice with T or B cells of defined specificity is usually accomplished by transgenesis or gene targeting strategies. Somatic cell nuclear transfer affords the opportunity to harness the specificity and characteristics of B and T cells without experimental alterations of the loci that encode antigen receptors. It further has the significant advantage that primary t or B cells can be used as the source of nuclei, without the need for selective propagation in vitro of lymphocytes of the desired specificity. This approach creates opportunities for new disease models through generation of transnuclear mice that carry pathogen-specific B or T cells. It also allows the re-examination of questions pertaining to lymphocyte development less readily captured by transgenic or knock-in mouse models Results obtained with these models will be presented.

LECTURES – **L.04**

DEVELOPMENT OF CHEMICAL PROBES FOR THE STUDY OF THE CB_1 AND CB_2 CANNABINOID RECEPTORS

Silvia Ortega-Gutiérrez,[1] Lidia Martín-Couce,[1] Mar Martín-Fontecha,[1] Arnau Cordomí,[2] Leyre Mestre,[3] Carmen Guaza,[3] Leonardo Pardo,[2] and María L. López-Rodríguez[1]

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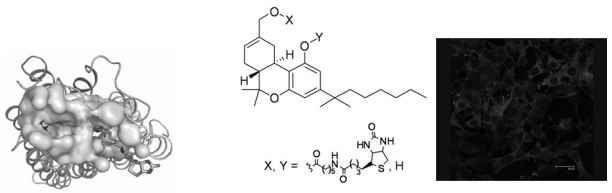
Functional proteomics has emerged as a powerful chemical proteomic strategy to characterize enzyme function directly in native biological systems on a global scale.[1] Hence, develop-

ment of probes to cover other fractions of the proteome currently constitutes an important challenge. To date, no probes have been developed for the study of G protein-coupled receptors (GPCRs), which constitute almost the 50% of the druggable genome.[2] In this context, we have started a project aimed at the development of a set of chemical probes bearing different tags that enable visualization, isolation, enrichment and/or identification of GPCRs.

We have focused our initial efforts on cannabinoid receptors (CBRs), which are involved in a number of (patho)physiological processes of importance.[3] However, there are still important questions that remain unanswered such as the existence of alternative CBRs different from the characterized CB_1 and CB_2. The development of tagged small-molecule probes would greatly improve our understanding of the endogenous cannabinoid system, its physiology and its therapeutic potential. Toward this aim, we have synthesized a set of probes based on the structure of the endocannabinoids anandamide and 2-arachidonoylglycerol as well as in the high-affinity synthetic ligands HU210 and HU308. In order to identify those regions in the molecule where the different tags can be introduced we have used β_2 adrenergic-based homology models of the receptor in complex with the selected scaffolds. Up to this moment, we have introduced different labeling moieties including biotin, benzophenone and alkyne. Some of the synthesized probes display high affinity for the CBRs and they enable their direct visualization in cell systems (Figure 1).[4] These results provide the basis for further development of these or other derivatives as probes for cannabinoid receptors in an approach that we are extending to other GPCRs.



A B C

Figure 1. (A) Computational model of the complex between a HU210-derived probe and CB_1R. (B) HU210-derived probes. (C) Visualization of CB_1 in HT-22 cells

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LECTURES – **L.05**

CANOSIMIBE - LOWERING OF ATHEROGENIC LDL BY A NON-SYSTEMIC CHOLESTEROL ABSORPTION INHIBITOR

Glombik, Heiner; Jaehne, Gerhard; Heuer, Hubert O.; Schaefer, Hans-Ludwig; Frick, Wendelin; Lindenschmidt, Andreas; Theis, Stefan; Kramer, Werner

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Elevated plasma LDL-cholesterol is a major risk factor for cardiovascular diseases. Despite the beneficial effects of a stand alone therapy with the widely used statins (reducing the endogenous synthesis of cholesterol by inhibiting HMG-CoA reductase) there is a persisting medical need for combination therapy to reach recommended treatment goals, especially in high risk patients. This can be achieved with compounds inhibiting the intestinal absorption of dietary and endogenous cholesterol (like e.g. Ezetimibe).

Biological targets in the small intestine have gained much interest in recent years, predominantly with respect to nutrient sensing and absorption, intestinal hormone release (e.g. GLP-1, GIP, PYY), and the potential treatment of metabolic diseases. If the target proteins are accessible at the luminal side of the intestinal mucosa, drugs addressing these targets do not need to be available systemically to exert a profound systemic effect. This principally offers the advantage of a high inherent compound safety, largely avoiding adverse systemic side effects, and minimizing hepatic drug-drug interaction potential. In addition, this implies the need to follow other than standard procedures in compound optimization and candidate development, and the concept of low absorption drugs was established for this purpose.

One application of this novel approach was to test the hypothesis that the target(s) of cholesterol absorption inhibitors might be accessible at the luminal side of the small intestine. By retaining the pharmacophore of ezetimibe and introducing linker-and spacer units as well as low absorption groups (LAGs) to different parts of the lead structure we probed the spatial acceptability of these modifications. As LAGs polar residues such as polyols, permanent cations or permanent anions were coupled to the pharmacophore via various functional group linkers and spacers of various lengths and structural types. The compounds were tested in vivo by oral application in a mouse model for their ability to inhibit cholesterol absorption and further optimized to meet the low absorption - non-systemic criteria in rat PK studies. Candidates were profiled to confirm their ability to inhibit intestinal cholesterol absorption and lower LDL-C in subchronic animal models of hypercholesterolemia. The design, synthesis, SAR, and in vivo activity of non-systemic inhibitors of cholesterol absorption, which led to the clinical candidate Canosimibe (AVE5530), will be discussed.

LECTURES – **L.06**

DISCOVERY OF RIOCIGUAT: A POTENT, ORAL STIMULATOR OF SOLUBLE GUANYLATE CYCLASE FOR THE TREATMENT OF PULMONARY HYPERTENSION

Joachim Mittendorf

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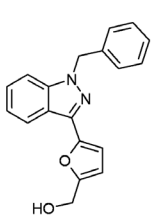
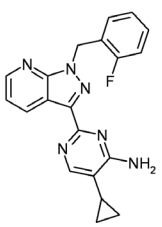
Soluble guanylate cyclase (sGC) is a key signal-transduction enzyme activated by nitric oxide (NO). Impairments of the NO-sGC-signaling pathway have been implicated in the pathogenesis of various cardiovascular and other diseases. Direct stimulation of sGC therefore represents a promising therapeutic strategy particularly for the treatment of pulmonary hypertension (PH), a devastating disease where a markedly impaired bioactivity of NO contributes to excessive pulmonary vasoconstriction.[1]

Direct NO-independent sGC stimulation was first demonstrated in 1994 when Ko and colleagues reported cGMP-stimulating properties for benzylindazole YC-1. Our initial chemical optimization program based on YC-1 as a lead structure resulted in the identification of sGC stimulators such as pyrazolopyridine BAY 41-2272 with approximately 2-3 orders of magnitude higher potency.

BAY 41-2272 demonstrated beneficial effects in experimental models of PH, but was associated with unfavorable drug metabolism and pharmacokinetic properties. We disclose an extended SAR exploration of this compound class addressing these issues.[2] Our efforts led to the identification of the potent sGC stimulator riociguat, which exhibits an improved DMPK profile and exerts strong effects on pulmonary hemodynamics and exercise capacity in patients with PH. Riociguat has the potential to overcome limitations of existing drugs and is currently being investigated in phase III clinical trials for the oral treatment of PH.



YC-1



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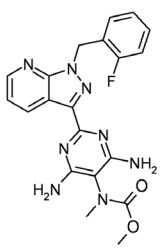

Riociguat

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LECTURES – **L.07**

DESIGN AND PREPARATION OF POTENT, NONPEPTIDIC, BIOAVAILABLE RENIN INHIBITORS

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The Renin-Angiotensin-Aldosterone System (RAAS) represents one of the major and most studied regulating systems of the arterial blood pressure in humans, and plays a primordial role in cardiovascular diseases, renal diseases, and other metabolic diseases.

After developing diazabicyclononene,[1] tetrahydropyridine, and other bicyclic derivatives,[2] we focused our efforts on the development of 3,4-disubstituted piperidines. Starting from the optimized substituents that were identified with **ACT-077825**,[1] we developed new, more polar benzyl amides (position 3) and phenyl substituents (position 4), leading to bioavailable renin inhibitors with sub-nanomolar potencies in human plasma. These compounds led to a sustained blood pressure decrease in the double transgenic rats (TGRs) at a dose of 1 mg/kg po.

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LECTURES – **L.08**

MOLECULAR OBESITY, POTENCY AND OTHER ADDICTIONS IN DRUG DISCOVERY

Mike Hann

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Platform Technology and Sciences, GSK, Stevenage, UK.

There have been many retrospective studies of attrition data over the last few years which have led to the emergence of new rules of thumb around drug-like and lead-like space and parameters that are linked to, for instance, toxicity. I will take a fresh look at some of the more recent data in the literature and combine them with some new thinking and observations based on our own experiences at GSK. In particular we introduce the term Molecular Obesity as a way to describe the risks associated with over large and fat candidate molecules, and their low likelihood of survival and progression. We look at the relationship of Molecular Obesity to the desire for Potency in molecules and why potency appears to behave like an addiction that controls the behaviour of project teams. We suggest ways in which this can be controlled.

LECTURES – **L.09**

DRUG GURU - ENCODING TACIT KNOWLEDGE FROM EXPERIENCED MEDICINAL CHEMISTS

Kent Stewart

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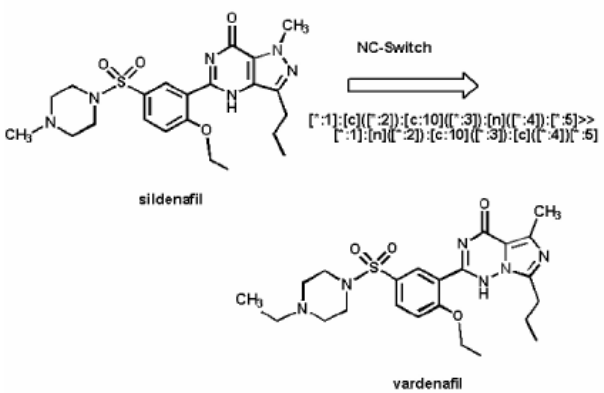
Drug Guru (Drug Generation Using Rules) is a computer program that applies medicinal chemistry "rules-of-thumb" to an input structure to design new analogs [K. D. Stewart et al., Bioorg. Med. Chem. 14, 7011-7022, 2006]. After entering the structure of interest, the chemist is presented with a list of output structures along with historical precedent. As examples, every benzene ring is converted to a thiophene ring and every amide is made into a retro-amide. Several hundred of these rules have been captured from medicinal chemistry research efforts from the last 50 years and programmed into a web-based software tool, Drug Guru, that is distributed corporate-wide within Abbott. Some rules, such as the carboxylate-to-tetrazole rule, correspond to well known isostere replacements. Other rules, such as ring modification, metabolism blocking, or solubility increasing rules are more complex. The rules are encoded using "SMIRKS" line code (interconverts SMILES strings; Daylight, Inc.) Calculated physical properties, incorporated directly into the software, are useful for prioritizing output. Output can be taken directly into other computer programs, such as docking software. A comparison is made between Drug Guru and related software programs BROOD (Open Eye), BIOSTER (Accelrys) and EMIL (CompuDrug). This work will be placed into the context of current research in "Compound Pairs Analysis".



LECTURES – **L.10**

BIOISOSTERES FROM LIGAND- AND STRUCTURE-BASED DATA ANALYSIS

James Mills

Pfizer, Canterbury, U.K.

The search for novel bioisosteres that either preserve or increase activity, whilst retaining or improving ADMET properties, is part of the day-to-day role of a medicinal chemist. Until recently, this was often achieved through word of mouth and anecdotal evidence. With the advent of combinatorial chemistry and higher-throughput screening, there is now a wealth of data to mine, either in the public domain or within pharmaceutical corporation databases. In this talk, some of the datamining approaches being applied to the identification of bioisosteres will be presented, including pairwise approaches for the replacement of functional groups, mining of multiple pairwise datasets to identify similar structure-activity relationships and the use of such methods not only in compound design but also e.g. monomer selection strategies.

Suggestions for bioisosteric replacements can also be derived from mining 3D data from the large number of ligand-protein co-crystal structures that we now have at our disposal. Although a smaller dataset, it can deliver novel suggestions, and also, when combined with the aforementioned SAR mining, is capable of delivering new insights into the analysis of both structural and activity data.

LECTURES – **L.11**

'BETTER COMPOUNDS FASTER' – THE DEVELOPMENT AND EXPLOITATION OF A DESKTOP PREDICTIVE CHEMISTRY TOOLKIT

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Today's drug designer has access to a vast quantity of data and a huge number of sophisticated computational methods. At the same time there is increasing pressure on the pharmaceutical industry to improve its productivity and reduce candidate drug attrition. In AstraZeneca we recognised both the need and the opportunity to build a strong Predictive Chemistry capability comprising an efficient and well-defined design process supported by highly integrated design and knowledge tools underpinned by the best predictive chemistry models. Here we describe the evolution of the AstraZeneca Predictive Chemistry integrated desktop toolkit and its exploitation by design teams to make better design decisions during the lead identification and lead optimisation phases.

The toolkit comprises a mixture of in-house developed and commercial applications for data visualisation, Structure-Activity Relationship (SAR) analysis, virtual compound generation, property prediction and target compound selection, linked to a collaborative annotation and knowledge capture environment.[1] The guiding principles for the development of the toolkit have been (a) to facilitate diverse design and analysis workflows in a flexible manner, (b) to use the best available validated computational and cheminformatic methods, (c) to develop user-friendly, robust and supportable desktop applications.

The exploitation of the toolkit is driven through extensive Awareness, Training and Utilisation activities including web portals, on-line guides, help pages and video demos, classroom hands-on training using real project examples and 'hot-desking' by informaticians with design teams. We will describe some of the challenges we have encountered along the way, both technical and cultural, and highlight the benefits of close team working between informatics, medicinal and computational chemistry functions.

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LECTURES – **L.12**

CARDIOVASCULAR DERIVATIVES OF EMBRYONIC STEM CELLS IN CARDIAC REPAIR AND DRUG DISCOVERY

Christine Mummery

Department of Anatomy and Embryology, Leiden University Medical Centre, The Netherlands

Derivation of heart and vascular endothelial cells from human pluripotent stem cells (embryonic stem cells or HESCs and induced pluripotency stem cells or hiPS cells) is an area of growing interest both as a route to cell therapy for the heart and as a platform for drug discovery and toxicity. Understanding the underlying developmental mechanisms that control differentiation of pluripotent cells to cardiac progenitors and their derivatives and mimicking these in defined culture conditions in vitro is now essential for moving the field forward. Culture conditions have now been sufficiently refined that cardiomyocyte and vascular differentiation is a fairly efficient and reproducible process. Genetically marked HESCs have been produced in which expression of the green fluorescent protein marker is expressed ubiquitously or driven by specific lineage markers. We have used these tagged lines to trace cardiomyocytes following transplantation into a mouse heart after myocardial infarction and select the progenitors of cardiomycytes, endothelial cells and smooth muscle cells. Long term survival of the cells and integration into the host heart has been observed and early improvements in cardiac function but these are not sustained. Cardiac repair using stem cell derived cardiomyocytes will likely require more than efficient cardiomyocytes production. More immediate applications of hESC- and hiPSC derived cardiomyocytes and vascular endothelial cells in drug discovery and disease are now close to implementation. Results of these studies, in particular drug responses of hESC-derived cardiomyocytes and an hiPSC model for vascular disease, will be shown.

LECTURES – **L.13**

DEVELOPMENT OF SMALL MOLECULE THERAPEUTICS FOR TREATING ENDOGENOUS CELLS FOR REGENERATION

Wei Xiao[1], Matthew Epperson[1], Frank Stappenbeck[1], Francine Farouz[1], Mariko Reilly[1], David Jenkins[1], Scott Thies[1], Farhad Parhami[2], Jeffrey Wang[3], Michael Jung[4], Khanhlinh Nguyen[4]

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Bone morphogenic proteins (BMPs) that induce osteoblast differentiation have been used successfully in humans in spinal fusion procedures and non-union fracture treatments. However, BMPs are restricted in clinical use due to safety risks and high costs. Therefore, the identification of small molecules acting as anabolic agents represents a need for orthopedic medicine, in particular for spinal fusion and fracture repair. In our search for new small molecule anabolic agents, we found that several naturally occurring oxysterols play a critical role during the differentiation of pluripotent mesenchymal stem cells to osteoblasts through activation of the Hedgehog signaling[1] pathway. Here, we report on the synthesis and characterization of several novel structural analogs of 20(S)-hydroxy cholesterol and confirm that these novel oxysterols can induce *in vivo* osteogenic differentiation of mesenchymal stem cells in a rat spinal fusion model.

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LECTURES – **L.15**

MOLECULAR THERAPIES FOR INFLAMMATORY AND AUTOIMMUNE DISEASES: ONGOING CLINICAL TRIALS AND FUTURE PROSPECTS

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Current pharmacologic treatments for inflammatory diseases and autoimmune diseases are largely palliative rather than curative. Most of them result in nonspecific immunosuppression. This can be associated with disruption of natural and induced immunity with significant, sometimes dramatic, adverse effects, which can be irreversible. Among the novel strategies that are under development, tools that target specific molecular pathways and cells, and more precisely modulate the immune system to restore normal tolerance mechanisms, for instance, are central. In these approaches, peptide therapeutics do constitute a novel class of agents. They can be produced and purified in large amount and controlled conditions for a relatively moderate cost. They possess a number of intrinsic properties that are favorable for long-term treatments. In particular free peptide display poor immunogenicity. They are also versatile components that can be easily modified to improve their capacities without affecting their bioactivity. They can be synthesized with modified amino-residues mimicking crucial post-translational modifications. Peptide-mediated immunotherapy has been evaluated in several appropriate experimental animal models, and a few peptides are currently evaluated in clinical trials for the treatment of human chronic inflammatory diseases. In the near future, therapeutic peptides might find important applications in addition to other strategies, which are more commonly put forward, such as gene and cellular therapies or therapies based on monoclonal antibodies. Beside the identification of unique sequences that specifically target autoreactive B and T cells or deviate central pathways involved in cell signalling, for example, future challenges include the optimization of peptide dosage and route of administration as well as the improvement of peptide stability for adequate bioavailability and specific targeting.

LECTURES – **L.16**

GENERATION AND DEVELOPMENT OF A SERIES OF MAPKAPK5 INHIBITORS FOR THE TREATMENT OF RHEUMATOID ARTHRITIS

M. Andrews[1], R. Brys[1], N. Vandeghinste[1], P. Pujuguet[2], F. Namour[2], M. Chambers[3], W. Schmidt[3], K. Hirst[3], G. Bar[1], G. Tricarico[1], A. Clase[1], V. Birault[3], F. Vanhoutte[1], J. Beetens[1] and G. Dixon[1,2]

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Rheumatoid arthritis (RA) is a chronic joint disease, characterized by inflammation leading to destruction and debilitation of the joints, which affects approximately 1% of the world's adult population. Methotrexate remains the standard disease-modifying anti-rheumatic drug (DMARD), and its use has recently been complemented by biological anti-TNFα therapeutics, such as Enbrel®, Remicade® and Humira®. However, due to the risk of side effects, there remains a need for safe, effective and orally available DMARDs. Through systematic RNA inhibition of druggable proteins in cells from rheumatoid arthritis patients, the protein kinase MAPKAPK5 was identified as a novel drug target in the RA disease process. Subsequently, a drug discovery campaign focused on identifying small molecule inhibitors of this kinase was undertaken.

In the first stage of hit identification, screening of the BioFocus SoftFocus® compounds against MAPKAPK5 led to the identification of three main series. Further analysis showed that one series in particular had the most promising activity in both the biochemical and phenotypic cell assays, in which a reduction of collagenases and inflammatory cytokines upon compound dosing was observed. This series of molecules was also found to show sufficiently good ADME properties to allow determination of PK, and *in vivo* activity in the murine collagen-induced arthritis (CIA) model.

To expand the chemistry of the project, a scaffold hopping exercise was undertaken and a closely related pharmacophore was generated. Compounds from this new series showed good activity in the biochemical and phenotypic assays, with general transferability of SAR, although clear areas of distinction were also found. This new core was shown to have good ADME properties and, subsequently, excellent pharmacokinetic properties. Compounds were then tested in models of arthritis, including the murine anti-collagen monoclonal antibody-induced arthritis and CIA models. In these *in vivo* models, a number of molecules showed significant activity related to both inflammation and bone damage. Analysis of bone was performed by both radiography and micro-computed tomography, and imaging techniques were also applied to confirm the effect of these compounds in reducing levels of joint degrading proteases in the paws of affected animals. One of these compounds has now completed Phase I clinical studies in healthy volunteers, showing good safety, and a PK profile consistent with once-daily oral dosing.

In summary, a series of compounds with good *in vitro* potency have been produced. Several compounds from this series showed encouraging ADME and PK profiles and activity in *in vivo* models of RA. One of these compounds has completed Phase I clinical trials and will enter Phase II studies in RA patients in 2010.

LECTURES – **L.17**

DISCOVERY AND OPTIMIZATION OF HIGHLY SELECTIVE BTK INHIBITORS THAT BIND TO AN INACTIVE ENZYME CONFORMATION AND ARE EFFICACIOUS IN ANIMAL MODELS OF ARTHRITIS AND LUPUS

Kevin S. Currie[1], James Barbosa[2], Peter Blomgren[1], Meire Bremer[4], Doug Davies[9], Steven Gallion[3], Tao Huang[5], Sarah Hymowitz[6], Randall Jones[2], Jeffrey E. Kropf[1], Tony S.H. Lee[1], Lichuan Liu[7], Jennifer Macaluso[2], Scott Mitchell[1], Karin Reif[5], Hong Rong[2], Ann Marie Rossi[2], Heleen Scheerens[4], Bart Staker[9], Harvey Wong[7], Wendy B. Young[8], and Julie A. DiPaolo[2]

Departments of Medicinal Chemistry[1], Biology[2], and Computational Chemistry[3], CGI Pharmaceuticals, Inc., 36 East Industrial Rd, Branford, CT, USA. Departments of PD Biomarkers[4], Immunology[5], Structural Biology[6], DMPK[7], and Chemistry[8], Genentech, 1 DNA Way, South San Francisco, CA, USA. [9]Emerald BioStructures, 7869 NE Day Rd W., Bainbridge Island, WA, USA

Bruton's tyrosine kinase (Btk) is expressed in multiple cell types implicated in the pathogenesis of autoimmune disease, including B-cells, monocytes, and macrophages. Btk is therefore a compelling target for small molecule inhibitors, but no adequately selective Btk inhibitors have been reported to date. We have discovered a novel series of potent, reversible, and specific Btk inhibitors, exemplified by the proof-of-concept molecule CGI1746 (IC_{50} =2nM). We solved co-crystal structures of CGI1746, PCI-32765 and dasatinib bound to human Btk that reveal significantly different binding modes. Data will be presented to show the impact of each binding mode on selectivity and pharmacology. CGI1746 binds to an inactive conformation of Btk in which the key activating tyrosine 551 is displaced ~18Å relative to the apo structure, creating a novel selectivity pocket that is exploited by the ligand. Consequently CGI1746 is >100-fold selective for Btk over all kinases tested (386) and inhibits both the auto- (Y223) and trans- (Y551) phosphorylation steps necessary for full activation of Btk. CGI1746 potently inhibits multiple B-cell functions *in vitro* and *in vivo*, and as well as cytokine release from monocytes. Furthermore, CGI1746 shows robust DMARD efficacy in rodent CIA models, and is efficacious in a mouse model of SLE. Optimization in this series led to the identification of CGI3272 which maintains the selectivity and biological profile of CGI1746, but has significantly improved pharmacokinetic properties and human whole blood potency. Together, our results provide (1) the first pharmacological tool enabling interrogation of Btk function with specific, reversible inhibition and (2) a foundation for the design and of highly selective bioavailable Btk inhibitors with potential application in autoimmune disease.

LECTURES – **L.18**

OPTIMISATION OF THE DIAMINOPYRIMIDINE CARBOXAMIDE SERIES LEADING TO HIGHLY POTENT, SELECTIVE AND ORALLY BIOAVAILABLE SYK INHIBITORS

Neil Garton, John Liddle, Mike Barker, Clement Douault, Vipul Patel, Tracy Shipley, Geoff Stemp, Ann Walker, Michael Woodrow, Alex Preston, Francis Atkinson, Don Somers, Peter Blencowe, Petter Jennison, Stephanie Gray

GlaxoSmithKline, ImmunoInflammation CEDD, Epinova DPU, Gunnels Wood Road, Stevenage, Herts SG1 2NY, UK

SYK (Spleen Tyrosine Kinase) is a non-receptor tyrosine kinase that is involved in coupling activated immunoreceptors to signal downstream events that mediate diverse cellular responses, including proliferation, differentiation, and phagocytosis. Inhibition of SYK mediated Ig Fc epsilon and gamma receptor and B-cell receptor signaling leads to mast cell, macrophage and B-cell inhibition. Accordingly, SYK kinase inhibitors have attracted interest in a number of therapeutic areas, including the treatment of rheumatoid arthritis, B-cell lymphoma and asthma / rhinitis.

The lead optimisation of the diaminopyrimidine carboxamide series from compound 1 will be described. The initial strategy focussed on optimising the physicochemical properties, particularly modification of pKa and PSA, to deliver adequate PK and low hERG activity. The series was subsequently optimised to overcome mutagenicity whilst maintaining SYK potency and a broad kinase selectivity.

LECTURES – **L.19**

VIRTUAL SCREENING – SUCCESS STORIES AND CHALLENGES

Jürgen Bajorath

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Computational approaches of different sophistication are successfully employed in the search for novel active compounds. Among those are various holistic similarity methods that often utilize only simple molecular representations. It is evident that the complexity of computational tools and molecular representations does not correlate with their success in virtual screening and scaffold hopping. Why is this so? Why do many different virtual screening methodologies frequently display comparably good or poor performance on given compound classes? The inability to rationalize virtual screening performance and predict

successes or failures presents one of the grand challenges in this field. Based on systematic analyses of structure-activity relationships, some answers to these still open questions can be offered. Furthermore, virtual screening case studies using different computational approaches and addressing different types of targets are presented that illustrate opportunities and limitations of current in silico screening efforts.

LECTURES – **L.20**

TARGETING THE DYNAMIC NATURE OF PROTEIN-PROTEIN INTERFACES

Alessandro Padova

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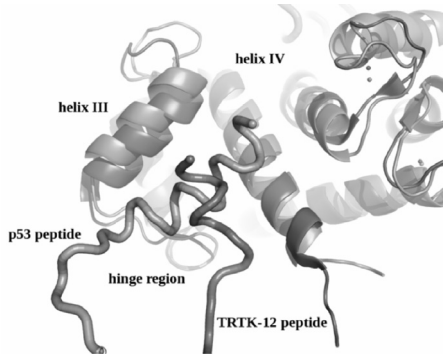
Protein-protein interactions play a key role in biological processes. In the past few years, there has been a significant increase in research activities aimed at identifying protein-protein interactions in the context of cellular function and detailed studying of these interactions at a molecular and atomic level.[1] However, whilst inhibitors have in some case been identified it has proved challenging to develop drugs capable of inhibiting these interactions, characterized in general by large, rather flat and potentially discontinuous contact epitopes.

After a general introduction, this seminar will focus on a critical assessment on novel strategies, based on in silico approaches, to inhibit such bimolecular interactions. Firstly, a ligand based approach aimed at identifying compounds containing α-helix recognition motifs will be described. A simple pharmacophore model has been derived from the analysis of several α-helix complexes deposited in the Protein Data Bank. Interestingly, whilst able to identify known ligands, the pharmacophore approach did not retrieve any suitable virtual hits from screening of a database of about 4.5 million commercial compounds, potentially revealing a chemical space gap in commercially available libraries. These results point towards the need to revise the virtual screening process used in hit identification phase and to expand "tractable" chemical space beyond "Lipinski world" during optimization. A possible solution could be targeting the adaptive nature of protein-protein interface by a combination of in silico and biophysical approaches that may reveal new way towards protein-protein inhibition.

As an example, the talk will describe an in silico fragment screening targeting the S100B–p53 binding interface taking into consideration an ensemble of S100B conformations derived from available NMR structures, followed by an NMR-driven fragment screening.[2]



Superimposition of the protein backbone for the complexes S100B-p53 (PDB ID: 1DT7 in green) and S100B-TRTK-12 (PDB ID: 1MWN, in magenta). The most representative frame is showed for each complex. Protein backbone is represented as cartoon, bound peptide as ribbon.

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LECTURES – **L.21**

CINDERELLA'S SHOE FOR VIRTUAL DRUG DISCOVERY SCREENING AND DESIGN

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A biological action of a drug involves its interaction with some subjects in our organism (ferments, DNA, water, membrane, etc.). Therefore, it is necessary to find potentially active centers of molecular interactions. It is possible to determine the potentials of electrostatic, Van der Waals interactions, hydrogen bonding, etc. at each point of the molecular surface. These potentials determine a molecular field. Basing on the complementarity conception we can suppose that the field of a good drug must be complementary to the receptor. The more complementary molecule to the receptor the more active it is. Therefore, the molecular field of an ideal drug is ideally complementary to the field of the receptor. Thus we can reconstruct the field of the receptor as a complementary one to the field of the ideal drug (as its mould, its negative). Then, it is possible to recognize a new prospective drug as complementary one to the obtained mould. We do not, however, have an ideal drug. Moreover, there seems little use in creating a new drug if one already has the ideal one. What we do have, however, are real drugs and it is necessary to reconstruct the field of the receptor using the fields of these real molecules. It should be noted that the field of a real molecule can define only a part of the receptor active site. Therefore, it can be assumed that a generalized set of active molecules can completely reconstruct the receptor active site as a complementary

field. We should have in view that a molecule includes active (pharmacophoric) fragments interacting with the receptor, non-active fragments that do not take part in the interaction and fragments disturbing the interaction (*e.g.*, providing steric barriers). In addition, we must also bear in mind the conformational and tautomeric flexibility of a molecule and the flexibility of the receptor. Consequently, not only does a molecule have to adjust to the receptor, but the receptor also has to adjust to the molecule, due to their inherent flexibilities.

So, the principles for 3D/4D classification are as follows:

- the geometry and the distribution of the interaction centers of an active molecule must be appropriate to the target;
- the classification method must find molecules with complementary geometry and active centers distribution provided by their conformational and tautomeric state;
- the "photograph" of a molecule must be representative. The method must recognize an active molecule, irrespective of its foreshortening, its conformational and tautomeric state.

Basing on this assumptions the new algorithm CiS ("Cinderella's Shoe") allows to model drugs and receptors flexibility is suggested. The method creates flexible pseudo-atomic receptor model and simulates a movement of drug to receptor through water and membrane. Thus, the method allows to predict bioactivity of compounds in dynamic conditions. All kinds of molecular movement (translational, rotational, vibrational, internal rotation) are taken into account. The method is used for detailed elucidation of action of dihydrofolate reductase inhibitors and anti-inflammatory COX-1, COX-2, LOX, p38 MAP kinase, dihydrofolate reductase, monoamine oxydase, HIV-1 reverse transcriptase inhibitors. The conformational and tautomeric forms of the compounds in water and in receptor pocket are determined. The obtained conformations in receptor pockets are in a good agreement with X-ray and NMR data. Using the proposed method new potential anti-inflammators was designed. Now the designed molecules are synthesized and successfully tested in vitro and in vivo. The algorithm is used for virtual screening of more than 40 kinds of bioactivities.

The work is fulfilled with the support of SKIF – GRID Supercomputer Initiative.

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LECTURES – **L.22**

DISCOVERY OF NEW ANTIMALARIAL LEADS THROUGH A VIRTUAL SCREENING APPROACH

Tiago Rodrigues,[1] Jiri Gut,[2] Philip J. Rosenthal,[2] Rui Moreira,[1] Francisca Lopes[1], Daniel dos Santos,[1] Rita C. Guedes[1]

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Malaria is one of the most devastating infectious diseases, affecting around 3 billion people and killing 1.5-2.7 million a year, especially in endemic countries [1]. *Plasmodium falciparum* is the most lethal of malaria parasites in humans and the emergence of drug resistance to conventional therapies and prophylaxis is a worldwide public health threat [2]. As a result, there is an urgent need for novel drugs, preferably acting on underexploited parasite targets in order to delay or overcome the selection of clinical resistance [3,4].

Cytochrome bc_1, from the mitochondrial electron transport chain, is one attractive and validated drug target, for which few efficient inhibitors are known today [5]. In an attempt to explore the chemical space of the bc_1 complex we designed a virtual screening protocol in order to find novel leads and scaffolds that might inhibit cytochrome bc_1. The drug-like compounds of both ZINC [6] and MOE [7] databases were sieved through a pharmacophore model and the resulting molecules subjected to three rounds of receptor-guided virtual screening with GOLD software [8]. Fifteen structurally different compounds were purchased and evaluated *in vitro* for their antimalarial activity against the *Plasmodium falciparum* W2 strain. The most active compounds included indole, amide and triazinoindole scaffolds and five of them presented activity between 10 and 50 μM. The protocol and results will be presented and discussed.

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LECTURES – **L.23**

DISCOVERY OF SMALL MOLECULES FOR MYOCARDIAL DIFFERENTIATION AND PROTECTION

Erik Willems, Paul J. Bushway, Fabio Cerignoli, Joaquim Teixeira, Marion Lanier, Karl J Okolotowicz, Zebin Xia, Jeffrey H. Price, Marcia I. Dawson, John Cashman and Mark Mercola

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A debilitating loss of cardiomyocytes can underlie heart failure. Since most current therapies do not address myocardial regeneration, there has been tremendous interest in defining natural and synthetic molecules to regenerate myocardium from exogenous or endogenous progenitors. We have been developing automated screens using mouse and human embryonic stem cells to discover novel small molecule and microRNA inducers of stem cell cardiogenesis. chemical compounds have been found to regulate diverse processes including production of committed cardiac progenitors and differentiation of cardiomyocytes. In cases where the targets are unknown, affinity versions of the compounds have led to candidate cellular protein targets that will be discussed. These chemical and microRNA probes increase knowledge of pathways important for stem cell cardiomyogenesis and deepen our understanding of the differentiation process. Moreover, based on biological mechanism of action, certain of the compounds might target signaling pathways that control endogenous regeneration and, therefore, could be considered as leads for drugs to stimulate myocardial regeneration in the failing heart. Secondly, we are using transgenic mouse models to validate a novel target for drug therapy that potentially confers cardioprotection via regulation of myocardial angiogenesis. Finally, high content screening algorithm, software and instrumentation have been developed for visualizing cardiomyocyte physiology in a moderate throughput, applicable to small-scale screening, secondary assays and cardiotoxicity assays. Validation of the platform for cardiotoxicity includes distinguishing toxic effects of drugs with known arrythymogenic effects.

LECTURES – **L.24**

ASK-DEPENDENT STRESS SIGNALING IN CELL DEATH, INFLAMMATION AND DISEASE

Hidenori Ichijo D.D.S., Ph.D.

Laboratory of Cell Signaling, Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1 Bunkyo-ku, Tokyo 113-0033, Japan

Actions of pro- and anti-apoptotic factors are often modulated by phosphorylation and dephosphorylation, and protein kinases and protein phosphatases thus contribute to the regulation of cell death decisions made in response to various stresses. Apoptosis Signal-regulating Kinase 1 (ASK1) is a member of the mitogen-activated protein (MAP) kinase kinase kinase family, which activates both the MKK4/MKK7-JNK and MKK3/MKK6-p38 MAP kinase pathways and constitutes a pivotal signaling pathway in various types of stress responses[1]. ASK1 plays crucial roles in oxidative stress[2]- and endoplasmic reticulum (ER) stress[3]-induced apoptosis, calcium signaling[4] and innate immunity[5] that are implicated in the pathophysiology of a broad range of human diseases. In this seminar, I will review our recent findings on the regulatory mechanisms and in vivo roles[6,7,8,9] of ASK family kinases in stress responses. I will also summarize the roles of stress-activated MAP kinase pathways and update our new findings on the pathophysiological roles of ASK family kinases in stress responses and disease.

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LECTURES – **L.25**

THE DISCOVERY OF BKM 120, A PAN CLASS I PI3K INHIBITOR IN PHASE I/II CLINICAL TRIALS

Sabina Pecchi, Matthew Burger, Mark Knapp, Saveur-Michel Maira, Gordana Atallah, Kevin Shoemaker, Hanne Merrit, Marion Wiesmann, Daniel Menezes, Kay Huh, and Charles Voliva

Novartis Institutes for Biomedical Research, Global Discovery Chemistry, Oncology and Exploratory Chemistry, Emeryville, Cambridge and Basel

A substantial number of epidemiological and experimental studies support an important role for PI3K in the biology of human cancer. The activation of PI3K, and its downstream effectors, has been clearly validated as an essential step for the initiation and maintenance of the tumorigenic phenotype. Parallel to the clinical development of our dual pan-PI3K/mTOR modulators (e.g., NVP-BEZ235), we continued our drug discovery activities to identify PI3K inhibitors with distinct biological and pharmacological profiles. Starting from potent 2-morpholino, 4-substituted, 6-(3-hydroxyphenyl) pyrimidines as lead series, a structure based design approach was adopted, with focus on

replacing the phenol moiety while maintaining potent target inhibition and improving *in vivo* properties. These efforts led to the identification of a new clinical candidate, NVP-BKM120. This 2,6-dimorpholino pyrimidine derivative is a potent pan-PI3K (e.g., IC_{50}= 35 nM, p110α) inhibitor that does not significantly inhibit other protein or lipid kinases (e.g., IC_{50}= 4.6 μM, mTOR). The compound exhibits antiproliferative activity against a broad panel of tumor cell lines by specifically blocking the biological function of PI3K signaling components (e.g. IC_{50} = 93 nM S473P-Akt in Rat1-p110α cells). NVP-BKM120 shows good oral bioavailability in preclinical species and demonstrates significant antitumor activity at tolerated doses in mouse xenograft models of diverse cancer lineage. Analyses of tumor tissues after acute dosing or at the end of efficacy studies, shows a good correlation between compound exposure, PI3K pathway blockade (reduction in P-Akt levels) and antitumor activity. NVP-BKM120 is currently undergoing Phase I/II human clinical trials for the treatment of solid tumors and hematological malignancies.

LECTURES – **L.26**

PHENOTYPIC DRUG DISCOVERY AT LILLY: MERGING IMAGES, INFORMATICS AND CHEMISTRY TO ENABLE SAR

Thomas A. Engler

Senior Research Advisor, Discovery Chemistry Research & Technologies, Lilly Research Laboratories, Eli Lilly & Company, Indianapolis IN, USA

Current drug discovery is highly target centric often starting with primary assays that measure effects of compound treatment on isolated proteins. Some limitations of target-based drug discovery include the consequences of removing target proteins from their cellular context, wherein modifications by, or interactions with, other biomolecules may play essential roles, and an inability to anticipate polypharmacology from a single assay. In addition, the readout from secondary cellular assays is often reduced to a single variable representing an average of the entire population, even though it is almost certainly a composite of several variables, known and unknown, from a heterogeneous population. Recent advances in imaging and data analysis allow simultaneous observation of multiple events in individual cells and subpopulations in response to compound treatment. This presentation focuses on efforts taking advantage of high-content imaging methods coupled with informatics tools to collect and analyze data from disease relevant cellular systems and their application to enable SAR. Comparisons between so-called targeted therapeutics suggest that these techniques provide an alternative way to assess selectivity and insights into potential polypharmacology.

LECTURES – **L.27**

IDENTIFICATION OF A PDE9 CLINICAL CANDIDATE FOR THE TREATMENT OF ALZHEIMER'S DISEASE UTILIZING PROSPECTIVE DESIGN AND NOVEL PROTOCOL DEVELOPMENT

Patrick Verhoest

Pfizer PharmaTx, Neuroscience Chemistry

Alzheimer's disease is a neurodegenerative disease with high unmet medical need. One hallmark of the disease is the loss of neuronal synapses which has been shown to most closely correlate with the cognitive decline seen in patients. We believe that inhibition of PDE9 will improve synaptic transmission and stabilize vulnerable synapses leading to an improvement in treating patients.

Our strategy involved utilizing prospective design, the development of novel library protocols and coupled with structure based drug design we were able to rapidly identify a lead series which built our confidence in rationale. By enabling the synthetic chemistry we were able to quickly improve selectivity over PDE1 and optimize physicochemical properties to identify a clinical candidate with good predicted pharmacokinetic properties and preclinical safety. We developed a deeper understanding of CNS drug property space, which we expanded by minimizing molecular weight, hydrogen bond donor count and adjusting fractional polar surface area. Our PDE9 clinical candidate has shown excellent human pharmacokinetic properties and coupled with its positive human biomarker data is poised to test the rationale of PDE9 inhibition in Alzheimer's patients.

LECTURES – **L.28**

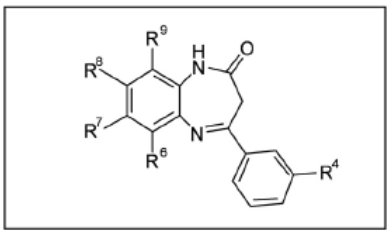
SYNTHESIS AND CHARACTERIZATION OF 1,3-DIHYDRO-BENZO[b][1,4]DIAZEPIN-2-ONE DERIVATIVES AS POTENT NON-COMPETITIVE METABOTROPIC GLUTAMATE RECEPTOR 2/3 ANTAGONISTS

T. J. Woltering, J. Wichmann, E. Goetschi, G. Adam, A. Alanine, J. N. C. Kew, V. Mutel, F. Knoflach, T. M. Ballard, S. Gatti

Pharmaceutical Division, Discovery Research CNS & Medicinal Chemistry. F. Hoffmann-La Roche Ltd., CH-4070 Basel, Switzerland

We have synthesized and characterized the properties of a series of 1,3-Dihydro-benzo[b][1,4]diazepin-2-one derivatives which are potent and selective non-competitive mGlu2/3 receptor antagonists. Attachment of an 8-(2-aryl)-ethynyl-moiety produced

compounds inhibiting the binding of [^{3}H]-LY354740 to rat mGlu2 with low nanomolar affinity and consistent functional effect at both mGlu2 and mGlu3 [1]. The selectivity of this new class of non-competitive mGlu2/3 receptor antagonists was demonstrated versus mGlu1, mGlu4 mGlu5 and mGlu8 receptors and ionotropic glutamate receptors.



Replacement of a cyano group by a 5- and 6-membered heterocycle at the position R4 and further modification to improve the physico-chemical properties led eventually to compounds with the ability to reverse LY354740-mediated inhibition of field excitatory postsynaptic potentials in the rat dentate gyrus [2]. Finally replacement of the (2-aryl)-ethynyl-moiety in 8-position with smaller less lipophilic substituents produced compounds where *in vivo* activity could be demonstrated by reversal of the LY354740-induced hypoactivity in mice after oral administration [3]. Selected compounds were further profiled in animal models of cognition [4].

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LECTURES – **L.29**

DISCOVERY OF LU AE51090, AN ALLOSTERIC MUSCARINIC M_1 AGONIST: PRO-COGNITIVE POTENTIAL ALONE AND AS ADD-ON TO ANTIPSYCHOTIC TREATMENT

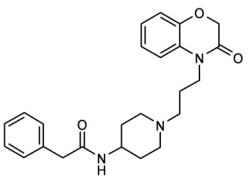
Anette Graven Sams, Gitte Kobberøe Mikkelsen, Krestian Larsen, Christoffer Bundgaard, Niels Plath, Claus Tornby Christoffersen, Morten Hentzer, Pascal Goethgebeur, Christina Kurre Olsen and Benny Bang-Andersen*

Lundbeck Research Denmark, H. Lundbeck A/S, Otilliavej 9, DK-2500 Valby, Denmark

The muscarinic M_1 receptor is the most abundant of the muscarinic receptor subtypes in the central nervous system, located in brain areas such as cerebral cortex and hippocampus that are important for learning and memory. Herein we report the structure-activity relationship of a series of allosteric muscarinic M_1 receptor agonists, leading to the discovery of the partial muscarinic M_1 receptor agonist Lu AE51090. The *in vitro* and *in vivo* pharmacological profile of Lu AE51090 is also discussed.

In vitro, Lu AE51090 is moderately potent at muscarinic M_1 receptors, and highly selective against a panel of other muscarinic receptor subtypes. Furthermore, Lu AE51090 displayed a high degree of selectivity when tested in a broad panel of G-protein coupled receptors, ion channels, transporters and enzymes, and Lu AE51090 showed an acceptable pharmacokinetic profile and sufficient brain exposure in rodents for *in vivo* characterisation.

In vivo, Lu AE51090 was efficacious in rodent models of working memory and executive functioning, the cognitive domains, which are most severely impaired in schizophrenic patients. For example, in a disease model of executive functioning, Lu AE51090 reversed a PCP-induced deficit, alone as well as in combination with a relevant dose of the antipsychotic haloperidol. Lu AE51090 was inactive in the conditioned avoidance response model, a rodent model predictive of antipsychotic potential, and did not induce catalepsy. Ferrets were used to evaluate Lu AE51090 for emetic potential. Vomiting was not induced at any of the tested doses, at or above exposure levels effective in the cognition models, suggesting Lu AE51090 to be effective in the treatment of cognitive impairment associated with schizophrenia (CIAS) without induction of muscarinic side effects.



Lu AE51090

LECTURES – **L.30**

IDENTIFICATION OF FUNCTIONALLY SELECTIVE ALPHA2C-AR AGONISTS AS POTENTIAL NEW DRUGS FOR TREATMENT OF PAIN

Eric Jnoff

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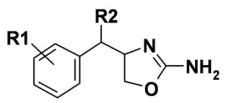
The neurotransmitter norepinephrine acts through different adrenergic receptors (ARs) including the α2-ARs belonging to the rhodopsine-like G-protein coupled receptor class. The α2-ARs are subdivided into three highly homologous subtypes (α2A-, α2B- and α2C-AR) [1-4] and are widely distributed in both the central and the peripheral nervous system. The α2-ARs play an important role in the regulation of many physiological

processes and are involved in mechanisms regulating among others blood pressure, sedation and antinociception. [5-6] So far, elucidating the subtype-specific functions of α2-AR has been hampered by the lack of subtype selective ligands. However, studies involving knock-out and transgenic rodents have highlighted some specific functions related to each subtypes. [3,7-8] The α2C-AR subtype is involved in many central nervous system processes and contributes to spinal analgesic actions and interactions with opioids . [9-10] Contrary to α2A-AR, the α2C-AR subtype does not play a major role in cardiovascular regulation. [11] In this context, new agonists discriminating α2C-AR from α2A-AR may open up the perspective of drug discovery aimed at clonidine-like robust analgesia without cardiovascular and sedative side-effects. [10] Chemical modulation around 4-benzyl-4,5-dihydro-oxazol-2-ylamine scaffold **1** led us to identify a set of compounds comprising functionally selective α2C-AR agonists. Ligands from this class display the interesting profile to behave as agonist at α2C-AR and as antagonist at α2A-AR. [12] Structure-activity relationships have been studied and the best compounds were tested in animal models of pain and evaluated in anaesthetized rat to monitor their cardiovascular effects.



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LECTURES – **L.31**

IN SILICO MODELS FOR TOXICITY: PREDICTING THE CHALLENGING ENDPOINTS

Mark T.D. Cronin

School of Pharmacy and Chemistry, Liverpool John Moores University, Byrom Street, Liverpool, L3 3AF, England.

In silico approaches for predicting toxicity include the use of existing data, chemical grouping and the formation of categories to facilitate read-across, in addition to (quantitative) structure-activity-relationships ((Q)SARs). These have been used to predict toxicological endpoints such as the mutagenicity, carcinogenicity and hERG binding of drugs for a number of years. Attention is now turning on how to make predictions for the "more difficult" toxicities such as repeated dose and reproductive effects. One approach to consider these challenging endpoints is through the grouping of compounds into robust categories. This approach relies on being able to group similar compounds together in a rational manner. Approaches to group chemicals together according to chemical similarity, which can be rationalised in terms of modes and mechanisms of action, will be presented. In order make best use of predictions, strategies to improve through integration are required and will be described in the context of the recently started EU eTox project. The funding of the European Union 7th Framework Programme IMI eTox Project (No. 115002) is gratefully acknowledged.

LECTURES – **L.33**

A MULTISCALE SIMULATION SYSTEM FOR THE PREDICTION OF DRUG CARDIOTOXICITY

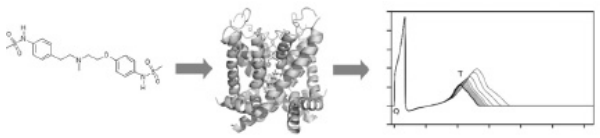
Manuel Pastor[1], Cristian Obiol-Pardo[1], Julio Gomis-Tena[2], Javier Saiz[2] and Ferran Sanz[3]

[1] Computer-Assisted Drug Design Laboratory, [3] Integrative Biomedical Informatics Laboratory, Research Programme on Biomedical Informatics (GRIB), IMIM, Universitat Pompeu Fabra, PRBB, Dr. Aiguader 88, E-08003 Barcelona (Spain) and [2] Grupo Bioelectronica I3BH, Universitat Politecnica de Valencia, Camino de Vera s/n, 46022 Valencia (Spain).

Cardiotoxicity and in particular drug-induced arrhythmia has been one of the main reasons responsible of late stage drug attritions during the last decades. Reliable assessment of drug cardiotoxicity often requires costly in vivo test based on electrocardiographic measurements in animal models and, for this reason, a predictive surrogate based on the in vitro hERG potassium channel blockade is now widely used in drug discovery or early drug development stages. Nevertheless, in spite of its convenience, the hERG binding affinity depicts a very narrow picture of the complex phenomena which are involved in drug-induced arrhythmia. As a consequence, the hERG binding test can yield false positive results (non-car-

diotoxic hERG blockers) which might discard valuable drug candidates or, even worse, false negative compounds (cardiotoxic non hERG blockers) which can enter development stages only for being detected as cardiotoxic far too late.

Here we present a computational prediction system for drug cardiotoxicity assessment based on a multiscale model of the drug effects on the cardiac repolarization. In this system, the effect of the compound is modeled at two different levels: at the *molecular level*, we predict the binding affinity of the compounds for several ion channels (hERG and others) using in tandem receptor-structure based methods and 3D-QSAR models. Then, at *physiological level*, these results are incorporated into a 1D electrophysiological model of guinea pig heart tissue, providing an integrated assessment of the drug effect on the cardiac repolarization, which can be read in terms of predicted QT segment elongation. All this system is fully integrated and can be run using a simple WEB interface, in which the user only needs to introduce the 2D structure of the compound for obtaining the final cardiotoxicity prediction.



As a proof of concept, the system has been applied to predict the cardiotoxic effects of diverse reference compounds, obtaining better results than hERG-based prediction systems. In particular, we predicted correctly a recently reported compound which appeared as false negative in the in vitro hERG assays but which produced a marked QT segment elongation and sudden animal death in later studies. All in all, the system reported here offers significant advantages with respect to state-of-the-art *in silico* predictions methods, due to the incorporation of several relevant ion channels and the integration of the binding affinities by an electrophysiological model into a more useful in vivo outcome. In this sense, our predictions can prove even more useful than those obtained with experimental in vitro hERG binding assays, since the output provides a more realistic and comprehensive description of the physiological phenomena involved in drug-induced cardiotoxic effects.

LECTURES – **L.34**

FROM GRADUATE STUDENT TO GROUP LEADER IN ACADEMIA

Lennart Bunch

Associate Professor, PhD. University of Copenhagen

In 2006 I was appointed Associate Professor and began to build-up my research group. Today it comprises 7 co-workers (Postdocs, PhD students, and MSc students). In this presentation I will explain why I chose to pursue an academic career and what it takes to be successful outlining which pitfalls to avoid. In more details, my talk will touch upon the following topics: Job insecurity, Freedom to successful - Freedom to fail, Internal funding - External funding, Grant applications – the *yea* and the *nay*, Your colleagues - Your competitors, Time management, Leadership in academia.

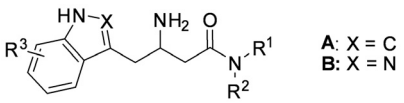
LECTURES – **L.35**

(R)-3-AMINO-4-(6,7-DIFLUORO-1H-INDAZOL-3-YL)-1-(3-(TRIFLUOROMETHYL)-5,6-DIHYDRO-[1,2,4]TRIAZOLO[4,3-A]PYRAZIN-7(8H)-YL)BUTAN-1-ONE, (PK44), A POTENT AND SELECTIVE DIPEPTIDYL PEPTIDASE IV INHIBITOR FOR THE TREATMENT OF TYPE 2 DIABETES

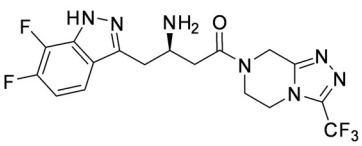
Rebecca Glen, Terance Hart, David Leese, Adrian Maddaford, Matthew Tozer, Robert Wybrow

Peakdale Molecular, Peakdale Science Park, Sheffield Road, Chapel-en-le-Frith, High Peak, SK23 0PG, UK

Two new series of β-aminoamides (**A** and **B**) have been developed as potent and selective inhibitors of dipeptidyl peptidase IV (DPP-IV), a proven target for the treatment of type 2 diabetes.



The β-aminoamide motif is shared with the sitagliptin (MK-0431), the first marketed DPP-IV inhibitor, and through careful design the indole and indazole groups afforded viable alternatives to the trifluorophenyl group of sitagliptin. Routes were devised to racemic mixtures of indoles (**A**) and indazoles (**B**), and the two series were initially progressed in parallel. From the latter set, PK44 emerged as the lead compound, for which a route to the single enantiomer was developed. PK44 is a potent inhibitor of DPP-IV (IC_{50} 15.8nM), with more than a thousand-fold selectivity over DPP-8 and DPP-9 and ten thousand-fold selectivity over FAP. DPP-8 and -9 have been associated with toxicity. It showed no adverse effects in hERG and cytoxicity screens. In a preliminary oral glucose tolerance assay in mice PK44 showed significant improvements in glucose tolerance.



PK44

LECTURES – **L.36**

SIZE MATTERS: WORKING AS A MEDICINAL CHEMIST IN BIG PHARMA

Dr. Simona M. Ceccarelli

Scientific Specialist, Team Leader, F. Hoffmann-La Roche Ltd., Pharmaceuticals Division, Bldg. 092/7.88, CH-4070 Basel, Switzerland. Tel. +41 (0)61 6884437. Fax. +41 (0)61 6886459

The presentation will detail my own experience of a career in medicinal chemistry, from lab head to group leader, focusing on the challenges and opportunities which are peculiar to large private research organizations Aspects covered will range from seizing the opportunity in an interview to making an impact in a multidisciplinary, multicultural and sometimes multinational team. Gender-specific issues and the combination of career and family will also be discussed.

LECTURES – **L.37**

CAREER EVOLUTION IN THE MED CHEM DEPARTMENT OF A SMALL DRUG DISCOVERY COMPANY

Riccardo Zanaletti

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My experience in medicinal chemistry started six years ago in Siena Biotech, Siena, Italy, where I live and work.

Siena Biotech is a small drug discovery company (150 employees) committed to the development of innovative small molecule therapies for CNS and orphan diseases.

For a medicinal chemist working in such an environment, this gives the opportunity to get in contact with all aspects of drug development; from target identification/validation to Phase1 studies. This is facilitated by the company's size: the bureaucracy is very limited and getting in touch with everyone within the company is very easy.

The career path for a medicinal chemist can be developed both in the lab and outside.

The starting point for a graduate or Ph.D usually starts at the junior scientist level and, with time and experience can progress to higher levels with increasing responsibility for projects and groups. Working in a smaller company involves being very flexible and willing to help with whatever needs to be done. This flexibility brings its own rewards in terms of frustration and excitement. In my talk I will go through these aspects typical of a career path in the medicinal chemistry department of a small drug discovery company.

LECTURES – **L.38**

MICROFLUIDICS IN CHEMISTRY AND BIOLOGY

David M Parry PhD

Cyclofluidic Ltd, BioPark, Welwyn Garden City, Hertfordshire, AL7 3AX UK

Microfluidics is presenting opportunities in discovery research that encompass both chemistry and biology. Synthesis in flow is emerging as a valuable technique for the organic chemist and can be readily carried out on the microfluidic scale. A range of biological assays are currently available on microfluidic platforms and there exists considerable scope for the optimisation of a wide range of conventional microtitre plate format assays to microfluidics.

Cyclofluidic is developing an integrated synthesis and biological assay platform which will enable a rapid serial approach to hit to lead activities within discovery research. This approach offers very considerable advantages in the speed with which SAR data may be generated. In this presentation an overview of some of the challenges of the microfluidic scale will be illustrated, the difficulties of integrating synthesis and biological assay, the inclusion of automated molecular design and selection methods and the very significant time savings that can be achieved utilising an integrated microfluidic approach.

LECTURES – **L.39**

ADVANCES IN MASS SPECTROMETRY BASED SCREENING TECHNOLOGIES

Dr John Crosby[1], Dr Matthew Crump[1], Hannah Maple[1], Dr Richard Taylor[2], Dr Alistair Henry[2], Dr Rachel Garlish[2].

[1]School of Chemistry, University of Bristol, Cantock's Close, Bristol BS8 1TS, UK. [2]UCB Celltech, 208 Bath Road, Slough, SL1 3WE, Berkshire, UK.

The speed and sensitivity of modern mass spectrometers makes them attractive tools for high throughput screening of compound libraries for potential drug candidates. In recent years mass spectrometric techniques that preserve non-covalent interactions have been developed, allowing the composition, stoichiometry, interactions, and dynamics of intact complexes to be examined. Analysing non-covalent protein-ligand interactions by nano-ESI-MS provides complementary information to other established biophysical methods (gel filtration, analytical ultracentrifugation, ITC, SPR, NMR, X-ray). No labeling or immobilisation of the protein or ligand of interest is required, direct stoichiometric measurements can be made, and suitable controls can be introduced to identify non-specific binding. As protein-ligand complexes retain a near native conformation in the gas phase dissociation constants can be accurately determined and compared with results obtained using other techniques.

In collaboration with UCB Celltech we initially screened small compound libraries against lymphocyte function-associated antigen-1 (LFA-1) using an Advion NanoMate linked to an Applied Biosystems QStar™ XL mass spectromer to validate the technique. LFA-1 binds intracellular adhesion molecule-1 and mediates the process of lymphocyte migration during an immune response. Blocking this interaction using small molecule inhibitors has potential for the treatment of rheumatoid arthritis, psoriasis and organ transplant rejection. We showed that binding affinities from low nM through to 250μM could be measured. Drug-like compounds could quickly be screened and then grouped as high, medium or low affinity binders.

Our aim, however, was to set up an automated system which could analyse several hundred compounds in a single run. Using the Advion TriVersa NanoMate we performed automated "on-deck mixing" of protein and ligand before this mixture was sprayed into a Waters LCT Premier™ XE mass spectrometer. Subsequent data collection and processing was automated using MassLynx™ and BiopharmaLynx™ software. Again collaborating with UCB Celltech, we used B-cell lymphoma-2 protein (Bcl-2) and its truncated form Bcl-xL, as protein targets. Both inhibit apoptosis by binding to pro-apoptotic proteins such as Bad and Bak. Binding inhibits the activation of intracellular proteases that are ultimately required for cell destruction. Preliminary MS analysis showed that Bcl-2 exists as a mixture of at least three folded and partially folded conformations, and that a low molecular weight ligand (with a nM K_d) bound to only two of these species. In collaboration with Waters we have run the same Bcl-2 sample on the Synapt HDMS™. This showed that in solution at least four distinct monomeric structures could be identified as well as a dimeric form which could not have been detected on the current MS set up. Using the Synapt HDMS™ we could also see differential ligand binding to both monomeric and dimeric proteins. Additionally, collisional cross section analysis, which provides information on the size and to some extent the globular structure of the proteins, showed that binding of a 16 residue Bak peptide and a 25 residue Bad peptide to Bcl-2 eliminated the unstructured conformations of this protein. To extend our screening studies a library of 150 fragment to scaffold sized compounds was screened against Bcl-xL. K_d values were estimated from the free protein/complex peak heights and ranged from μM to low mM levels. Further work is in progress to calculate K_d values using orthogonal techniques for comparison.

Our research demonstrates the potential of MS based techniques to rapidly screen fragments or drug-like compounds with up to mM binding affinities. This technique has the potential to provide complemetary information to other biophysical methods.

LECTURES – **L.41**

NANOTOOLS FOR BIOMEDICAL TARGETS – NANOBODIES STABILIZE ACTIVE STATE OF G-PROTEIN COUPLED RECEPTORS

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The complex behavior of GPCRs in respons to natural or synthetic ligands and proteins can be attributed to the receptor's structural plasticity manifested in multiple functionally distinct conformational states. Obtaining crystals of GPCRs in an agonist bound, active-state has proven to be challenging due to the instability of this state in the absence of G protein. Here we describe the generation of camelid single-domain antibody fragments (nanobodies) that have G protein like properties towards the human β2 adrenergic receptor (β2AR). To generate receptor specific nanobodies, a llama was immunized with purified agonist bound β2AR reconstituted at high density into phospholipid vesicles. A library of nanobody clones was generated and screened against agonist bound β2AR. Seven clones were identified that recognize the agonist bound but not the inverse agonist bound receptor. One of these nanobodies was found to increase the affinity of β2AR for agonists and to induce conformational changes at TM6, two effects that are indistinguishable from those observed upon adding G protein to the receptor. This nanobody that faithfully mimics the effects of Gs binding was used to obtain diffraction quality crystals and to solve the first structure of an active agonist-bound state of the human β2 adrenergic receptor.

LECTURES – **L.42**

BA SIGNALING AND THE CONTROL OF METABOLISM

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Over the last 5 years, the field of bile acid (BA) research has undergone a considerable evolution (Thomas et al, Nature Rev. Drug Discovery, 2007). Besides their well-established roles in dietary lipid absorption and cholesterol homeostasis, it has recently become clear that BAs are also biological signaling molecules. The first indication in this direction came from the

fact that BAs control their own synthesis in the liver through a feedback inhibitory pathway involving the nuclear receptors farnesoid X receptor (FXR) and short heterodimeric partner (SHP) (Lu et al, Mol Cell, 2000). In addition, we have demonstrated that BAs decrease the hepatic production of triglycerides and very low density lipoproteins (VLDL) via the activation of the same nuclear receptor signaling pathway (Watanabe et al, JCI, 2004). The fact that bile acids could signal beyond the enterohepatic axis came from our work that demonstrated that BAs increase energy expenditure systemically by increasing triiodothyronine levels in rodent brown adipose tissue and human skeletal muscle, thereby preventing obesity and insulin resistance (Watanabe et al, Nature, 2006). This effect is mediated by TGR5, a G protein-coupled receptor, which upon activation by BAs stimulates cAMP production and type 2 deiodinase enzyme activity. These observations build a strong case that BAs have effects beyond the enterohepatic axis and function as systemic metabolic integrators.

In enteroendocrine cells, TGR5 has been associated with BA-mediated secretion of glucagon-like peptide 1 (GLP-1). GLP-1 is an incretin with potent antidiabetic effects, due to its ability to enhance pancreatic β-cell function. Although the link of TGR5 in BA-mediated GLP-1 secretion has been established *in vitro*, no evidence exists at present that this is also important *in vivo*. We therefore hypothesized that TGR5, in addition to its role in energy balance, may also have a role in the control of glucose homeostasis. To evaluate the role of TGR5 in glucose metabolism and its contribution to type 2 diabetes (T2D), we generated both mice that are overexpressing TGR5 by BAC transgenesis (TG1-TGR5 mice) and mice with a germline deficiency of the TGR5 gene (TGR5-KO mice), and performed a comprehensive metabolic profiling on both control (CT), TG1-TGR5, and TGR5-KO cohorts fed a normal chow (CD) or a high-fat (HFD) diet. Our results demonstrate that TG1-TGR5 mice are protected against diet-induced glucose intolerance, whereas TGR5-KO mice are glucose intolerant under these conditions. The improved glucose tolerance observed in HFD challenged TG1-TGR5 mice cannot be attributed to a decrease in body fat mass, but is rather the result of improved insulin release. We further show that this effect is mediated by GLP-1, which is increased after an oral challenge with glucose or a high glucose/high lipid meal. In the pancreas, β-cell function of TG1-TGR5 mice is improved compared to those derived from control littermates. Most of these beneficial metabolic effects seen in the TG1-TGR5 transgenic mice are recapitulated upon the administration of 6α ethyl, 23(S)-methyl Cholic Acid, a semi-synthetic BA that selectively and potently activates TGR5, and are lost in TGR5-KO mice (Thomas et al, Cell Metabolism, 2009).

Collectively, these data demonstrate that the TGR5 locus is a critical region to maintain normal pancreatic β-cell function and identify TGR5 as an attractive and potential target for the treatment of T2D.

LECTURES – **L.43**

TESTING THE CONCEPT OF ACC INHIBITION FOR THE TREATMENT OF METABOLIC DISEASE

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Acetyl-CoA carboxylases (ACC1 and ACC2) play an important role in lipid pathways. ACC1 is primarily expressed in lipogenic tissues (liver and adipose), whereas ACC2 is highly expressed in oxidative tissues (muscle, heart, and liver) and recently also found to be abundant in human white adipose tissue. ACCs catalyze the carboxylation of acetyl-CoA to produce malonyl-CoA, a metabolic control signal acting as both a substrate for *de novo* lipogenesis and as an allosteric inhibitor of carnitine palmitoyltransferase1 (CPT-1) thereby blocking fatty acid entry into the mitochondria for oxidation. Consequently, inhibition of ACC is expected to lower malonyl-CoA levels resulting in reduced fatty acid synthesis and increased fatty acid oxidation, leading to weight loss and improved insulin sensitivity. Hence, we sought to discover inhibitors of ACC2 to increase oxidation of fatty acids in mitochondria. A high throughput screen of the AstraZeneca in house compound collection resulted in the identification of several weak inhibitors of ACC2. Exploration of the initial hits led to a lead compound with greatly improved potency. Further efforts to optimise the series by modulating lipophilicity to improve the physical property and ADME profile whilst maintaining potency will be described. Finally, the ability of these compounds to inhibit ACC2 and reduce malonyl-CoA levels *in vivo* and the relationship between the pharmacokinetic and pharmacodynamic profile will be shown.

LECTURES – **L.44**

DISCOVERY OF A NOVEL GUT-TARGETED MICROSOMAL TRIGLYCERIDE TRANSFER PROTEIN INHIBITOR (JNJ-16269110) TO TREAT OBESITY: PROOF OF CONCEPT

L. Meerpoel, L. Backx, L. Van der Veken, M. Viellevoye, E. Fraiponts, K. De Waepenaert, E. Clessens, H. Borghys, P. Moelans, K. Wuyts, R. Gilissen, N. Bode, A. Van Peer, G. Mannens, L Lammens, D. Verstraeten, E. Wajs, F. Vercruysse, L. Van Nueten, D. De Chaffoy de Courcelles and P. Roevens*.*

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Microsomal Triglyceride Transfer Protein (MTP) is a lipid transfer protein that is involved in the assembly of very low density lipoproteins (VLDL) in the liver and chylomicrons in the gut wall. Genetic loss of functional MTP is the basis of the human recessive disorder called abetalipoproteinemia. This disease results from an inability of both the liver and intestine to secrete respectively VLDL and chylomicrons leading to extremely low plasma lipid levels and malabsorption of dietary fat.

JNJ-16269110 (R256918) is a methylester that has been chemically designed to remain intact in the gut wall and to inhibit lipid absorption, but to be rapidly degraded into inactive metabolites in the liver. It was hypothesised that this might help to avoid fat accumulation in the liver, a class effect observed with slowly metabolized MTP inhibitors. JNJ-16269110 is a potent and selective inhibitor of MTP *in vitro*, inhibiting human, dog, and rat MTP with IC_{50} values of 3.4, 3.3, and 2.8 nM, respectively. JNJ-16269110 also displays good cellular potency inhibiting apolipoprotein B (ApoB) secretion from HepG2 cells with an IC_{50} of 15.9 nM. Its acid and N-dealkyl metabolite do not inhibit MTP. With the aim to minimize inhibitory activity in the liver, the selection of JNJ-16269110 was based on its weak metabolic stability *in vitro* in human and dog hepatic S9 fractions, but high metabolic stability in corresponding intestinal S9 fractions.

A dose-dependent inhibition of the postprandial increase of plasma triglycerides (PPTG) was observed in dogs after dosing with JNJ-16269110. Selectivity for gut wall versus liver fat accumulation was shown histologically using Oil-Red-O staining of intracellular lipid droplets.

Because of its unique pharmacodynamic, pharmacokinetic and safety profile, JNJ-16269110 is currently investigated in clinical trials as a novel treatment of obesity. To determine the clinically relevant dose range, inhibition of PPTG was documented after a high-fat meal in ascending dose Phase I studies. Subsequently, two 12 weeks, double blind, randomised, placebo controlled, multicenter, parallel group Phase 2 studies conducted in obese/overweight subjects (BMI > 30 kg/m^2 and < 50 kg/m^2) demonstrated a dose-dependent weight loss. Importantly, in one of these studies the primary efficacy endpoint was mean changes in hepatic triglyceride content (HTGC) from baseline to weeks 6 and 12, measured by 1H magnetic resonance spectroscopy (MRS), following treatment with placebo or 10 or 15 mg JNJ-16269110 twice daily. The point estimates for mean change from baseline in HTGC were -2.78, -2.81, and -3.83% in the placebo and 10 and 15 mg JNJ-16269110 dose groups, respectively, at week 12. The changes in HTGC were not statistically significant (p=0.968 and p=0.116 for mean difference versus placebo for the 10 mg and 15 mg dose groups, respectively). The association of weight loss with HTGC reduction was apparent in all the treatment groups.

These results demonstrate clinically that JNJ-16269110 in contrast to slowly metabolised MTP inhibitors can indeed block intestinal MTP resulting in weight loss, without inducing hepatic fat accumulation. It is expected that this will help to reduce fat-mediated adverse events in the liver.

LECTURES – **L.45**

DISCOVERY OF A SECOND GENERATION FBPASE INHIBITOR, MB07803, WITH IMPROVED ORAL BIOAVAILABILITY

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MB06322 (CS-917, a prodrug of FBPase inhibitor MB05032) significantly lowered fasting plasma glucose levels in patients with type 2 diabetes (T2DM) during two phase 2a clinical trials. The second generation program sought to improve a number of properties of the prodrug including oral bioavailability and efficiency of conversion of to the active phosphonic acid. Optimization of prodrugs of a novel lead compound in the thiazole FBPase inhibitor series (MB07729, human FBPase IC_{50} 21 nM) led to phosphonic diamide MB07803, which demonstrated good to high OBAV in rats and monkeys (30-40% and 50-60%, respectively) and efficient conversion to MB07729. MB07803 elicited marked glucose-lowering effects in rodent models of T2DM (e.g. db/db mice and ZDF rats, MED of 10-30 mg/kg) and in normal fasted monkeys (MED = 3 mg/kg). In a phase 1 clinical trial, MB07803 was well tolerated and led to dose-linear exposure of the active FBPase inhibitor MB07729.

LECTURES – **L.46**

EXAMPLES FOR RECENT SUCCESSES IN DRUG DISCOVERY

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The implementation of dynamic and complementary Lead Identification strategies are a prerequisite for successful design and selection of clinical candidate molecules which will ultimately enable clinical outcome trials. At Roche, multiple approaches are used in an unbiased manner. This talk will outline those strategies in greater detail. Two project examples will be given that yielded development candidates which are currently undergoing clinical phase 3 trials. A final outlook into future directions of Discovery Chemistry and alternative lead finding approaches will be given for small molecules that may enable i) intracellular delivery of macromolecules into cytosol, ii) RNA as drug target for successful gene regulation, and iii) regenerative medicine through differentiation of pluripotent stem cells.

LECTURES – **L.47**

FRAGMENT-BASED LEAD DISCOVERY FOR LECTIN TARGETS

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Although the enormous potential of carbohydrates as lead structures for drug discovery has been uncovered within the last two decades, carbohydrates are still a relatively untapped source of new drugs and therefore offer exciting new therapeutic opportunities.

The reasons for the disappointingly slow progress are manifold. First, physiological carbohydrate-lectin interactions are notoriously weak, *i.e.* in the μM to mM range. Second, the molecular dynamics of the binding process of carbohydrates to lectins is still poorly understood. Furthermore, the pharmacokinetic hurdles, which are an inherent problem of carbohydrates and mimetics thereof, have been completely neglected. Finally, the development of drugs from carbohydrate leads suffers from complex syntheses, which hampers lead optimization and scale-up of potential drug candidates.

In the first part of the lecture, the pitfalls of the classical lead-to-candidate search for carbohydrate-based antagonists are discussed using the example of a MAG (myelin-associated glycoprotein) antagonist. In the second part, a new *fragment-based in situ combinatorial approach* leading to high affinity ligands of siglecs and selectins is presented. Finally, the problem of the drug unlikeness of the pharmacokinetic properties of most carbohydrates and mimetics thereof is addressed.

LECTURES – **L.48**

ENSEMBLINS™: A NEW CLASS OF THERAPEUTIC MACROCYCLES

Nicholas K. Terrett

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Macrocycles are found widely in nature where they fulfill numerous specific biological functions. However they have been generally underexploited as drug molecules, as they are larger than more conventional 'Rule of 5'-compliant molecules and their synthesis and screening has been considered a challenge.[1] Consequently, most pharmaceutical companies have very few macrocycles in their screening files, and yet these compounds can have potent and selective pharmacological activity, and exhibit drug-like properties.

In order to permit the further investigation of macrocyclic compounds, Ensemble Discovery has developed two complementary platforms for the rapid synthesis and screening of Ensemblins and are currently using these macrocycles for the discovery of lead molecules for challenging protein-protein interaction targets. The platform incorporates both a DNA-programmed chemistry (DPC) approach to compound libraries as well as more conventional synthetic and medicinal chemistry. DPC permits the synthesis of hundreds of thousands of macrocyclic molecules using predefined DNA sequences to control individual synthetic steps. The process allows for the step by step purification and analysis of complex mixtures of intermediates and final macrocyclic products.

The macrocycle collections have been used successfully for the discovery of compounds that interact with a number of important drug discovery targets, including the oncology target BCL-XL. Ensemble Discovery has shown that these small molecule macrocycles have good drug-like properties including solubility, membrane permeability and oral bioavailability.

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LECTURES – **L.49**

FERROQUINE, THE ARCHETYPE COMPOUND OF BIOORGANOMETALLIC CHEMISTRY

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The most known natural organometallic molecule is vitamin B12, a porphyrin containing a cobalt atom, useful for several enzymatic transformations. Based on the remarkable properties of this class of compounds, a new area of medicinal research was developed. Gérard Jaouen was the first to introduce the term of "bioorganometallic chemistry" in 1985. Bioorganometallic chemistry consists on the synthesis and the study of organometallic complexes, complexes with at least one metal-carbon bond, in a biological and medicinal interest.

Ferroquine (FQ, SSR97193) is a unique ferrocenic drug candidate emerging from a french collaborative discovery project. This collaboration of chemists and biologists has investigated a number of new compounds that could be developed as potential antimalarials. FQ is currently the most advanced project on malaria drugs developed by Sanofi Aventis. This organometallic compound is extremely active against both chloroquine-sensitive and chloroquine-resistant *Plasmodium falciparum* and is currently in phase II of clinical trials.

This oral presentation will focus on the innovative metallo-drug design which has led to the discovery of FQ. Then, discussion will be directed towards its mechanism(s) of action. Finally, we will comment on the impossibility to develop a resistance to FQ.

LECTURES – **L.50**

TARGETED NANOMEDICINES - RECENT PROGRESS IN EC-SPONSORED RESEARCH

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A major limitation impeding the entry of targeted drug delivery systems into the clinic, is that new concepts and innovative research ideas within academia are not being developed and exploited in collaboration with the pharmaceutical industry. To improve that situation, MEDITRANS is a multidisciplinary Integrated Project sponsored by the European Commission for applied research on targeted drug nanoparticulate delivery systems. The MEDITRANS consortium consists of 30 partners from 9 EU member states (including 1 new member state) and 3 associated states, and includes 13 industrial companies, 11 universities and 6 research institutes. Within this project with a total budget of over 16 Million euro (the EC grant being 11 Million euro), platform technologies are being developed with broad applicability to disease treatment, as exemplified by the choice for chronic inflammatory disorders (i.e. rheumatoid arthritis, Crohn's disease and multiple sclerosis) and cancer as target pathologies. The main objectives of this large FP6 project are I) to design and develop novel and highly efficient delivery systems for drugs and imaging agents; II) to evaluate the targeting behaviour and the efficacy of these systems both in vitro and in vivo; and III) to advance several selected formulations towards industrial exploitation and clinical application.

Research efforts within MediTrans have been structured around nanocarrier materials classified as Emerging Materials, Candidate Materials and Established Materials. In this lecture, we summarize some of the progress made as part of the MEDITRANS project in the past three years. In addition, we describe some of the experimental evidence obtained with regard to systems selected for preclinical toxicity analysis and industrial exploitation, i.e. core-crosslinked polymeric micelles, liposomal corticosteroids, gadolinium-containing liposomes and iron oxide nanoparticles. With its integrated 'bench-to-clinic' approach - realised within a structural collaboration between industry and academia - MEDITRANS is well on its way to promote the progression of several selected nanomedicine formulations towards clinical application.

LECTURES – **L.51**

THERAPEUTIC NANOPARTICLES FOR IMPROVED AND SAFE DELIVERY OF DRUGS

L. Juillerat-Jeanneret et al.

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The targeted delivery of chemotherapeutic agents to defined cells across biological barriers is presently one of the main challenge in the development of treatment strategies, including the use of nanoparticles in nanomedicine. Some biocompatible polymer-coated nanoparticles encapsulating the therapeutics in their hydrophobic/hydrophilic core for the delivery of therapeutic agents or for disease imaging are in clinical use. The next goals in the field of targeted therapeutic nanoparticles will be to better understand the interaction of nanovectors with living tissues and achieve selective delivery of drugs to defined cell targets. This presentation will first review what is presently available as therapeutic nanovectors and functionalized drug vectors under development able to target defined cells via specific recognition mechanisms. Then the properties of the polymers used to deliver drugs will be discussed from the point of view of their fate in cells, including the problems associated with the little information of their pathways of degradation and the potential cytotoxicity of their degradation products. Finally, the recent development, not yet in clinical use, that are being explored to further increase the selectivity and safety of delivery of drugs with nanoparticles will be presented using two examples of chemical functionalization of drug nanovector systems, covalent drug-functionalization of superparamagnetic iron oxide nanoparticles and photosensitizing polymeric nanogels, that we have developed for targeted delivery of therapeutic agents and the analysis of the impact of the vectors on cell functions.

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LECTURES – **L.52**

THIOMER MICRO- AND NANOPARTICLES IN DRUG DELIVERY

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Thiolated polymers or designated thiomers are gained by immobilization of sulhydryl-bearing ligands on the polymeric backbone of well-established polymers such as chitosan and poly(acrylates). This functionalization leads to significantly improved properties compared with the corresponding unmodified polymers. Mucoadhesive properties are strongly improved by the formation

of disulfide bonds between thiol groups of the thiomer and cysteine-rich glycoproteins of the mucus gel layer. Moreover, strongly improved enzyme- and efflux-pump inhibitory and permeation enhancing properties are gained by thiolation. These thiomers can be formulated to micro- and nano-particlulate delivery systems via various techniques, such as in situ gelation and subsequent covalent crosslinking, radical emulsion polymerization, emulsification/solvent evaporation, high pressure homogenization or air jet milling. As thiomer micro- and nano-particles were shown to exhibit the same features as thiolated polymers per se, their great potential in particular for non-invasive delivery of hydrophilic macromolecular drugs such as therapeutic peptides and efflux pump substates such as anticancer drugs could be demonstrated in numerous in vivo studies.

LECTURES – **L.53**

THE DISCOVERY OF A SMALL MOLECULE INHIBITOR OF IGF-1R

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Considerable interest in the pharmaceutical industry has been generated around the challenges of developing inhibitors of IGF-1R. As monoclonal antibodies targeting the extra-cellular binding domain of IGF-1R have advanced, the clinical potential of this target is being realized. Following closely on the heels of these advances, small molecule inhibitors are also being investigated in the clinic. This presentation will describe the lead opitimization efforts to address the development issues surrounding a series of 1*H*-(Benzoimidazol-2-yl)-1*H*-pyridin-2-ones pyridine inhibitors and how a discovery strategy was developed that eventually led to the clinical candidate, BMS-754807. The preclinical characterization of this novel pyrrolo [1,2-f][1,2,4]triazine will be presented along with efforts to identify a potential backup.

LECTURES – **L.54**

INHIBITORS OF THE MDM2-p53 INTERACTION

Lutz Weber

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p53 has been in the centre of advertency for drug design since the discovery of its growth suppressive and proapoptotic activity. In this talk we report the design and characterization of a new classes of competitive protein-protein interaction inhibitors for the MDM2-p53 interaction.

Our identification of drug-like and selective inhibitors of this protein-protein interaction included: a straightforward in silico compound selection process, a recently reported NMR spectroscopy approach for studying the MDM2-p53 interaction, as well as selectivity screening assays using cells with the same genetic background. The selected inhibitors were all able to induce apoptosis and the expression of p53 related genes, but only few inhibitors stabilized p53.

Our NMR experiments give a persuading explanation for these results, showing that isoquinolin-1-one derivates are able to dissociate the preformed MDM2-p53 complex in vitro, releasing a folded and soluble p53. The joint application of these methods provides a framework for the discovery of protein interaction inhibitors as a promising starting point for further drug design.

Highly potent and selective MDM2-p53 inhibitors have also been used to study more extensively the biological role of both Mdm2 as well as p53. Especially during the last 5 years the picture has become more complicated than estimated in the beginning: MdmX can replace Mdm2, p73 can partly take over the role of p53. Interesting connections have been observed with the Rb axes of cell cycle control and apoptosis. We will try to give an updated picture on these interactions and their potential implications for developing MDM2-p53 inhibitors as novel therapeutic agents.

LECTURES – **L.55**

DISCOVERY OF A POTENT AND SELECTIVE SMO INHIBITOR

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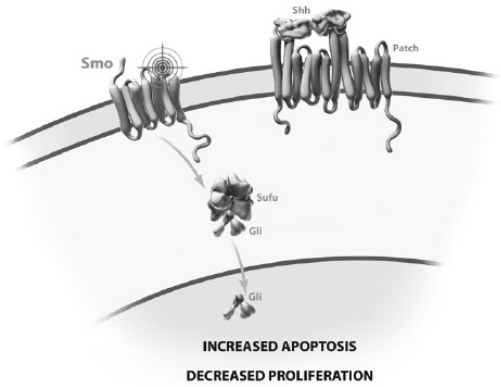
The Hedgehog (Hh) signaling pathway plays a critical role in the development and homeostasis of many organs and tissues. Research in recent years shows that this pathway can have a crucial role in tumorigenesis when reactivated in adult tissues by genetic activation or other mechanisms. Genetic activation of the Hh pathway at or upstream of Smoothened is implicated in cancers such as basal call carcinoma and medulloblastoma. Several academic groups and pharmaceutical companies are targeting this pathway and developing Hh inhibitors into cancer therapeutics.



Cell-based phenotypic screens performed in our laboratories identified multiple new classes of Hh inhibitors via antagonism of the Smoothened (Smo) receptor. Structure-activity relationship studies led to the discovery of potent and selective Smo antagonists which were profiled in vivo. Selected inhibitors displayed a good pharmacokinetic profile and showed efficacy in genetic mouse models of medulloblastoma. Regression was observed in both a subcutaneous and orthotopic medulloblastoma model. One of them, LDE225, is currently in clinical development for the topical treatment of superficial basal cell carcinoma (BCC) and the systemic treatment of BCC and medulloblastoma. Continued clinical development of LDE225 is supported by early evidence for its activity.

LECTURES – **L.56**

FROM NATURAL PRODUCTS TO MITOCHONDRIAL TARGETING AGENTS

Peter Wipf

Center for Chemical Methodologies and Library Development, University of Pittsburgh, Pittsburgh, PA

This seminar will highlight our current small molecule discovery and development approaches in neurodegeneration, inflammation and radiation protection based on mitochondrial targeting agents.

Mitochondria are key organelles that perform essential cellular functions and play pivotal roles in cell death and survival signaling. Hence, they represent an attractive target for drugs to treat metabolic, degenerative and hyperproliferative diseases. Targeting mitochondria with organelle-specific agents or prodrugs has proven to be an effective therapeutic strategy. More specifically, controlling the cellular ROS balance *via* selective delivery of an antioxidant "payload" into mitochondria is an elegant emerging therapeutic concept. Based on the natural antibiotic gramicidin S, we have developed several mitochondrial targeting scaffolds, to which we have attached active agents that modulate mitochondrial biochemistry. This talk will present the recent medicinal chemistry and clinical data of these exploratory strategies which should point the way for future generations of therapeutics.

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LECTURES – **L.57**

MARINE NATURAL PRODUCTS AS DRUG SOURCE

Sveta Savina, Paolo Bruno, Pau Ruiz-Sanchis, Xavier Just, Adriadna Lorente, Fernando Albericio, Mercedes Álvarez

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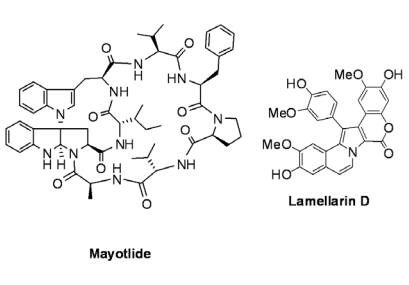
Marine organisms have developed complex biological mechanisms for survival, such as production of potent natural chemicals that may be used in their defense. The sea, with its immense biological diversity, offers a rich hunting ground for the identification of chemical compounds for the effective treatment of cancer among other diseases. Around 10 % of the extracts of these marine invertebrates exhibit cytotoxic activity against different tumour cell lines. Because only small quantities of natural product can be produced by isolation procedures, there is a need for the development of efficient methods for the synthesis of these interesting compounds, to facilitate their biological evaluation.

Our work in this area is focused on developing new synthetic strategies for the preparation of active peptide and polyheterocyclic nitrogen-containing marine natural products. The synthetic route should be versatile for the preparation of derivatives for pharmacological evaluation. In this sense our recent synthetic achievement in the total synthesis of natural products as mayotlide, lamellarines, CH0035 or thiopeptides will be presented.



Several aspects of drug delivery we have been focusing during the last years, as well as, the mechanism of action of the most potent compounds will be remarked.

LECTURES – **L.58**

ANTIBIOTIC CLUSTERS

Pál Herczegh

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Starting from ristocetin aglycon and teicoplanin pseudoaglycon (N-acetyl-D-glucosaminyl aglycoteicoplanin) lipophilic derivatives have been prepared using a diazo transfer-1,3-dipolar cycloaddition route. Some of the new teicoplanin derivatives showed good antibacterial activity against Gram positive strains including vanA enterococci. This excellent activity can be explained by the aggregation, cluster forming ability of such antibiotics. This phenomenon has been studied with various physical methods.

New sugar derivatives of aglycoristocetin displayed not only high antibacterial activity but some of them were found effective antivirals against H1N1 subtype of influenza A virus.

LECTURES – **L.59**

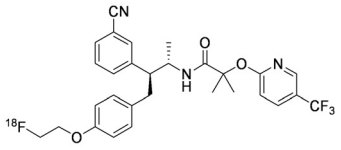
PET IMAGING IN THE DISCOVERY OF NEW THERAPEUTICS

Terence G Hamill

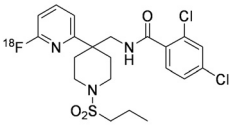
Molecular Imaging Dept., Merck Research Laboratories, West Point, PA, USA

Positron Emission Tomography (PET) is a quantitative, noninvasive imaging technique that can directly measure target engagement. This *in vivo* imaging modality is directly translatable from preclinical to clinical applications, and can be used to guide clinical drug development. Molecules labelled with short-lived, positron emitting isotopes are required and two of the more common isotopes used are carbon-11 (20 minute half life) and fluorine-18 (110 minute half life). With limited reagents available to the PET radiochemist and the time constraints inherent in working with these isotopes, the types of labelling chemistry that are possible and the types of functional groups that can be labelled are restricted. Designing routes for labelling PET tracers must take these and other constraints into consideration. Despite these limitations, a wide variety of molecules can be labelled with either carbon-11 or fluorine-18.

PET can be used to guide drug development by measuring the level of target (ie receptor, enzyme, transporter) occupancy by a therapeutic candidate. This ensures that adequate target engagement is achieved for testing the fundamental hypothesis in Phase II clinical trials so that Go/No Go decisions can be made with confidence about the compound or the mechanism. The design, radiosynthesis and application of the cannabinoid CB1 PET tracer, [^{18}F]MK-9470 and the glycine transporter 1 PET tracer, [^{18}F]MK-6577 in clinical studies of CNS drug candidates will be discussed.



[^{18}F]MK-9470



[^{18}F]MK-6577

LECTURES – **L.60**

THE NEXT GENERATION OF PET CARDIAC IMAGING AGENTS: DISCOVERY OF FLURPIRIDAZ F 18 FOR DETECTION OF CORONARY DISEASE AND LMI1195 FOR SYMPATHETIC NEURONAL IMAGING

Simon Robinson

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Coronary artery disease, heart failure or arrhythmia affects millions of people each year. Myocardial perfusion imaging with SPECT agents has revolutionized the detection of cardiac disease with millions of scans performed each year. Positron Emission Tomography (PET) is a very powerful non invasive imaging technique that has been used for oncology, CNS and cardiovascular disease detection and diagnosis. Some short lived positron emitting agents have demonstrated that PET imaging can further improve myocardial perfusion assessment, but their rapid decay prevents wide spread use.

In the first part of the presentation I will briefly outline the principle of perfusion imaging and the status and limitations of the current agents followed by a summary our new [^{18}F] fluorinated small molecule, flurpiridaz F 18. This will include the SAR for flurpiridaz F 18, the improved cardiac extraction and distribution characteristics and initial clinical evaluation.

In the second part of the presentation the development of an 18F labeled substrate for the norepinephrine transporter (NET) will be described. This will include non-clinical studies to demonstrate selectivity for the sympathetic nervous system and ability to image regional cardiac denervation. Initial clinical evaluation will also be described.

LECTURES – **L.61**

PET IMAGING FOR ONCOLOGY

Christer Halldin

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PET (positron emission tomography) is a unique molecular imaging technique, which is noninvasive and provides quantitative in vivo assessment of physiological and biological processes. PET has been accepted in common practice for the management of various cancers. Though fluorine-18 fluorodeoxyglucose (FDG) is the most widely used tracer in clinical oncology (more than 95% of the molecular imaging procedures make use of FDG at present), there are many non-FDG PET radiotracers, which has shown promising results in the managements of various cancers where FDG has a limited role. These radiotracers are more specific as their mechanism of uptake is based on other than glucose metabolism. In the near future, more non-FDG PET tracers will likely be investigated exploring various tumor pathophysiology. Oncological non-FDG PET tracers can be divided into three major groups: those labeled with fluorine-18, carbon-11 and other non-FDG tracers. Fluorine-18 and carbon-11 are labeling the following targets: different aminoacids, substrates involved in fatty acid synthesis, protein synthesis, amino acid transport substrate, and tracers linked to nucleic acid synthesis as well as specific tracers for receptor imaging. Non-FDG radiotracers can be labeled with Gallium-68, Cupper-64, etc and are aimed to detect cell hypoxia, bone metabolism and receptors. These tracers have more specific mechanism of uptake and is likely to be investigated more in the near future. PET and during the recent years PET/CT have stood out in the management of many malignancies. The future will bring advances in the daily use of this part of molecular imaging, by the addition of non-FDG tracers, and by technological improvements such as improved detectors allowing faster image acquisition and new applications.

LECTURES – **L.62**

ADVANCES IN BACTERIAL FATTY ACID SYNTHESIS INHIBITORS FOR USE AGAINST *TOXOPLASMA GONDII* AND *BACILLUS ANTHRACIS*

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The problem of increasing bacterial resistance to the current generation of antibiotics is well documented. Known resistant pathogens such as methicillin-resistant Staphylococcus aureus are becoming more prevalent, while the potential exists for developing drug-resistant pathogens for use as bioweapons, such as Bacillus anthracis. The biphenyl ether antibacterial agent, triclosan, exhibits broad-spectrum activity by targeting the fatty acid biosynthetic pathway through inhibition of enoyl-acyl carrier protein reductase (ENR) and provides a potential scaffold for the development of new, broad-spectrum antibiotics. We used a structure-based approach to develop novel aryl ether analogues of triclosan that target ENR, the product of the fabI gene, from B. anthracis (BaENR). Structure-based design methods were used for the expansion of the compound series including X-ray crystal structure determination, molecular docking, and QSAR methods. Structural modifications were made to both phenyl rings of the 2-phenoxyphenyl core. A number of compounds exhibited improved potency against BaENR and increased efficacy against both the Sterne strain of B. anthracis and the methicillin-resistant strain of S. aureus. X-ray crystal structures of BaENR in complex with triclosan and two other compounds help explain the improved efficacy of the new compounds and suggest future rounds of optimization that might be used to improve their potency.

Moreover, Toxoplasmosis (Figure 1A) has long been known to cause substantial morbidity and mortality, especially in persons who are congenitally infected or immune-compromised, and this parasite is the most frequent infectious cause of uveitis. *T. gondii* is acquired as a sporozoite from oocysts formed in cats or bradyzoites from cysts in meat. In humans, this parasite has a simple life cycle consisting of two stages; tachyzoites and bradyzoites. This presentation will discuss the extension of the BaENR work to toxoplasmosis, as two of the triclosan related compounds were found to show low toxicity and activity in the low nM range. A promising adaptation to the triclosan scaffold is the addition of an *n*-propyl group at the 4-position, which was designed to exploit an increase in space in the parasitic ENR binding pocket (Figure 1B). The idea was validated by a co-crystal structure of one of the analogs in complex with TgENR, which was determined to a resolution of 2.6Å. Lastly, an entirely new series of NCEs for treating toxoplasmosis was discovered through HTS, and these structures and their activity will be presented here.

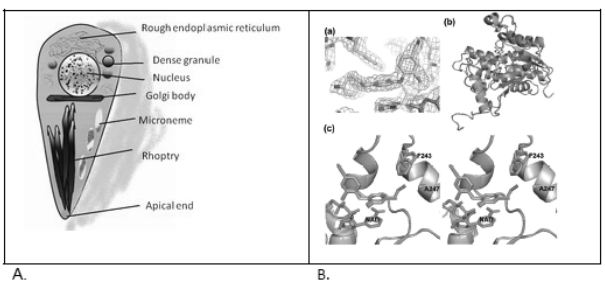


Figure 1. A) Diagram of Toxoplasma gondii structure (from Wikipedia); B) Co-crystal structure of a triclosan analog in complex with TgENR

LECTURES – **L.63**

BENZOXABOROLES AS ANTI-INFECTIVE AGENTS

SJ. Baker[1], YK Zhang[1], V Hernandez[1], T Akama[1], A Lau[1], H Zhou[1], JJ Plattner[1], FL Rock[1], W Mao[1], MRK Alley[1], YR Freund[1], V Sanders[1], K Maples[1], K Hold[1], SJ Benkovic[2], L Shapiro[3], T Crépin[4], S Cusack[4], X Hui[5], HI Maibach[5], R. Mendes[6], D. Biedenbach[6], A. O'Leary[7]

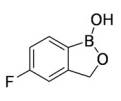
[1]Anacor Pharmaceuticals, Inc, Palo Alto, CA, USA. [2]Pennsylvania State University, University Park, PA, USA. [3]Stanford University, Stanford, CA 94305, USA. [4]European Molecular Biology Laboratory, Grenoble, France. [5]UCSF, San Francisco, CA, USA, [6]JMI Laboratories, North Liberty, IA, USA, [7]Ricerca Biosciences, Concord, OH, USA

By exploiting their unique chemical properties, novel boron-containing small molecules are being developed as anti-fungals, anti-inflammatories and antibiotics.

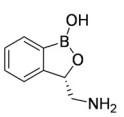
Benzoxaboroles AN2690 and ABX inhibit leucyl-tRNA synthetase (LeuRS) in microorganisms resulting in inhibition of protein synthesis and cell growth. The boron forms a spiro complex with 2'- and 3'-hydroxyl groups of the terminal adenosine of substrate tRNA in the editing active site thus trapping tRNA onto the enzyme. Inhibition of LeuRS by binding to the editing active site is an unprecedented mechanism of action for an antimicrobial agent.

This presentation will first describe the research effort that led to AN2690, a novel antifungal agent currently in late-stage clinical development for the topical treatment of onychomycosis. AN2690 was determined to be an inhibitor of fungal cytoplasmic LeuRS and X-ray crystallography with a bacterial homologue revealed how it bound in the editing active site trapping substrate tRNA. Using this co-crystal structure of AN2690 to the bacterial LeuRS, compound ABX was subsequently designed and this compound exhibited good Gram-negative antibacterial activity. In part due to its novel mechanism, it has good activity against known clinical resistant strains including multi-drug resistant bacteria. ABX has demonstrated *in vivo* efficacy in mouse models of *E. coli* and *K. pneumoniae* infection. This research effort has now led to a LeuRS inhibitor in clinical development for bacterial infections.

Boron-containing small molecule inhibitors of microbial leucyl tRNA synthetase (LeuRS)



AN2690
A Novel Antifungal Agent in Clinical Development for Onychomycosis



ABX
A Novel Gram Negative Antibacterial Agent

LECTURES – **L.64**

DISCOVERY OF TMC-207, A NOVEL ANTITUBERCULOSIS AGENT

Jérôme Guillemont

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Tuberculosis (TB) is the second leading cause of death from infectious disease after HIV/AIDS. More than 2 billion people of the world's population currently have latent tuberculosis and 10% of this population will develop active tuberculosis. In 2008, the World Health Organization (WHO) estimated that there were 9.4 million new TB cases and 1.8 million deaths from TB around the world particularly in Asia and Africa. The current daily treatment for TB is a combination of 3 or 4 antibiotics comprising isoniazid (H), rifampin (R), pyrazinamide (Z) and /or ethambutol (E) for two months followed by 4 months of daily doses of (H) and (R). The lack of compliance led to the emergence of multi drug resistant (MDR) and extensively-drug resistant (XDR) tuberculosis strains. In addition to MDR and XDR impact, the co-infection with human immune deficiency virus (HIV) has increased the urgency to treat this chronic disease. Since the introduction of rifampicin in the 1960s, no breakthrough development has occurred in the four last decades.

We discovered and optimized at Johnson & Johnson Pharmaceutical Research & Development (J&J-PRD) a novel class of antimycobacterial agents, the diarylquinolines (DARQ), highly active against both drug-susceptible *M.tuberculosis* and MDR-TB. Structure-activity relationship of DARQs led to the selection of TMC207 which is currently in phase IIb clinical trials for MDR-TB[(1)]. TMC207 (R207910) has a unique mechanism of action; it acts by specifically inhibiting mycobacterial ATP synthase [(2)].

The purpose of this presentation is to discuss the key steps of TMC207 discovery.

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LECTURES – **L.65**

HDAC INHIBITORS ABLE TO REDUCE ACQUIRED ANTIFUNGAL RESISTANCE

Antonello Mai

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After the approval of vorinostat (suberoylanilide hydroxamic acid, SAHA) and romidepsin (depsipeptide, FK-228) for the treatment of cutaneous T cell lymphoma (CTCL), a number of HDAC inhibitors (HDACi) are currently in Phase II or III clinical trials (as single agent or in combination) for the treatment of a great number of tumors. In addition to these cancer uses, HDACi can be successfully used in non-cancer diseases. Here we focused on the uses of HDACi in some infectious diseases, such as *C. albicans*, HIV-1, and *P. falciparum* infections. In *C. albicans* cultures, HDACi increased the frequency of cell switching (a relevant virulence trait) in the white-to-opaque transition, reduced the azole trailing effect through reduction in azole-dependent upregulation of *CDR* and *ERG* genes, and inhibited the fluconazole-dependent resistance induction. Moreover, they inhibited germination in several strains, and caused 90% reduction in the adherence of *C. albicans* to human cultured pneumocytes. In HIV-1-infected cells, the treatment with HDACi reactivates the HIV-1 expression in latent cellular reservoirs. Thus, the use of HDACi as adjuvant to highly active antiretroviral therapy (HAART) can represent a new potential therapeutic strategy to eradicate the viral infection. A number of HDACi have been reported as active against *P. falciparum* infection. Two recent papers show some 2-aminosuberic acid-based compounds as well as a series of phenylthiazolyl suberoyl hydroxamates as very potent and selective antimalarial agents. These non-cancer applications of HDACi will be discussed at the meeting.

LECTURES – **L.66**

RENAISSANCE OF COVALENT INHIBITORS IN DRUG DISCOVERY

Tjeerd Barf

MSD, Oss, The Netherlands

Based on historical achievements, covalent binding inhibitors deserve more attention. Numerous drugs based on this principle have successfully conquered the market and benefit patients with a variety of disorders. Much of the concerns associated with covalent binding are slowly being abandoned, and the pharmaceutical industry is cautiously embracing this concept again. The general concept of covalent inhibition can be widely applied to the majority of the protein target families, and is no longer limited to enzyme families with activated nucleophilic amino acid residues like in proteases.

Two major classes can be discerned: covalent reversible and covalent irreversible inhibitors. These inhibitors can offer distinct advantages in terms of potency, selectivity and (if irreversible) prolonged in vivo efficacy since the duration of action becomes a function of target protein turnover, rather than the pharmacokinetic profile of the inhibitor. Potential liabilities such as immunogenic- and toxic events are a serious downside, and have to be considered when embarking on the covalent binding principle. The choice of the appropriate warhead is of paramount importance, for a covalent binding drug will only be successful if warhead-related side effects can be avoided.

LECTURES – **L.67**

HARNESSING THE POWER OF REVERSIBLE, COVALENT INHIBITORS: THE DISCOVERY OF ODANACATIB

W. Cameron Black

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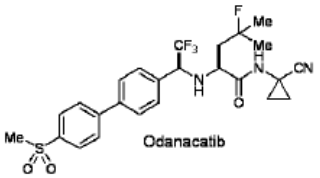
Cathepsin K (Cat K) is a lysosomal cysteine protease that is highly expressed in osteoclasts and plays a critical role in the degradation of bone collagen. It is postulated that an inhibitor of Cat K will be an effective anti-resorptive therapy for the treatment of osteoporosis.

Inhibitors of cysteine proteases are usually characterized by their ability to form a covalent bond to the active site cysteine. So long as this covalent bond is fully reversible, inhibitors with drug-like properties can be prepared. The nature of the electrophile used in these inhibitors is of critical importance: a weak electrophile will make it difficult to reach the required level of potency, while a strong electrophile will give poor selectivity over related enzymes and may exhibit irreversible binding behaviour. We have found that α-aminonitriles have an optimal degree of electrophilicity that allows for the preparation of potent and selective Cat K inhibitors.

Selectivity is of critical importance to inhibitors of Cat K. Related cathepsins have essential physiological functions that should not be interfered with in a drug for osteoporosis. Designing a neutral inhibitor is just as important to achieving a high degree of selectivity as is choosing an appropriate electrophile. This is due to the property of lysosomotropism in which a basic drug can accumulate 10-100 fold in the acidic lysosomes of a cell. Since off-target cathepsins reside in lysosomes, a dramatic loss of selectivity is observed for basic inhibitors in whole cell assays.

Taking these two essential design elements into account, we have prepared potent and selective nitrile-based Cat K inhibitors that do not require the basic substituent found in many other inhibitors of this enzyme. By blocking metabolically labile positions on the molecule we succeeded in identifying odanacatib, a 0.2 nM inhibitor of human Cat K with >300-fold selectivity over

off-target cathepsins. Odanacatib has an excellent pharmacokinetic profile and is efficacious in preclinical models of osteoporosis. Odanacatib is currently in Phase III development for the treatment of post-menopausal osteoporosis.



LECTURES – **L.68**

TARGETING DRUG RESISTANCE IN PROTEIN KINASES WITH IRREVERSIBLE INHIBITORS

Dr. Daniel Rauh

Chemical Genomics Centre of the Max Planck Society, Dortmund

Targeting protein kinases in cancer therapy with irreversible small molecule inhibitors is moving to the forefront of kinase inhibitor research and is thought to be an effective means of overcoming mutation-associated drug resistance in epidermal growth factor receptor kinase (EGFR). Here we systematically analyzed the determinants of the activity and selectivity of irreversible EGFR inhibitors. A focused library of irreversible as well as structurally corresponding reversible EGFR-inhibitors was synthesized for chemogenomic profiling involving genetically defined NSCLC and EGFR-dependent cell lines. Our results show that the growth-inhibitory potency of all irreversible inhibitors against the EGFR(T790M) resistance mutation was limited by reduced target inhibition, linked to decreased binding velocity to the mutant kinase. The obtained results are discussed together with structural biology and biochemical studies of catalytic activity in both wild type and gatekeeper mutated kinase variants to draw conclusions about the impact of steric hindrance and increased catalytic activity in drug resistant kinase variants.

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LECTURES – **L.69**

SUNSET SESSION ON "ACADEMIC CURRICULAE AND INDUSTRIAL CAREERS" "FIT FOR AN INDUSTRIAL CAREER?"

Klaus Müller

F. Hoffmann-La Roche AG, Basel, Switzerland

Therapeutic research is highly complex and interdisciplinary. Chemists play a central role in this endeavor, not only because they synthesize novel compounds, but also because they have (or should have) the capacity to integrate the concepts and data from all collaborative disciplines of biology, pharmacology, analytics, medicine, physics, and informatics. Chemistry, i.e., the understanding at the molecular level, is the unifying conceptual platform par excellence.

For this reason, chemical education has to be as broad as possible, integrating key aspects from neighboring disciplines when and wherever possible; however, without jeopardizing the education in profound theoretical and practical chemistry.

While chemical synthetic competence is key, understanding the relationships between structure and property of compounds is equally vital, not only for chemists in the life sciences, but also in material sciences. Current academic curricula should improve the balance between structure-reactivity and synthesis teaching, on the one hand, and teaching of molecular conformation, structure and property relations, non-bonded interactions and molecular recognition, on the other hand. The thinking in terms of structural analogies is hardly ever educated and requires a thorough understanding of molecular conformation and property aspects.

The academic curriculum optimally should educate chemists that are both creative in designing novel molecules and productive in synthesizing them. Chemists who can design, but not synthesize, or chemists who can synthesize, but don't know what, are each half of the game.

The academic curriculum should also open vistas into emerging fields in the life, environmental, and material sciences so as to sensitize and stimulate new chemist generations for upcoming challenges and opportunities.

LECTURES – **L.70**

SCALE-UP OF API PROCESSES - CHALLENGES OF SOLID STATE PROPERTIES: CASE STUDIES

Roland Thieme, Pirmin Hidber, Ralph Diodone, Urs Schwitter

F. HOFFMANN-LA ROCHE Ltd, Basel, Switzerland

Bioavailability, stability, the route of administration and many other factors are determining the selection of a drug formulation

principle, which in turn requires specific solid state properties from the corresponding active pharmaceutical ingredient (API). Decisions about the composition of the API (free base/acid, salt, cocrystal) and the polymorphic form have to be taken at an early stage of development. The timely characterization and selection of the most appropriate salt and polymorph of a given API provides the basis for development and robust scale-up of both chemistry and formulations processes. These activities have to be performed with very limited amounts of API and under significant time pressure, calling for automated and miniaturized equipment to perform a large number of experiments with a minimum of material in a reasonable time frame.

After verification of the feasibility and performance of a chosen formulation principle on small-scale, both formulation development and chemical development have to scale-up their respective processes. These activities have to be pursued in close collaboration of the involved disciplines in order to carefully address the interdependencies. Integrated Drug Development is the Roche nomenclature for this cross-functional approach which will be discussed along some examples.

Performance and manufacturability of solid drug products are significantly affected by solid state properties of an API like particle size distribution, morphology, flowability, bulk density etc. These physical quality attributes of APIs are frequently very dependent on the equipment and corresponding process parameters selected for the scale-up from early clinical development until commercial production. Examples covering a range of solid state investigations will be discussed and resulting conclusions shared.

In order to increase bioavailability of drugs the amorphous state of APIs is of special interest. A case study is presented which visualizes the formation and scale-up of a micro-precipitated bulk powder approach to form and stabilize an amorphous API in an excipient matrix.

LECTURES – **L.71**

SALT SELECTION FOR SCALE UP & DEVELOPMENT "THE 100 MG –APPROACH"

*Stefan Balbach**

Sanofi-aventis, Frankfurt/Main, Germany

Early development candidates are often selected for pre-clinical and clinical development based primarily on pharmacological and toxicological data. In order to choose the best compounds or it´s physical form (salt, polymorph) from a biopharmaceutical and pharmaceutical-technical point of view (e.g. selecting the right candidate for an oral dosage form), physicochemical parameters such as solubility, dissolution rate, hygroscopicity, lipophilicity, pK_a, stability, polymorphism, and particle characteristics need to be evaluated as early as possible and above all with the highest accuracy. However, poor batch purity and the low amounts of drug substance available in early development often compromise data quality, and, therefore, hamper an early in-depth drugability assessment. This presentation summarizes the principles of the so-called "100mg - approach" on early biopharmaceutical profiling with the aim of providing a high quality drugability assessment requiring not more than 100 mg of drug substance. In particular, the evaluation criteria, process, and miniaturized analytical technology that can be applied for this purpose are discussed. In addition, case studies are provided which highlight how development issues can be predicted early on and how an early biopharmaceutical profiling can contribute to candidate development.

Keywords: Physicochemical characterisation, pharmaceutical evaluation, salt selection, miniaturization, preformulation

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LECTURES – **L.73**

DAPAGLIFLOZIN, A NOVEL SGLT2 INHIBITOR

William N. Washburn

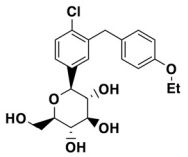
Metabolic Diseases Chemistry, Research and Development, Bristol-Myers Squibb Co.

Diabetes mellitus type 2 (T2DM) is a major ever-increasing public health problem in developed and developing nations alike. In 2007, the number of people worldwide with diabetes mellitus was estimated to be 246 million. As a consequence of ~10% of the adult population being diabetic in the United States, the annual economic burden exceeds $150 billion in direct medical costs, disability, work loss, and premature death. Diabetes increases the risk of cardiovascular disease, stroke, nephropathy, retinopathy, renal failure, and amputations of the extremities. Although these complications result from multiple metabolic derangements, hyperglycemia plays a central role central in both the vascular consequences of diabetes and the progressive nature of the disease itself.

The kidney is a critical component for maintenance of glucose homeostasis. Healthy individuals filter and recover upwards of 180g per day of glucose. Two sodium dependent glucose cotransporters SGLT1 and SGLT2 mediate glucose recovery. The major renal contributor is SGLT2; whereas SGLT1 is responsible absorption of glucose/galactose from the small intestine. Development of selective SGLT2 inhibitors is of current interest for medicinal chemistry particularly as this approach would provide an unique insulin-independent means of controlling hyperglycemia. Since the risk of hypoglycemia is low, SGLT2 inhibitors could be utilized as firstline therapy or in combination.

The focus of the talk will be the discovery, synthesis and characterization of the C-aryl glucoside-derived renal sodium-dependent glucose cotransporter-2 (SGLT2) inhibitor, dapagliflozin which is undergoing evaluation for treatment of Type 2 diabetes

in Phase 3 clinical trials. The SAR evolution from O-glucoside to the first C-glucoside derived SGLT2 inhibitors will be reviewed. The talk will discuss aspects of the pharmacological profile of dapagliflozin in diabetic rat models prior to briefly summarizing selected clinical results.



Dapagliflozin

LECTURES – **L.74**

A NOVEL CHK1 INHIBITOR IN ADVANCED CLINICAL TRIALS

Timothy Guzi

Director, Medicinal Chemistry, Schering-Plough Research Institute/Merck Research Laboratory, Cambridge, Massachusetts [timothy.guzi@spcorp.com]

Following exposure to DNA antimetabolite drugs, the CHK1 kinase is activated as part of the replication checkpoint and is essential for tumor cell viability in this context. RNAi-mediated knockdown of CHK1 during DNA antimetabolite exposure leads to several signature phenotypes, notably the rapid and irreversible induction of dsDNA breaks, which can be quantitatively assayed via the γ-H2AX marker of DNA damage. CHK2 status does not impact replication checkpoint override phenotypes, implying a non-redundant role for CHK1 during DNA antimetabolite exposure. These observations refined the target profile for a putative CHK1 inhibitor, specifically establishing a requirement for minimal CHK2 cross-reactivity.

High throughput screening led to the identification of novel pyrazolo[1,5-a]pyrimidines as a potential lead structures for the development of novel CHK1 inhibitors. Key to the SAR development program was the development of high-throughput functional screens of replication checkpoint override. These tracked mechanism-based accumulation of γ-H2AX, were highly discriminatory and led to the discovery of SCH 900776.

SCH 900776 is a potent, selective inhibitor of CHK1 (IC50 = 0.002 uM) and binds with high affinity to the CHK1 kinase domain. SCH 900776 does not significantly inhibit other kinases (including CHK2) and exhibits selectivity versus a range of other enzymes and ion channels. SCH 900776 induces rapid override of the replication checkpoint induced by multiple DNA antimetabolites, causing accumulation of DNA damage. Using the γ-H2AX assay, replication checkpoint override following SCH 900776 exposure is dose-dependent (EC50 = 0.125 uM), readily apparent following short exposures and occurs in multiple tumor cell lines. Cellular responses to SCH 900776 exposure were similar to those observed following siRNA-mediated depletion of CHK1. Importantly, similar DNA damage phenotypes were not observed following replication checkpoint override in proliferating normal fibroblasts, suggesting this mechanism of action may selectively impact tumor cell lines.

The in vitro biomarkers of replication checkpoint engagement and override were translated in vivo (mouse, rat and dog) and facilitated accurate calibration of mechanism-based activity, relative to onset of dose-limiting toxicities. In combination with gemcitabine, SCH 900776 rapidly induced biomarker responses in xenograft tumor tissue and surrogate skin samples which were correlated with enhanced efficacy. Doses of SCH 900776 required for mechanism-based activity in vivo were well below those associated with toxicity, suggesting this compound has a wide therapeutic index. Importantly, efficacious doses of SCH 900776 in combination with gemcitabine did not adversely alter the nadir or rebound kinetics of hematological parameters in Balb/C mice, relative to gemcitabine monotherapy.

In summary, SAR development facilitated by high-content functional screening led to the discovery of SCH 900776, a selective CHK1 inhibitor with potent, mechanism-based activity against the replication checkpoint induced by multiple DNA antimetabolite drugs.

LECTURES – **L.75**

TMC 435, A NOVEL HCV PROTEASE INHIBITOR IN ADVANCED CLINICAL TRIALS

Pierre Raboisson

Ph.D.; Pharmacist Research Fellow – HCV Disease Area Chemistry Head; Tibotec BVBA (Belgium)

Hepatitis C virus (HCV)-encoded NS3/4A protease is essential for viral replication and represents an attractive target for therapeutic intervention in HCV-infected patients. Recent studies demonstrated that NS3/4A protease inhibitors are able to decrease HCV viremia, either as monotherapy or in addition to the current standard of care therapy. Lead optimization of a novel series of cyclopentane-containing macrocyclic NS3/4A serine protease inhibitors resulted in the discovery of the clinical candidate TMC435, a potent and selective inhibitor of HCV replication in genotype 1b replicon cells with an EC_{50} value of 8 nM. The selectivity index (SI) was over 2000 in a panel of different cell lines as well as in a representative panel of RNA and DNA viruses. The binding mode of TMC435 to the HCV protease was confirmed by X-ray crystallography analysis of TMC435 bound to NS3. Furthermore, TMC435 exhibited a synergistic effect with interferon-α (IFN-α), some NS5B polymerase and NS5A inhibitors in reducing HCV replicon RNA and in suppressing the emergence of drug resistant replicon colonies. Pharmacokinetic and safety pharmacology assessment prompted the selection of TMC435 as the lead candidate currently being evaluated in a phase IIb clinical trial in HCV-infected patients.

LECTURES – **L.76**

DISCOVERY OF A NOVEL OREXIN RECEPTOR ANTAGONIST FOR THE TREATMENT OF SLEEP DISORDERS

Paul J. Coleman, Christopher D. Cox, Michael J. Breslin, John D. Schreier, Anthony J. Roecker, David B. Whitman, Swati P. Mercer, Jeffrey P. Bergman, Karen M. Brashear, Georgia B. McGaughey, Rodney A. Bednar, Wei Lemaire, Scott M. Doran, Steve V. Fox, Susan L. Garson, C. Meacham Harrell, Richard L. Kraus, Kathy Murphy, Duane R. Reiss, Dan Cui, Chunze Li, Thomayant Prueksaritanont, Shane Roller, Cuyue Tang, Kelly Yee, Joanne Stevens, Pam Tannenbaum, Steven D. Young, Ken S. Koblan, W. Dexter Kennedy, W. Joseph Herring, Christopher J. Winrow, George D. Hartman, and John J. Renger

Departments of Medicinal Chemistry, Molecular Systems , In Vitro Sciences, Neuroscience Research, and Drug Metabolism, Clinical Pharmacology, Clinical Neuroscience
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Orexins are excitatory neuropeptides secreted by hypothalamic neurons that project into regions of the brain that modulate sleep and arousal. Two G-protein coupled receptors respond to orexin signaling, Orexin 1 Receptor (OX1R) and Orexin 2 Receptor (OX2R) with partially overlapping brain distributions. Genetic and pharmacological studies suggest orexin receptor antagonists could provide therapeutic benefit for insomnia and other disorders in which sleep/wake cycles are disrupted.

We have identified MK-4305 as a potent, dual orexin receptor antagonist with excellent brain penetration and robust preclinical in vivo activity. The development and optimization of lead compounds along with the profile of clinical candidate MK-4305 will be presented.

LECTURES – **L.77**

MULTI-TARGETED DRUG DISCOVERY: STRATEGIES AND CHALLENGES FOR MEDICINAL CHEMISTS

Richard Morphy

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The inherent redundancy and robustness that exists within biological networks means that drugs acting on single target may not produce the desired therapeutic efficacy, whilst at the same time minimising adverse side effects. Indeed many complex diseases such as schizophrenia and cancer remain inadequately treated. Increasing interest in the design of drugs with superior efficacy and safety profiles has lead to a rapid evolution of the field of multi-target drug discovery (MTDD) in recent years. The discovery of multi-target drugs is following an increasingly rational path compared to the historical non-selective agents that were mostly discovered serendipitously.[1] However the premeditated design of compounds with a predefined multi-target profile (i.e. "designed" multiple ligands or DMLs) can prove to be a challenging task and an early assessment of the feasibility of designing ligands for a particular target combination is essential.

Historically, there have been two quite different methods of generating lead compounds, screening approaches that rely largely upon serendipity and knowledge-based approaches that exploit information either from the general literature or proprietary information from within an organization. Considering both approaches is often a sensible strategy in order to improve the overall chance of success. The three principal challenges presented to medicinal chemists during lead optimisation relate to the need to balance the desired activities, obtain wider selectivity over undesired targets and attain the pharmacokinetic profile required for an oral drug.

Looking to the future, certainly new approaches to help medicinal chemists discover the next generation of DMLs are needed. One possible way of generating leads is to use a fragment-based approach.[2] The growing field of network pharmacology has the potential to identify novel disease relevant target combinations.[3] Despite the many challenges, the opportunity to discover novel and superior medicines should continue to drive MTDD forward.

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LECTURES – **L.78**

SEROTONIN/NORADRENALINE REUPTAKE INHIBITORS: A CASE HISTORY IN THE CHALLENGES OF MULTIPHARMACOLOGY DRUG DISCOVERY

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Many drug design programs focus on identifying molecules that selectively modulate a single biological target. However, this approach may not deliver the required clinical efficacy due to inherent redundancy in complex biological pathways. Another

drug discovery approach involves the rational design of molecules which interact with multiple biological targets, and this strategy has the potential to deliver increased efficacy against complex diseases.

This talk will highlight the SNRI medicinal chemistry program at Pfizer, and exemplifies the challenges involved in balancing multiple pharmacological activities whilst retaining good drug-like properties. 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.

LECTURES – **L.79**

DESIGN, SYNTHESIS AND EVALUATION OF A SERIES OF BIPHENYLSULFONAMIDES AS POTENT DUAL ANGIOTENSIN II AND ENDOTHELIN A RECEPTOR ANTAGONISTS

Natesan Murugesan (to be presented by Bill Washburn)

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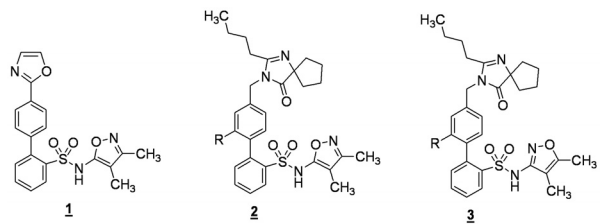

We have previously shown that 4′-oxazolyl biphenylsulfonamide derivatives represented by **1** are potent and selective ET_A antagonists. BMS-193884 shares the same biphenyl core as a large number of AT_1 receptor antagonists, including irbesartan. Thus, it was hypothesized that merging the structural elements of **1** with those of the biphenyl AT_1 antagonists (e.g., irbesartan) would yield a compound with dual activity for both receptors. This strategy led to the design, synthesis, and discovery of a series of biphenylsulfonamide derivatives (**2**) as potent and orally active dual antagonists of both AT_1 and ET_A receptors.

We have also found that replacement of the 5-aminoisoxazole in **2** with the metabolically more stable 3-aminoisoxazole resulted in a series of analogs (**3**) with improved binding activity against both receptors as well as much improved pharmacokinetic profile. **3a** (R = CH_2OEt) was then selected from this group for additional preclinical studies on the basis of its optimal binding activity and superior pharmacokinetic properties.

LECTURES – **L.80**

NEW G PROTEIN-COUPLED RECEPTOR CRYSTAL STRUCTURES: INSIGHTS AND LIMITATIONS

Prof. Gebhard F.X. Schertler

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The structures of a stabilised adrenergic receptor with agonist and antagonist ligands bound will be described and implications for ligand selectivity and signaling will be discussed. We have introduced constitutively activating mutations into rhodopsin using an engineered disulfide bridge that stabilises rhodopsin. We obtained crystals of the constitutively active receptor in presence of a peptide derived from the C-terminal peptide of the G Protein. The structure contains the receptor in an active like sate the covalent bond between retinal and Lys 296 and the retinal is hydrolyzed but retinal is present in the structure and the beta ionone ring is inserted between helix 5 and 6 of the receptor. We observe an internal water mediated hydrogen bond network that involves many highly conserved amino acids of the GPCR. There is a direct interaction of the arginin of the highly conserved DRY motive with an exposed backbone carbonyl of the G-protein backbone. We are able to compare the rearrangement of the internal hydrogen bond network between the inactive and active like state. Highly conserved Tyrosines in helix 5 and 6 are Important for stabilising the active and inactive conformation. The X-ray structures of the inactive ground state and of the peptide bond active state represent a static picture of GPCR activation; in the future we need to develop a more dynamic description of receptor activation that also takes other possible conformations of GPCRs into account.

LECTURES – **L.81**

ACTIVATION MECHANISM OF CLASS C GPCRS

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G-protein coupled receptors (GPCRs) are encoded by the largest gene family in mammalian genomes. This complexity is likely even larger if one consider the possible assembly of these 7TM proteins into dimeric or oligomeric entities with specific properties. However, the existence and physiological relevance of such oligomers remains elusive.

Among the large GPCR family, class C GPCRs, that include metabotropic glutamate and GABA receptors, are well recognized as constitutive dimers, and as such represent an interesting model to unravel the functional significance of GPCR dimerization and oligomerization.

Using new cell surface labeling methods in combination with the use of time-ressolved FRET fluorophores, we have been able to examine the stoechiometry of class C GPCR complexes at the cell surface. We show that whereas mGlu receptors only form strict dimers, GABAB receptor can assemble into dimers of heterodimers.

Using various approaches, we examined the molecular dynamics involved in the activation and allosteric modulation of these receptors at the structural level. We show that a relative movement of one subunit compared to the other is needed for agonist activation of the receptor. Surprising, we found that such movement lead to G-protein activation by a single subunit within the dimer.

Our data bring new information regarding the functioning of class C GPCRs, and revealed a possible combination of asymmetric versus symmetric functioning of GPCR dimers, opening new views to understand the multiple signaling pathways activated by one GPCR.

LECTURES – **L.82**

DUALSTERIC GPCR TARGETING: A NOVEL ROUTE TO SUBTYPE SELECTIVE AGONISTS AND ANTAGONISTS OF THE MUSCARINIC RECEPTORS

Ulrike Holzgrabe,[1] Marco De Amici,[2] Evi Kostenis,[3] and Klaus Mohr[3]

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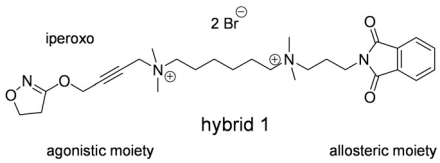
All types of G-protein-coupled receptors are characterized by the expression of a variety of subtypes which impart different messages upon activation of an endogenous ligand. Thus, exogenous small-molecule GPCR activators with receptor subtype selectivity and signaling pathway specificity are urgently needed as tools for a target modulation of biological function and therapeutic purposes. Recently, we developed a subtype-selective agonist of the muscarinic M_2 receptor by means of the message-address concept. The highly active, non-selective agonist iperoxo (= message) was linked via a hexamethonium moiety to highly selective allosteric moiety (= address) resulting in hybrid 1 which acts not only subtype-selective but also shows a pathway-specific signaling [1]. The selectivity could be attributed to a dualsteric binding mode, i. e. binding to the highly conserved orthosteric and to the less conserved allosteric site. Variations of the isoxazole moiety, the length of the middle chain and the contralateral imido skeleton influences both affinity to the different muscarinic receptor subtypes (and selectivity) and the intrinsic activity.



The message-address concept was taken one step forward and the agonist message replaced with antagonist message using atropine and scopolamine. Even though the selectivity of the resulting antagonist hybrids is not as pronounced as with the agonist hybrid 1 a dualsteric binding mode could be shown.

Structure-activity relationships of the allosteric/orthosteric agonists and antagonists will be discussed with regard to affinity, binding mode, and intrinsic activity.

Acknowledgement: Thanks are due to the Deutsche Forschungsgemeinschaft for financial support.

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LECTURES – **L.83**

STRUCTURAL DESIGN AND OPTIMIZATION OF ORALLY AVAILABLE RENIN INHIBITORS

David A. Claremon

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VTP-27999, a direct renin inhibitor (DRI) in early phase clinical development, was discovered by computer aided drug design (CADD). The inhibition of renin is an attractive mechanism for an antihypertensive since it interferes in the first and rate limiting step of the Renin Angiotensin System (RAS). Also, both animal and clinical studies with aliskiren, a marketed DRI, have suggested that drugs acting by this mechanism offer renal protection advantages over other modalities for interfering with the RAS. Historically, key impediments faced by the majority of DRIs were high molecular weight, poor water solubility, weak plasma renin inhibitory potency, poor bioavailability and pharmacokinetics, unacceptable interference with metabolic enzymes, and challenging chemical complexity. Some of these issues were surmounted by aliskiren, however, the very low human oral bioavailability (~2%) and chemical complexity are two key shortcomings that are unmet and represent an opportunity for a second generation DRI to provide an advantage over aliskiren. The goal of our design strategy was to identify a chemically accessible DRI with good bioavailability that meets all of the criteria for a safe and effective antihypertensive. The pharmacology of VTP-

27999, the lead molecule in one of several classes identified at Vitae Pharmaceuticals by CADD, demonstrated blood pressure lowering efficacy with a single oral administration in a double transgenic rat model expressing human renin and angiotensinogen genes, and a sodium depleted cynomolgus monkey model. It achieved good oral bioavailability in three species, and its terminal half life predicts once a day dosing in man. The hurdles overcome by lead optimization, and the profile of VTP-27999 in animal pharmacology and Phase 1 clinical studies will be disclosed.

LECTURES – **L.84**

BGG492, A COMPETITIVE AMPA/KAINATE ANTAGONIST IN CLINICAL DEVELOPMENT FOR THE TREATMENT OF MIGRAINE AND EPILEPSY

Henri Mattes, David Orain, Manuel Koller, Kurt Lingenhoehl, Jürg Kallen, Mario Pozza, Markus Schmutz, Sandrine Desrayaud, Stephan Urwyler, Johanne Renaud and Yves P. Auberson, on behalf of the BGG492 discovery and development team.

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With few exceptions, earlier attempts to develop AMPA/kainate antagonists resulted from an extensive SAR exploration of substituted quinoxalinediones. Several clinical development compounds were identified (e.g. YM-90K, MPQX, or AMP397), but none of them proved suitable for full development and so far, no competitive AMPA/kainate antagonist made it to the market. In contrast to the previous candidates, BGG492, an orally active AMPA/kainate antagonist, results from the optimization of quinazolinedione sulfonamides, which have a similar SAR but improved overall properties.

BGG492 shows anticonvulsant activity in several animal models of epilepsy, including electroshock and chemically-induced seizures in rodents, WAG/Rij rats (a genetic model of absence epilepsy), the rat amygdala kindling model (indicating a potential anti-epileptogenic effect), and in fully kindled rats (a model of therapy-resistant partial seizures in human). It is well understood that properties required for high affinity at AMPA receptors are contrary to those required for oral bioavailability. As a compromise, BGG492 has moderate binding affinity for rat and human AMPA receptors (IC_{50} = 0.19 and 0.2 μM), but >100-fold selectivity with regards to the glycine-binding site of NMDA receptors and no significant affinity in a 150-target safety panel. BGG492 is only metabolized to a limited extent, and does not inhibit CYP450 enzymes. Its very favorable safety profile is evidence by a lack of cardiovascular, phototoxic or teratogenic potential, as well as by results of in vivo toxicology studies in rats, dogs and monkeys, where only minor and reversible effects were observed. Dose-limiting adverse effects were related to the classical signs of exaggerated pharmacology for AMPA/kainate receptor antagonism, mostly ataxia and decreased locomotor activity. BGG492 is currently in clinical evaluation in epileptic and migraine patients.

LECTURES – **L.85**

DISCOVERY OF A POTENT, SELECTIVE AND ORALLY BIOAVAILABLE ACIDIC 11-BETA-HYDROXYSTEROID DEHYDROGENASE TYPE 1 (11BETA-HSD1) INHIBITOR

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Inhibition of 11beta-HSD1 is increasingly seen as an attractive mechanism with the potential for treatment of obesity and other elements of the metabolic syndrome. We will describe the discovery of a nicotinic amide derived carboxylic acid class of inhibitors that has good potency, selectivity and pharmacokinetic characteristics, culminating in the identification of the clinical development candidate AZD6925.

LECTURES – **L.86**

MK-1903: A POTENT NIACIN RECEPTOR (GPR109a/ HCA2) AGONIST

P. Douglas Boatman, Thomas O. Schrader, Michelle Kasem, Benjamin R. Johnson, Philip J. Skinner, Jae-Kyu Jung, Jerry Xu, Martin C. Cherrier, Peter J. Webb, Graeme Semple, Carleton R. Sage, Jens Knudsen, Ruoping Chen, Andrew K. Taggart, Ester Carballo-Jane, Jeremy G. Richman

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Niacin has been an established treatment for dyslipidemia and cardiovascular diseases for over 50 years due to its positive effects on serum lipids. Niacin increases high density lipoprotein-cholesterol (HDL-c) more effectively than other drugs currently in use, has a positive effect on other plasma lipids and has been shown to effectively reduce the number of cardiac events and all cause mortality. Subsequently, combinations of niacin with the LDL-lowering statins have been shown to slow the progression of atherosclerosis, decrease the number of cardiac events and provide a therapeutic benefit beyond that of statins alone.

At therapeutic doses, a number of adverse side effects are associated with the use of niacin, most notably, a cutaneous flushing effect which limits patient compliance. Following identification of GPR109a as a molecular target for niacin, Arena

Pharmaceuticals in collaboration with Merck Research Laboratories initiated Medicinal Chemistry programs to produce potent agonists of the niacin receptor that would increase HDL cholesterol but were devoid of the flushing response generated by niacin. MK-0354 is an example of a biased agonist that activates antilipolytic pathways in adipose cells but does not signal via the ERK 1/2 pathway that leads to prostaglandin production and vasodilation.[1,2] In a Phase II clinical trial, MK-0354 decreased plasma free fatty acids and did not induce the flush associated with niacin; however, MK-0354 did not have a statistically significant effect on HDL, LDL or triglycerides.[3]

The absence of an increase in HDL cholesterol produced by MK-0354 in the clinic led to two separate hypotheses: 1) more potent analogues would result in beneficial effects on serum lipids and triglycerides, 2) separation of signaling pathways that inhibit cAMP production from those that activate MAP kinase influences both the effects on lipids and the flushing side effect. Both of these hypotheses were investigated and the result of these investigations was selection of MK-1903 to advance to human clinical trials. The structure of MK-1903, the preclinical data leading to its selection and data from clinical trials will be disclosed for the first time in this presentation.

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LECTURES – **L.87**

PROTEIN-PROTEIN INTERACTIONS AND APOPTOSIS IN CANCER CELLS

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Apoptosis, or programmed cell death, is a cell suicide mechanism with a major role in development and homeostasis in vertebrates and invertebrates. Inhibition of apoptosis can lead to the absence of physiological cell death and contribute to development and progression of various malignancies. Apoptotic cell death can be initiated through the engagement of cell-surface pro-apoptotic receptors by their specific ligands or by changes in internal cellular integrity. Both of these pathways converge at the activation of effector caspases. Blockage of programmed cell death enhances cell survival and contributes to escape from cytotoxic therapies. This well regulated process is governed by a series of protein/protein interactions including Bcl-2 family proteins and inhibitor of apoptosis (IAP) proteins. Specific interactions that are amenable to small molecule intervention will be presented. Examples will include small molecule mimetics of the second mitochondrial activator of caspases (Smac) that have been developed as clinical candidates for our oncology program. Evaluation of these compounds against a number of cancer cell lines indicated that they caused single agent cell killing by inducing apoptosis. The work leading to the discovery of clinical compound GDC-0152 will be presented. Our current efforts are focused on creating isoform-selective back-up leads in order to investigate the efficacy/toxicity profile versus GDC-0152. These selective compounds also give us insight into the mechanism of action of IAP inhibition. These data suggest that regulation of apoptosis may represent a useful approach to improve the current management of this serious disease.

LECTURES – **L.88**

SMALL MOLECULE INHIBITORS OF THE NEUROPILIN-1 VEGF-A INTERACTION

Ashley Jarvis,[1] Charles Allerston,[2] Haiyan Jia,[3,6] Birger Herzog,[3,6] Acely Garza-Garcia,[4] Lili Cheng,[3,6] Malini Menon,[3,6] Michelle Tickner,[3,6] Snezana Djordjevic,[5] Paul C. Driscoll,[4] Ian Zachary,[6] David L. Selwood,[7]

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We report the molecular design and synthesis of EG00229, the first small molecule ligand for the VEGF-A receptor neuropilin 1 (NRP1) and the structural characterization of NRP1-ligand complexes by NMR spectroscopy and X-ray crystallography. Mutagenesis studies localized VEGF-A binding in the NRP1 b1 domain and a peptide fragment of VEGF-A was shown to bind at the same site by NMR, providing the basis for small molecule design. EG00229 demonstrated inhibition of VEGF-A binding to NRP1 and attenuated VEGFR2 phosphorylation in endothelial cells. Inhibition of migration of endothelial cells was also observed. The viability of A549 lung carcinoma cells was reduced by EG00229, and it increased the potency of the cytotoxic agents paclitaxel and 5-fluorouracil when given in combination. These studies provide the basis for design of specific small molecule inhibitors of ligand binding to NRP1.

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LECTURES – **L.89**

TARGETING PDZ DOMAINS: FROM COMPUTATIONAL LIBRARY DESIGN, TO NMR AND X-RAY, TO *IN VIVO* STUDIES

Dmytro Kovalskyy[1], Maxim Platonov[1], Nestor Kamdem[2], Alexei Balinskyy[1], Yvette Roske[3], Walter Birchmeier[3], Hartmut Oschkinat[2]

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Protein-protein interactions (PPI) are promising but yet challenging targets for therapeutic intervention. Recent studies suggest PDZ domains as putative PPI targets for cancer and pain treatments. Unfortunately, only a few small molecule ligands were reported to bind PDZ domains. Here we report on the high precision design and evaluation of a targeted library showing broad activity on PDZ domains.

To consider for as many PDZ domains as possible upon the library design we classified them by binding sites. The binding sites of all PDZ domains available from Protein Data Bank were aligned followed by clusterization of their shapes. PDZ structures corresponding to the centroids from each cluster were docked against the Enamine compound collection. Putative virtual hits were identified by the original MultiFilter program. The generated library was evaluated in NMR screening studies against PDZ domains from DVL, PSD-95, Shank3 and AF-6. Discovered hits appeared to belong to different chemotypes, thus providing good starting point for selectivity among PDZ domains.

To show the potential of the library and elaborate discovered hits we solved the crystal structure of the complex of DVL3 with hit compound GA-14. The crystalographic data was used for structure based lead optimisation. The best derivatives of GA-14 exhibit low micromolar Kd estimated by NMR titration. Finally, inhibition of Wnt pathway with *in vitro* and *in vivo* models has confirmed the potency of discovered PDZ ligands of DVL for therapeutic purposes.

LECTURES – **L.90**

THE DISCOVERY OF MARAVIROC, AN ALLOSTERIC CCR5 ANTAGONIST FOR HIV INFECTION

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The approval of maraviroc (Celsentri™/Selzentry™) for the treatment of HIV-1 infection was the culmination of a complex R & D program at Pfizer that translated a genetic observation into an approved medicine. In 1996, it was reported that individuals with a 32 b.p. deletion in their CCR5 ORF were remarkably resistant to HIV-1 infection, yet were of normal phenotype. This led to studies showing CCR5 to be an essential and predominant co-receptor for HIV-1 entry. This prompted widespread search for novel antagonists of CCR5 to block HIV-1 entry into CD4+ cells to combat infection. The guiding biological assays for the medicinal chemistry program, pharmacological characterisation and preclinical-clinical translational studies that progressed maraviroc from discovery to approval will be presented. In addition, the program to identify second generation candidates and alternative indications for CCR5 antagonists will be described.

LECTURES – **L.91**

ALLOSTERIC, SMALL MOLECULE AGONISTS OF THE GLP-1 RECEPTOR FOR DIABETES

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The peptide hormone GLP-1 have important actions resulting in efficient glucose lowering along with lowering of body weight in patients with type 2 diabetes. As a peptide hormone, GLP-1 has to be administered by injection. As native GLP-1 is very short-acting, a longer-acting analogue was developed first, and then later on an analogue working for 24h/day was developed. Currently, exenatide is approved as a twice daily injection therapy, and recently liraglutide was approved as a once-daily injection therapy. Preclinical literature suggests that GLP-1 may also have a role in cardiovascular and brain protection, making this drug class even more interesting. Only few small molecule agonists to peptide hormone receptors have been described - none in the G-protein coupled B family of receptors to which the GLP-1 receptor belongs. The GLP-1 receptor is coupled to the adenylate cyclase activating pathway. We have discovered a series of small molecule so-called ago-allosteric modulators selective for the human GLP-1 receptor. These compounds act both as allosteric activators of the receptor and are also independent agonists. Potency of GLP-1 was not changed by the allosteric agonists, but affinity of GLP-1 for the receptor was increased. The most potent compound identified stimulates glucose-dependent insulin release from normal mouse islets, but importantly not from GLP-1 receptor knock-out mice. These compounds are not in themselves drug-like structures, but they represent an important tool for further understanding of the GLP-1 receptor.

LECTURES – **L.92**

FOLLICLE STIMULATING HORMONE RECEPTOR NEGATIVE ALLOSTERIC MODULATORS (FSHR NAM): DISCOVERY AND DEVELOPMENT OF A NEW SERIES OF ORALLY BIOAVAILABLE DIMETHOXY-BENZAMIDES

Jean-Philippe Rocher

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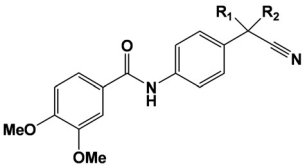
Normal function of both the ovary and the testis is long recognized to be dependent on the pituitary-synthesized gonadotropins Luteinizing hormone (LH), Thyrotropin hormone (TSH) and FSH.

These pituitary hormones are glycoprotein dimers: they have a common α-subunit, and a specific β-subunit, with an average molecular weight of ~30 kDa. Both hormone subunits are involved in the receptor binding specificity, which is achieved through a large number of complex molecular interactions and conformational changes. Allosteric modulation of the FSHR offers an opportunity to develop selective, orally available small molecular weight, non peptidic molecules which have a good developability profile, i.e. drug like properties.

Through a HTS campaign, Addex has identified ADX19420 as a hit (hFSHR cAMP production, IC_{50} = 949 nM). This compound has opened the avenue to a novel chemical class of FSHR NAMs. A simple chemical modification led to ADX61623 (IC_{50} = 696 nM) which showed good metabolic stability. A full lead optimization program addressed the potency on the target, the selectivity vs. other targets, in particular TSH, and the optimization of ADME-PK properties. Three potent FSHR NAM subseries were discovered and profiled. Finally, ADX68692 was selected as a clinical candidate; this compound was able to inhibit the production of progesterone and estradiol induced by FSH in rat granulosa cells *in vitro* and inhibited the FSH-induced follicle development in immature female rats after oral administration.

Repeated oral administration of ADX68692 to female rats caused significant disruption of the estrous cycle and histopathological effects which were suggestive of an anti-estrogenic effect in mature female rats. In male rats, circulating testosterone levels were reduced and prostate weight were affected, suggesting an anti-androgenic activity.

The *in vivo* effects of ADX68692 suggest that FSH NAMs may be effective treatments in estrogen related disorders such as endometriosis, uterin fibroids, polycystic breast cancer and ovarian cancer in women, and benign prostate hyperplasia and prostate cancer in man.



ADX19420 (R_1-R_2= -$(CH_2)_4$-)
ADX61623 (R_1=R_2= CH_3)

LECTURES – **L.93**

THE MEDICINAL CHEMISTRY OF GLUCOKINASE ACTIVATORS: PROPERTY-BASED DRUG DISCOVERY

Craig Johnstone, Mike Waring, Darren McKerrecher and Kurt Pike*

Cardiovascular and Gastrointestinal Research Area, AstraZeneca Pharmaceuticals, Mereside, Alderley Park, UK;
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We have previously described [1] the discovery and optimisation of a series of pyridine acid activators of the glucokinase enzyme, a target with the potential to improve glucose regulation in Type 2 Diabetes.

We would now like to disclose the shortcomings of the pyridine acids, and describe the design approach which led to the creation of a new series of neutral glucokinase activators.

The optimisation of this new series to compounds of candidate drug quality will be described, including the application of property-based design in achieving oral exposure in the neutral compounds and the optimisation of contradictory parameters of potency, oral exposure and hERG activity, culminating in the identification of the development candidate AZD1092.

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LECTURES – **L.94**

LEAD DEVELOPMENT STRATEGIES UTILIZED FOR THE DISCOVERY OF DGAT-1 INHIBITORS

David W. Piotrowski, Robert L. Dow, Bernard Hulin, Markus Boehm, Mayda Castrodad, Constantin Neagu, Jana Polivokva, Shawn Cabral

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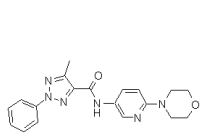
Acyl CoA:diacylglycerol acyltransferase (DGAT) is a microsomal enzyme in the endoplasmic reticulum (ER) that catalyzes the final step of triglyceride (TG) biosynthesis. DGAT-1 is highly expressed in small intestine and adipose tissue, with lower levels in other tissues, including liver and skeletal muscle. Inhibiting tissue TG synthesis should reduce the TG content in insulin-sensitive tissues to improve insulin action and restore glucose and lipid homeostasis in type 2 diabetes mellitus. Additionally, DGAT inhibition may decrease intestinal fat absorption and fat accumulation in adipose tissue making it useful as a target for the treatment of obesity.

Discovery of lead matter that progressed to a candidate was enabled through analysis of literature compounds, development of parallel-chemistry protocols and use of parallel prototyping in initial phases of our DGAT-1 medicinal chemistry program. Two chemical series, represented by **1** and PF-04094667, will be discussed in terms of *in vitro* potency, assessment of *in vitro* ADME characteristics and *in vivo* PK. The early strategic decisions that influenced the discovery of Phase I development candidate PF-04620110 will be highlighted.[1] Preclinical data for representative compounds will be presented alongside data for the clinical compound, PF-04620110.



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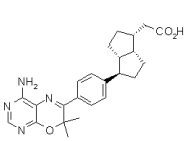

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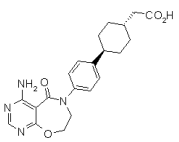

PF-04620110

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LECTURES – **L.95**

TOWARDS THE CLINIC : THE FRAGMENT-BASED DISCOVERY AND DEVELOPMENT OF AT13387AU. A POTENT INHIBITOR OF THE MOLECULAR CHAPERONE HSP90

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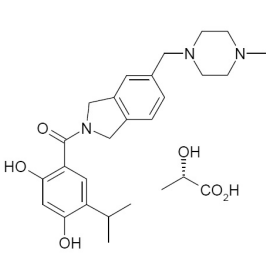
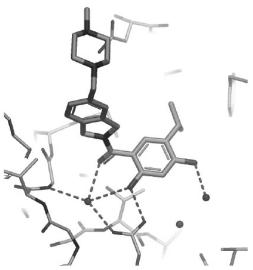
Heat shock protein 90 (Hsp90) is one of a family of molecular chaperones which are produced in response to a range of cellular stresses. It is involved in the activation and maintenance of a large number of regulatory and signalling proteins, by acting as a scaffold to allow these proteins to fold into their correct functional states. As well as their roles in normal cellular function, many of these client proteins are also directly implicated in cancer progression, such that inhibition of Hsp90 may simultaneously target many oncogenic pathways. Inhibitors of Hsp90 are currently generating significant interest in the clinic[1] as potential treatments for cancer.

We describe Astex's Pyramid™ approach and its application to fragment screening against Hsp90. Using a combination of NMR and high-throughput X-ray crystallography we discovered a number of low affinity fragment hits against the N-terminal ATPase domain of the protein. Structure aided drug design allowed for the rapid optimization of a key fragment hit into a lead compound with inhibitory activity in the low nM range.[2] Subsequent fine tuning of the physicochemical properties[3] of the hit series allowed for the identification of AT13387. This compound shows good efficacy in animal tumour models and has now progressed through pre-clinical development.[4] AT13387AU, the L-lactic acid salt, is currently being tested in man against a range of cancers.



AT13387AU



X-ray crystallographic structure of AT13387 bound to the N-terminal ATPase domain of Hsp90

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LECTURES – **L.96**

PHTHALAZINONE PYRAZOLES AS POTENT, SELECTIVE AND ORALLY BIO-AVAILABLE INHIBITORS OF AURORA-A KINASE

Michael Prime[1], Andrew Boyd[1], Frederick Brookfield[1], Stephen Courtney[1], Guy Georges[2], Bernhard Goller[2], Anja Limberg[2], Abdi Maie[1], Richard Marston[1], Petra Rueger[2], Matthias Rueth[2], Jonathan Snow[1], Wolfgang von der Saal[2], Victoria Walker[1].

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The Aurora kinases (consisting of Aurora A, B and C) are a family of serine/threonine kinases believed to play a key role in the protein phosphorylation events that are essential for the completion of essential mitotic events. Aurora A localizes predominantly to the centrosomes (Figure 1) and is highly expressed in many tumor types. Human tumor cell lines depleted of Aurora A transcripts arrest in mitosis and leads to apoptosis of tumor cells.

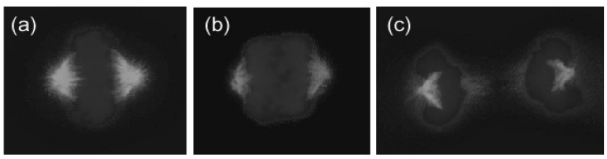

Figure 1 – Aurora A localisation within the cell

A novel series of low molecular weight inhibitors of Aurora A Kinase were identified. Optimisation of this inhibitor series was carried out culminating in the identification of a highly potent advanced lead series with a good pharmacokinetic profile that confers growth inhibition to tumor cells.

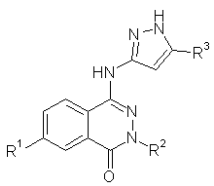

Figure 2 – Phthalazinone Aurora Kinase inhibitors

LECTURES – **L.97**

IMMUNOTHERAPY OF CANCER WITH TRIFUNCTIONAL ANTIBODIES: FROM BENCH TO APPROVAL

Dr. Horst Lindhofer

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Trifunctional bispecific antibodies (Triomab®) represent a growing family of promising therapeutics that open up novel treatment opportunities against malignant diseases. Currently, one member of this family – catumaxomab - actually represents the first approved trifunctional bispecific biopharmaceutical worldwide. Catumaxomab consists of two independent antigen binding sites for EpCAM and CD3 as well as the Fc region.[1] The outstanding Triomab® antibody format enables the formation of tricell complexes with tumour cells, T cells and accessory cells (e.g. monocytes, macrophages, natural killers or dendritic cells). The immediate vicinity of polyclonal T cells and accessory cells within these ternary cell complexes finally induces tumour cell killing elicited by both types of immunological effector cells.

Catumaxomab (anti-EpCAM x anti-CD3) showed strong antitumour efficacy and a clinically significant prolongation of puncture-free survival in a pivotal phase II/III trial with patients suffering from malignant ascites (MA), an advanced disease manifestation of e.g. ovarian, breast or gastric cancer. The clinically relevant primary and several other secondary endpoints were reached with high statistical significance resulting in a clear clinical benefit for the MA patients.[2] Additionally, a preliminary monitoring study showed that putative cancer stems cells ($CD133^+/EpCAM^+$) were present in peritoneal fluids of 62 % of analysed MA patients with different underlying primary tumour entities before treatment onset. Analysis demonstrated that during and after catumaxomab therapy these cells were efficiently destroyed within peritoneal fluids.[3] Consequently, catumaxomab-based therapeutic measures may offer an additional treatment option to eliminate cancer stems cells in malignancies with EpCAM expression.

Furthermore, Triomab® antibodies have the capacity to induce long-lasting cell-mediated immunity by combining passive antibody application regimes directly with active *in situ* immunization for tumour therapy. [4]

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LECTURES – **L.98**

NANOBODIES, FROM CAMELS TO THERAPEUTIC PROTEINS

Hilde Revets

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Nanobodies® are antibody-derived therapeutic proteins that contain the unique structural and functional properties of natu-

rally-occurring heavy-chain antibodies. The Nanobody® technology was originally developed following the discovery that camelidae (camels and llamas) possess fully functional antibodies that lack light chains. These heavy-chain antibodies contain a single variable domain (VHH) and two constant domains (C_H2 and C_H3). Importantly, the cloned and isolated VHH domain is a perfectly stable polypeptide harbouring the full antigen-binding capacity of the original heavy-chain antibody.

Nanobodies® combine the advantages of conventional antibodies with important features of small molecule drugs. Like conventional antibodies, Nanobodies® show high target specificity, high affinity for their target and low inherent toxicity. However, like small molecule drugs they have the opportunity to inhibit enzymes and readily access receptor clefts due to their unique 3-dimensional structure. These cavity structures are largely inaccessible to conventional antibodies but can be readily recognised by a long and protruding peptide loop as found in many Nanobodies®. Importantly, while enzyme and receptor clefts are also binding sites for many small molecule drugs, these drugs typically engage such targets with a low affinity and low selectivity. This can result in unwanted side-effects and lack of potency. In contrast, Nanobodies® have the potential to engage cavity structures with the same high affinity and selectivity as typically protein-protein interactions mediated by conventional antibodies. This is expected to yield highly potent molecules and minimise the likelihood of side-effects.

To date, Nanobodies® have been generated against more than 190 protein targets, including some complex targets and classes of targets such as GPCRs and ion channels, many of which are difficult to access with mAbs.

The Nanobody® platform allows the ability to design modular drugs based on Nanobody® building blocks combined with each other or with other protein domains or other molecules or drugs. These can combine more than one function in the final drug format. Nanobodies® have been combined in a wide range of formats, including unique multivalent (multiple identical binding sites for the same antigen), biparatopic (two Nanobodies® binding neighbouring epitopes on the same antigen), bispecific (Nanobodies® binding to two different antigens) and bi-functional molecules. These formats are easy to construct and the modular proteins can often be expressed at high levels in bacteria or yeast. As a result of this formatting flexibility, the range of therapeutic applications for Nanobodies® appears to be beyond that possible for conventional antibodies and antibody fragments.

Non-engineered Nanobodies® sually have a short half-life in plasma of hours rather than days. A variety of engineering methods can be used to tailor for a half-life varying from a few hours to 3 weeks. This versatility increases the range of therapeutic options available to Nanobodies® ranging from acute to chronic indications.

Nanobodies® have biophysical properties including resistance to heat, pH and enzymatic cleavage, which offer the potential for other routes of administration rather than just injection.

This presentation will highlight some of the key properties of these next-generation biologics and provide examples of how protein engineering and formatting can transform these Nanobodies® into novel molecular designs, which have now progressed into the clinic.

LECTURES – **L.99**

ENGINEERED PROTEIN SCAFFOLDS AS NEXT GENERATION THERAPEUTICS

Dr. Arne Skerra

Pieris AG & Professor of Biological Chemistry, Technische Universität München, Freising-Weihenstephan, Germany

The generation of engineered binding proteins based on scaffolds outside the immunoglobulin (Ig) family is a rapidly growing area that promises many applications in medical diagnostics, therapy, biotechnology, and basic research [1]. This technology goes hand in hand with our expanding knowledge about the molecular pathologies of cancer, immunological, and infectious diseases. While many protein scaffolds have been proposed during the past years, this field shows a trend towards consolidation, with a smaller set of systems that are being applied against multiple targets and in different settings. Current emphasis is on the development of drug candidates for therapy or *in vivo* diagnostics based on several structurally independent protein scaffolds: Adnectins, Affibodies, Anticalins, DARPins, engineered Kunitz-type inhibitors, and some others.

Among those, Anticalins are derived from the lipocalins, a widespread family of compact and robust proteins that usually serve for the transport or storage of vitamins, hormones, and metabolites in many organisms [2]. Their molecular architecture is dominated by a central β-barrel of eight antiparallel strands, which is open to the solvent at one end. There, four structurally variable loops form the entrance to the ligand pocket, similarly as the six CDRs of an antibody that make up the antigen-binding site. Yet, compared with Igs lipocalins have a much smaller size (160-180 residues), comprise a single polypeptide chain, and they can be produced at high yields in microbial host cells.

Anticalins with novel specificities have been engineered for the high affinity (pM) complexation of low molecular weight compounds as well as protein antigens. An Anticalin that recognizes a rare earth metal chelate complex opens applications in radioimmuno therapy [3]. An Anticalin that binds the T-cell coreceptor CTLA-4 in an antagonistic manner provides a promising drug candidate for immune stimulation in the treatment of cancer or infectious diseases [4]. Other Anticalins have been developed to neutralize the Alzheimer Aβ peptide and to target the neoangeogenesis marker ED-B. An Anticalin that exhibits strong antagonistic activity towards vascular endothelial growth factor (VEGF) effectively suppresses neoangiogenesis in xenograft tumour models and is currently subject to phase I clinical trials, offering an alternative to full size antibodies for the treatment of solid cancers.

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