

Opening of the conference - Keynote Lecture I

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The impact of deep sequencing on drug discovery and development

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Our understanding of human genetic variation, and gene expression patterns in human tissues, is undergoing dramatic expansion. This "second genomics" revolution is being enabled by the advent of massively parallel DNA sequencing technology (also termed next-generation sequencing). The extension of this powerful technology into pharmaceutical discovery and development offers unparalleled opportunity to improve the success rates for both small-molecule and biologics drug therapy. A technical overview of next-generation sequencing will be covered, followed by how this technology will provide valued information on target selection, pharmacodynamics, and profiling of clinical trial cohorts.

Drug Discovery Technologies I - Automated Image and Assay Analysis

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Knowledge and context-based image analysis for drug discovery

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In most cases, understanding the content of drug discovery-relevant images is a very complex task requiring a high degree of expert knowledge. Accordingly, the inspection and evaluation of these images is still typically performed by human experts. Human evaluations are sometimes subjective and can depend on the mood of the expert. Additionally the manual extraction of well defined statistical data, such as feature-properties of objects within images, can be very time consuming and monotonous. The automation of such a process has the potential to both considerably reduce the involved costs and evaluation time, and to enhance the quality of the results.

A prerequisite, however, for the automated analysis of complex images is that expert knowledge can be formulated mathematically. This is not a trivial task as expert knowledge represents a complex network of dependencies. The Cognition Network Language, CNL, enables a user to formulate expert knowledge in a natural way. Furthermore, this relatively new technique supports a new form of image analysis. The basic principles of CNL, which are inspired by human cognition capabilities, will be presented.

Image analysis is traditionally split into two parts. Firstly, the image is segmented into regions and objects. Secondly, the objects are classified and their properties are measured. In most drug discovery-related cases, the detection, i.e. the segmentation, of relevant objects is more difficult than the classification. For very complex problems the conventional procedure of applying a sequence of pixel filters combined with thresholds does not solve the problem.

By illustrating a number of examples, it is demonstrated that the method of stepwise evolution of objects can lead reliably to very precise results even for complex analysis problems. The core of this method is the detection of intermediate objects that are easier to segment and classify than the final objects of interest. The intermediate objects provide the necessary context to iteratively find more-complex objects until the final target objects are identified. In this way a stepwise development of context information enables a meaningful image analysis of complex images. As this procedure is knowledge driven and in certain ways reflects human cognition procedures, it could even be considered automated image understanding.

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Automated quantification of SPECT/CT information with Definiens Cognition Network Technology

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Introduction: Fast, accurate extraction of quantitative anatomical and functional information from 3D data sets is a critical step in analyzing biological questions through in vivo small animal imaging. This work examines the development of an image analysis solution to extract and quantify major anatomical features from SPECT/CT (single photon emission computed tomography) data using the Definiens Cognition Network Technology. The automation helps to reduce analysis time; increases reproducibility, and decreases observer variability. An application for early stage tumor quantification is shown.

Methods: Using the commercially available software platform Definiens Developer, we developed an image analysis script to segment major organs from co-registered, dual-modality SPECT and CT image. The organs are detected in using a context-dependent approach which evolves the extracted mouse anatomical model from simple structures (e.g. body and lung) towards more challenging organs (e.g. kidney and heart). The analysis uses the CT information to extract the anatomy which provides further context for the SPECT analysis. The radiopharmaceutical uptake of the tumor was measured in a region refined by the anatomical model to reduce partial volume effects. The Definiens platform provided a rapid prototyping environment which reduced the development time for the image analysis solution to less than one week.

Results: In a first evaluation, the body, bones, brain, kidneys, lungs, and bladder were successfully extracted using one single image analysis script. The uptake was measured automatically and exported to a database. The preliminary results are

promising, in particular with respect of reproducibility within a single animal. This aspect is particular important for multiple time points pharmacokinetic studies.
Conclusions: An image analysis routine was developed to enable automatic segmentation and measurement of many important anatomical features in pre-clinical SPECT/CT data sets encompassing a wide range of applications in oncology.



Result of the automatic extraction of major anatomical features in SPECT/CT data using Definiens Developer software. Image courtesy of Bioscan Inc.

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Integrated analysis of kinetics data: how to analyze curve shapes systematically and optimize identification of actives

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Time-dependent responses are measured in a variety of assay technologies, including calcium flux assays and scalable label-free detection methods. Conventionally, such responses are integrated over time on the instruments and then reduced to one or just a few output variables, which are considered to quantify the compound activity.

Unfortunately, this procedure eliminates a large amount of the information contained in the observed time traces, their amplitude and shape. Such information could be very useful for separating intended effects from artifacts, distinguishing multiple biological mechanisms, and optimizing quantification of responses on the level of complete screens. Currently, accessing this information is extremely tedious, since it involves multiple applications, manual reformatting and transfer of large data sets.

In our case study, we demonstrate a framework which can overcome these limitations: Combining trace visualization, definition of time series aggregation and plate-based QC in a single tool allows fast review and interactive re-analysis cycles on kinetics assays. We show how this can be used to classify different curve shapes automatically and distinguish real actives from artifacts. By using all the available information in time-series data, the quality of hits is increased and the verification rate rises considerably.

Biopharmaceuticals I - Therapeutic Antibodies and Beyond: Antibody-based Therapeutics

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Selecting targets for anti-cancer antibodies: what have we learnt so far?*M.J. Glennie*, S.A. Beers, S. Lim, R.R. French, P.P.W. Johnson, M.S. Cragg
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Monoclonal antibodies (mAb) have provided some of the most exciting anti-cancer drugs to emerge in the last decade. Their success is generally attributed to high specificity, long half-life and diverse effector mechanisms, which include anti-tumour transmembrane signaling and cytotoxic effector cell recruitment. In this presentation we will show, using anti-CD20 mAb as a prototypic reagent, how Fc R-expressing effector cells, particularly macrophages, appear to control mAb efficacy. In addition, the importance of antigenic modulation will be discussed and whether the ability of mAb to cause the internalisation of CD20 might be an important factor in explaining the relative potency of different types of reagent.

CD20 is generally considered to be an ideal antibody target on B cells, due to its high level of expression, close proximity to the plasma membrane, and apparent lack of antigenic modulation preventing loss of surface CD20 molecules. As a result, unlike many B-cell target molecules (eg CD19 and CD22) which can internalise when bound by mAb, anti-CD20 appears to persist at the cell surface, allowing sustained immunological attack from recruited natural effectors. However, the relative resistance of CD20 to surface clearance has recently been thrown into question. First, data from Taylor and colleagues (University of Charlottesville) has shown that in the presence of Fc R-expressing cells, particularly monocytes, anti-CD20:CD20 complexes can be plucked from the target cells in a process called 'shaving', which leaves them viable but CD20 negative. Second, we find that when bound by certain so-called Type I CD20 mAb, such as rituximab and ofatumumab, cell surface CD20 is lost by internalisation both in vivo and in vitro, from normal and also certain neoplastic B cells. Thus, the importance of antigenic modulation in determining anti-CD20 mAb efficacy remains to be fully resolved, particularly as its negative influence on killing by Fc R-expressing effectors may not be the same as its effect on the transmembrane signalling necessary to induce direct programmed cell death.

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Development of a portfolio of antibody based therapeutics for cancer therapy*K. Bosslet***MedImmune (Cambridge, UK)*

Within the last 15 years, 8 antibodies either directed against targets on tumour cells, endothelial cells or neutralizing the activity of soluble factors were approved by FDA for the treatment of cancer. This great success, which was mainly based on the R+D activities of Biotech companies, has induced greater efforts within the pharmaceutical industry to generate therapeutic antibodies. Right now, more than 100 therapeutic antibodies are in clinical trials for various cancer indications.

This presentation will cover the following aspects of MedImmune's efforts in the field of therapeutic anti cancer antibodies:

- MedImmune's technologies to generate therapeutic antibodies
- Short overview on MedImmune's clinical oncology development pipeline
- Presentation on the molecular and biological characteristics of MedImmune's anti IGF1/2, anti PDGFR and anti Ang2 antibodies

Finally, a short preview about the future of therapeutic antibodies in cancer therapy will be given.

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Vascular targeting antibodies in cancer and arthritis: from the bench to the clinic*D. Neri***ETH (Zurich, CH)*

One avenue towards the development of more selective anti-cancer drugs consists in the targeted delivery of bioactive molecules (drugs, cytokines, procoagulant factors, photosensitizers, radionuclides, etc.) to the tumor environment by means of human monoclonal antibodies specific to tumor-associated markers. In this context, the targeted delivery of therapeutic agents to newly-formed blood vessels ("vascular targeting") is particularly attractive, because of the dependence of tumors on new blood vessels to sustain growth and invasion, and because of the accessibility of neo-vascular structures for therapeutic agents injected intravenously. Certain antibody-based vascular targeting approaches are also applicable to non-tumoral angiogenesis-related conditions, which may benefit from the targeted delivery of therapeutic agents to the abnormal blood vessels at the site of disease.

In collaboration with Philogen SpA and Bayer Schering Pharma, my laboratory has developed human monoclonal antibodies, capable of selective targeting of neo-vascular structures in solid tumors and in a number of angiogenesis-related diseases. Seven derivatives of these antibodies are currently being investigated in numerous multicenter clinical trials in Italy, Germany and UK.

Joint Keynote Lecture MipTec/BioValley Life Sciences Week

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Lgr5 intestinal stem cells in self-renewal and cancer*H. Clevers***Hubrecht Institute (Utrecht, NL)*

The intestinal epithelium is the most rapidly self-renewing tissue in adult mammals. Current models state that 4-6 crypt stem cells reside at the +4 position immediately above the Paneth cells in the small intestine; colon stem cells remain undefined. Lgr5/Gpr49 was selected from a panel of intestinal Wnt target genes for its restricted crypt expression. Two knock-in alleles revealed exclusive expression of Lgr5 in cycling, columnar cells at the crypt base. In addition, Lgr5 was expressed in rare cells in several other tissues. Using an inducible Cre knock-in allele and the Rosa26-LacZ reporter strain, lineage tracing experiments were performed in adult mice. The Lgr5+ve crypt base columnar cell (CBC) generated all epithelial lineages over a 14 month period, implying that it represents the stem cell of the small intestine and colon. The expression pattern of Lgr5 suggests that it marks stem cells in multiple adult tissues and cancers.

We have now established long-term culture conditions under which single crypts undergo multiple crypt fission events, whilst simultaneously generating villus-like epithelial domains in which all differentiated cell types are present. Single sorted Lgr5+ve stem cells can also initiate these crypt-villus organoids. Tracing experiments indicate that the Lgr5+ve stem cell hierarchy is maintained in organoids. We conclude that intestinal crypt-villus units are self-organizing structures, which can be built from a single stem cell in the absence of a non-epithelial cellular niche.

Intestinal cancer is initiated by Wnt pathway-activating mutations in genes such as APC. As in most cancers, the cell of origin has remained elusive. Deletion of APC in Lgr5+ve stem cells leads to their transformation within days. Transformed stem cells remain located at crypt bottoms, while fueling a growing microadenoma. These microadenomas display unimpeded growth and develop into macroscopic adenomas within 4-6 weeks. When APC is deleted in short-lived Transit Amplifying (TA) cells using a different Cre mouse, the growth of the induced microadenomas rapidly stalls. Even after 30 weeks, large adenomas are very rare in these mice. We conclude that stem cell-specific loss of APC results in progressively growing neoplasia. Moreover, a stem cell/progenitor cell hierarchy is maintained in early stem cell-derived adenomas, lending support to the "cancer stem cell"-concept.

Biological Space: Targets and Tools I - Biology of Sense receptors

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Taste and flavour: examples for the biological relevance of chemical sense receptors

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Good flavour and taste is mandatory for the success of food and beverage products. Especially for more healthy products, i.e. reduced in fat, sugar, and salt content or enriched with healthy ingredients, the maintenance of taste and flavour is a huge challenge. In most cases the acceptance of the products is reduced. In the last years some new flavour compounds and concepts based on flavour and/or taste modulation were developed. These new compounds do not necessarily show an own taste or flavour but can change the intensity of other taste qualities, e.g. sweet enhancers, bitter maskers etc. A further class of compounds is on the borderline between aroma and taste and elicit trigeminal responses, such as cooling, tingling, and warming or pungency.

Whereas sensory screening has played the most important role for the discovery of flavour modulators in the past, nowadays most of the receptors for the known taste qualities are well described and some of them are used as screening tools to select more promising candidates from natural or synthetic libraries. Nevertheless, sensory tools are absolutely mandatory for validation of the results due to the taste detection dynamics, bioavailability, and "side effects" in the sense of off-tastes. A recent development to speed up the "classical" testing protocols is e.g. the LC Taste[®] method.

In order to give some examples, the sensory activity of some flavanons such as homoeriodictyol and related molecules as bitter maskers, some gingerdiones and relatives as sweet enhancers, as well as some menthyl derivatives as umami compounds will be shown. In some cases we have tried to show correlations to the biological receptors, e.g. for the umami compounds.

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Detection of food-borne molecules by taste receptors in the mouth and in the gut

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The chemical nature of food is first assessed in the mouth by the taste system, and a decision to ingest or reject the food is made based on this assessment. The functional units of the taste system are onion-shaped clusters of sensory cells called taste buds grouped in papillae at the surface of the tongue and the soft palate. The main taste modalities are sweet, bitter, salty, sour and umami, the taste of monosodium glutamate. Other "minor" modalities, which are still controversial, include metallic and fat tastes. The receptors for sweet, bitter and umami have been identified. The sweet-responsive receptor is a heterodimer of T1R2 and T1R3, two related G-protein coupled receptors. The umami receptor is a heterodimer of T1R3 and T1R1 and is narrowly tuned to glutamate in humans and broadly tuned to L amino acids in rodents. The bitter receptors are members of a family of 25 G-protein coupled receptors, the T2Rs. Candidate receptors for sour and fat have been proposed. Once the content of the mouth is ingested, its chemical nature must continue to be monitored by the gastrointestinal tract (GI) so that the digestive system adapts its physiological status to its contents. We and others have found in the GI cells that morphologically resemble taste cells and express taste receptors and taste signalling molecules, suggesting that they may be the GI chemosensory cells. These cells likely carry out multiple functions including the modulation of glucose uptake and possibly the regulation of intestinal inflammation. Molecules that modulate the activity of taste receptors not only impact taste but may also impart health benefits.

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Taste hunters: a nice TR(I)P to discover bioactive compounds in traditional food

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The relationship between the molecular activation of GPCR and TRP ion channels involved in gustation and the mechanisms of food preference and choice are extremely interesting. Recent findings showed that some tastes are particularly important for their implications in health. Among these there are the bitter taste and the so called chemesthetic sensations. The bitter taste is mediated by GPCR protein receptors of the T2Rs family; its significance in nature is that of protection against potentially toxic substances, but a low level of bitterness is usually associated to plants that have curative properties, so that bitter plants are often described as "food-medicine". Beside this, there is an increasing interest for the chemesthetic (also called trigeminal or somatosensory) sensations, those associated to the "unusual" or "strong" gustative sensations found in many herbs and spices used in traditional food. These sensations are mediated by TRP ion channels, a large family of receptor involved in many important processes such as chemical sensing and nociception. The seminar will describe our integrated approach to:

- 1) identify and isolate new compounds from traditional food and food plants with specific activity on GPCR and TRP receptors;
- 2) evaluate the biological activity by means of both in vitro tests with cloned receptors, sensory evaluation and molecular modelling;
- 3) study their applications related to food (gastronomy, food industry etc), medicine (herbal remedy, analgesic properties etc.) and agriculture (crop and foodstuff protection).

SystemsX in MipTec

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High-throughput metabolomics for functional drug toxicity classification

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One key problem in drug development is to find drugs that are highly specific on their target with minimal off-target effects that might lead to highly undesired toxic effects. With skyrocketing cost for drug discovery and development, it becomes increasingly important to detect off-target effects early on the drug development process to reduce late-stage drug attrition. What would be an effective and sensitive measure of off-target effects? I will describe here monitoring of small chemical compounds within cells by metabolomics as a potential key measure that monitors effects on the functional level. Specifically, we developed two high-throughput mass spectrometric methods for targeted (MS/MS) and untargeted (TOF) metabolomics. By using flow injection, we avoid chromatographic separation such that 100 – 1500 metabolites can be detected in a given sample per minute. As a proof-of-concept, we demonstrate that applicability of this method by a drug-metabolism interaction screen in the yeast *Saccharomyces cerevisiae*.

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Evolution towards disease: combating a moving target

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Many human diseases are the result of evolutionary processes on time scales much shorter than the human lifetime. Prominent examples of pathogenic, measurably evolving populations are cancer cells in a tumor and infectious parasites, such as bacteria and viruses. Treatment of these constantly changing ensembles of individuals is complicated by evolutionary escape from the selective pressure of drugs and immune responses. We present mathematical models for the evolutionary dynamics of escape and discuss applications to the genetic progression of cancer and to the development of drug resistance in HIV. We show that the evolutionary potential of individual viruses is a strong predictor of therapeutic success in a clinical setting.

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Principles of post-transcriptional gene regulation by miRNAs

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miRNAs are 21-23 nucleotides long RNAs that are involved in the post-transcriptional regulation of gene expression. While initially it was believed that they regulate the translation rate of mRNAs, more recent studies revealed that they also induce substantial degradation of the mRNA targets. Although miRNAs appear to be involved in a broad range of biological processes, starting from development to cell division and metabolism, the targets of most miRNAs that have been discovered to date are not known. Here I will present various approaches that have been taken in order to unravel the determinants and the factors that modulate miRNA-target recognition.

Drug Discovery Technologies II - Automating Complex Assays

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Bacterial mutagenicity screening: automation of the Ames Test*S. Kirchner*, W. Muster, E. Brandt, R. Schmitt, P. laiza, D. Voegelin, T. Zumstein, T. Kissling, R. Bosshart, C. Fattinger
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The bacterial reverse mutagenicity test on *Salmonella typhimurium*, known as the Ames Test, is generally used to assess the mutagenic potential of chemicals and is part of standard regulatory testing for non clinical safety assessment of new pharmaceuticals. As mutagenic activity is very critical for the development of potentially new drug candidates, reliable information on Ames Test outcome is needed early on.

Non-regulatory miniaturised versions of the Ames Test generally show higher predictivity for the outcome of the OECD 471 guideline assay compared to bacterial indicator assays. However requirements on manual work and incubation time are similar for the miniaturized and the regulatory Ames Test.

In order to enable screening of more compounds at the low quantities available in early drug discovery with excellent predictivity, we automated a small volume version of the regulatory Ames Test that allows a significant reduction of the quantity of test substance needed (30 mg), but remains applicable to all *Salmonella* strains used in the regulatory protocol. This robotic system processes all five tester strains in parallel and performs - for the first time - all steps of a complete Ames Test automatically. The system encompasses automated preparation of the compound and bacteria mixtures into 4 microtiter plates including all dispensing and incubation steps. The system also includes processing of Petri dishes and growth media, as well as parallel pipetting of the bacterial mixtures onto pre-cast agar dishes and stacking of the dishes in easy-to-handle racks for incubation. We analyzed the accuracy and the reliability of the system for more than 100 runs in continuous operation. We compared the Ames Test results obtained on the automated system with those obtained by the manual procedure and confirmed identical dose response curves at similar or better reproducibility.

Adapting the plate preparation on the system to handling of larger compound volumes would allow to utilize the robotic system for the OECD 471 guideline protocol used in regulatory testing.

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Driving drug discovery through comprehensive automation of ADME-tox assays*J.M. Kolb***Bristol-Myers Squibb (Wallingford, US)*

Traditionally, ADME-Tox assays have been performed outside a high-throughput environment. Lead Profiling at Bristol-Myers Squibb was originally established ten years ago within the high-throughput screening group to bring cutting-edge automation technology to a panel of safety assays for hit evaluation. As the Lead Profiling group evolved, classic assays such as metabolic stability, CYP inhibition and Caco-2 permeability were included, and continuous improvement and innovation have resulted in significant growth in the depth and breadth of the Lead Profiling portfolio. The success of an automated ADME-Tox approach in advancing drug discovery is inherently tied to two major tenets: (1) reliable, reproducible gold-standard assays, and (2) fast data turnaround, ensuring concurrent delivery of liability data with activity data. Discussion will include specific examples of assay automation and evolution including quality assurance criteria, as well as a comparison of ADME-Tox automation with HTS approaches, the importance of leveraging diverse technologies to merge quality and speed, balancing demand with capacity, and risk mitigation.

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SEC/RPA (size exclusion chromatography/reverse phase protein arrays): a novel methodology for high throughput profiling of plasma lipoproteins*G. Dernick*, E. Niesor, C. Maugeais**F. Hoffmann-La Roche Ltd. (Basel, CH)*

Historically, lipoprotein particles were classified by their sedimentation in plasma samples by gradient ultracentrifugation and termed high density lipoproteins (HDL), low-, intermediate-, and very low density lipoproteins (LDL, IDL, VLDL). Besides this purely descriptive physical property, their amount is typically measured by the cholesterol concentration of the respective particle populations, expressed as LDL-cholesterol and HDL-cholesterol.

Although the correlation of LDL-cholesterol and HDL-cholesterol to cardiovascular risk has been shown in many studies, this neglects both the presence of proteins in the particles as well as their function. However, a paradigm shift towards an increased interest in the function of the particles and the proteins is currently underway in the lipoprotein research field. This has also been fueled by proteomics studies, which identified many more proteins associated to HDL than initially assumed. Particularly in the context of studying the mode of action of lipoprotein particle modulating drugs, a better understanding of the function could provide valuable knowledge. For a minimal disturbance of the protein association with the particles, experimental conditions close to the native state are desirable.

In the past two decades, particle separation by size exclusion chromatography (SEC) has been established as a more practical alternative to ultracentrifugation. While ultracentrifugation is still widely accepted for the analysis of the lipid constituents, concerns have been raised that the high salt concentrations may disrupt the protein associations to the particles or may change the conformations of the proteins. The low salt concentrations employed in SEC should thus be beneficial for protein analysis of lipoprotein particles.

We now developed reverse phase protein arrays (RPA) from fractions, which were obtained using size-exclusion separation of plasma and termed this combination SEC/RPA. The arrays were probed with various antibodies against lipoproteins and other plasma proteins. In this way we measured the relative abundance of the individual proteins in each fraction from which we generated "protein chromatograms". This technique allows to analyze thousands of fractions for tens of proteins in a fast and cost-effective manner.

We studied the modulation of lipoprotein particle composition by drugs or proteins with SEC/RPA. We have shown that this tool indeed captures remodeling of lipoprotein particle composition.

Pharmacodynamics/Biomarkers I

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Protein microarrays for biomarker discovery

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Array-based assay systems allow the determination of hundreds of molecular parameters in a single experiment. Within the last decade protein microarray technologies achieved robust analytical performance and enable to screen for a multitude of parameters using minimal amounts of sample material. Protein microarray-based assays can be grouped according to formats and types of applications. Currently, forward-phase protein microarray assays are the most frequently used format. Using an array of well-defined capture molecules allows the simultaneous analysis of large numbers of different parameters from one sample. Examples of forward-phase microarray assays include protein profiling arrays using multiplexed immunoassays to identify and quantitate target proteins of interest. The other format, affinity arrays used immobilized recombinant proteins allowing to study interactions between proteins and immobilized binding molecules such as proteins, peptides, low molecular weight compounds, oligosaccharides, or DNA. The other protein array format, the reverse-phase protein arrays, immobilizes in a microarray format a multitude of different samples such as tissue or cell lysates. Each microspot contains the whole proteome repertoire of the tissue or cell. Highly specific antibodies are used to simultaneously screen these spots for the presence or absence of distinct target proteins. Using replicates of these microarrays large number of parameters in large collections of tissue or cell samples can be determined (1-4).

Examples will be given to discover disease specific biomarkers using focused protein expression analysis of tumour samples or to profile plasma samples using miniaturized and parallelized sandwich immunoassays. These findings for biomarker discovery will be discussed in the context of the FDA's critical path initiative and the Europe-based public-private Innovative Medicines Initiative (IMI) (5,6).

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Application of pharmacokinetic-pharmacodynamic (PKPD) modelling and simulation in translational biomarker research

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Although PKPD modelling and simulation approaches have formed part of clinical R&D since the 1980s, 'model-based drug development' paradigms are a far more recent phenomenon, being increasingly advocated by both regulatory agencies and pharmaceutical research organizations as a way of improving efficiency and productivity. Indeed, many companies and the FDA have formed groups dedicated to the discipline of 'Pharmacometrics'. This is the science of developing and applying mathematical and statistical methods to characterize, understand and predict a drug's behaviour in terms of its pharmacokinetics, pharmacodynamics, and biomarker-outcomes. More recently, it has also been suggested that PKPD modelling and simulation can play a significant role in early preclinical drug discovery and can provide a framework for 'translational' research which links, in a quantitative manner, the interactions between a drug (or combination of drugs), pharmacological targets, physiological pathways and, ultimately, integrated disease systems. An important application of preclinical PKPD is that a quantitative understanding of in vivo pharmacological exposure-response relationship can aid the identification and selection of suitable biomarkers in early exploratory clinical development.

Until recently, translational PKPD, a relatively new area in drug discovery, was mainly restricted to academic research. However, it is increasingly being recognized that successful implementation of PKPD reasoning in early drug discovery could have at least as much impact on the overall efficiency and success of pharmaceutical research as comparable investments in late-stage modelling and simulation. This is because arguably the most significant challenge facing the pharmaceutical industry is compound attrition resulting from the failure of preclinical efficacy and safety model data to translate into human proof of mechanism/concept studies. With increased interest in its relevance to preclinical research, PKPD has evolved towards a more mechanistic approach. Recently, this has led to the emergence of 'systems pharmacology' which bridges systems biology and PKPD as a multidisciplinary approach to the quantitative analysis of the dynamic interactions between drug(s) and a biological system.

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A multidisciplinary hunt for prostate cancer biomarkers and therapeutical targets

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Prostate cancer is usually indolent during its progression to clinically relevant cancers and a non-invasive and efficient screening procedure for its detection is still lacking. The identification of aggressive tumors or responders to targeted therapy at the time of diagnosis might offers further preventive therapeutical options to the patient. We present a multidisciplinary strategy for biomarker discovery, where a quantitative proteomics screen for N-linked glycosylated proteins is applied to prostate tissue and serum of the PTEN conditional knockout mouse model for prostate cancer progression. The knowledge derived by this approach is then filtered for candidate biomarkers verification and validation in both serum and tissue samples belonging to patients harboring localized prostate cancer or benign prostatic hyperplasia. Candidates are currently further screened for in vitro functional assays, in order to evaluate their potential role as easily accessible therapeutical targets.

Biological Space: Targets and Tools II - Impact of Stem Cells on Drug Discovery

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High-throughput screening in human embryonic stem cells for drug discovery

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High-throughput screening of chemical libraries has become an indispensable tool in modern basic biology and drug discovery. However, its implementation and the adaptation of high-content assays to human embryonic stem cells (hESCs) have been hampered by multiple technical challenges. hESCs can be difficult to grow in large amounts and to keep in an undifferentiated state in formats necessary for high-throughput screening procedures. We have developed a strategy to adapt hESCs to high-throughput screening conditions, resulting in an efficient assay for the discovery of small molecules that drive hESC self-renewal or differentiation. The use of this new assay has led to the identification of several drugs and natural compounds promoting short-term hESC maintenance and compounds directing early lineage choice during differentiation. We performed a global gene-expression analysis upon drug treatment, which allowed us to identify known and novel pathways correlated to hESC self-renewal and differentiation.

Our results demonstrate feasibility of hESC-based high-throughput screening and enhance the repertoire of chemical compounds for manipulating hESC fate. The availability of high content assays should accelerate progress in basic and translational hESC biology.

Ethical issues related to the use of hESCs led us to adapt this technology to a more suitable type of human cells: the keratinocytes. These cells are an excellent model for transdifferentiation. They can readily be reprogrammed to induced pluripotent stem cells.

We are now able to transdifferentiate them to neural progenitors, which should allow for efficient screening strategy for small molecules that could be used for regenerative medicine.

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Assay development in primary cells: What can we learn from bladder smooth muscle?

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Most early stage drug discovery programs use recombinant cell lines, often with highly overexpressed receptors in a non-relevant cell background. Ideally, cell models used for early stage SAR generation would be the same as used in ex vivo or in vivo models, and more predictive of true drug potency in patients.

Human-derived primary cells are assumed to have a more physiological expression profile, with the correct auxiliary proteins, and should therefore be a more predictive cell model than recombinant cells. If the challenge of developing and prosecuting a robust assay with cultured primary cells could be overcome, many researchers might choose to replace the recombinant cells they commonly use for screening.

In the current study primary bladder smooth muscle cells (BSMCs) from three human donors were commercially sourced from Lonza and Promocell. These cells were assessed for their potential to be used as a high throughput screening model for genitourinary diseases such as overactive bladder.

The work was divided into two stages: 1) characterisation of gene expression profiles using qRT-PCR and 2) functional analysis of key bladder receptors using conventional Ca²⁺ flux assays and label free technology. Culture media with or without growth factors that promote cellular proliferation was tested to assess how forcing the cells to proliferate would affect their gene expression profile and function.

The current study created more questions than answers; high quality antagonist assays were set up using both conventional and label free technologies, highlighting the possibility of using cultured primary cells for SAR generation. However, the mRNA expression of the key receptors in the hBSM cell model was not as close to human tissue as might be required, therefore challenging the idea of these cells being more predictive of drug potency in human.

It is likely that similar approaches to thoroughly check the gene expression and function may be needed for stem cell applications to make sure the data generated is predictive of tissue or in vivo models.

Biopharmaceuticals II - Therapeutic Antibodies and Beyond: Next Generation Antibodies

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Anticalins[®] and other non-Ig protein scaffolds as next-generation therapeutics

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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].

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 beta-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 form the antigen-binding site. Yet, 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 both low molecular weight compounds and protein antigens. An Anticalin that recognizes a rare earth metal chelate complex opens applications in radio-immuno 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]. Another Anticalin with strong antagonistic activity towards vascular endothelial growth factor (VEGF) effectively suppresses neoangiogenesis and is scheduled for clinical trials as an alternative to full size antibodies to treat cancer.

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T Cell-engaging BiTE[®] antibodies for therapy of cancer

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Micromet Inc. (Bethesda, US)

Bispecific antibodies can transiently link tumor cells with otherwise inactive T cells for induction of a target antigen-dependent redirected lysis of tumor cells. One example is MT103 (MEDI-538), a CD19/-CD3-bispecific BiTE for the treatment of human B cell malignancies. MT103 showed high anti-tumor activity in immunodeficient mouse models, and showed biological activity in a non-human primate. MT103 and other BiTE antibodies were shown to activate T cells in a highly conditionally manner that is entirely dependent on the presence of target cells. MT103 is in phase 1 dose escalation study for the treatment of patients with therapy-refractory non-Hodgkin's lymphoma (NHL), and in a phase 2 study in patients with B-precursor acute lymphocytic leukemia (ALL). Centrally confirmed complete and partial responses have been observed with MT103 in NHL patients, and clearance of bone marrow in ALL patients.

A novel BiTE antibody called MT110 is recognizing the pan-carcinoma antigen EpCAM (CD326), which is expressed on a great variety of human adenocarcinoma, and on so-called cancer stem cells. A murine EpCAM/CD3-specific version of the BiTE antibody, called muS110, has shown a robust therapeutic window in mice with no damage to EpCAM-expressing normal epithelia (11).

A new BiTE platform has been developed using an anti-CD3 single-chain antibody of human sequence, and shows crossreactivity with non-human primates. BiTE antibodies specific for CD33, EGFR, Her-2, IgE and melanoma target antigen MCSP have been generated on this platform and shown to have a very high potency of redirected target cell lysis. Primate studies with the MCSP- and CD33-specific BiTE antibodies showed efficacy (CD33) and good tolerability (CD33 and MCSP). Conversion of trastuzumab, cetuximab, panitumumab and omalizumab in BiTE antibodies was successful and generated molecules engaging at high potency T cells for target cell lysis.

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Nanobodies[™]: Antibody-based next generation therapeutics

G. Beste*

Ablynx NV (Zwijnaarde, BE)

Ablynx focuses on the discovery and development of Nanobodies[™] as next generation therapeutics for the treatment of human diseases. Nanobodies[™] are antibody-derived therapeutic proteins. They consist of the smallest antigen-binding fragments of naturally occurring heavy-chain only antibodies from camelids.

Nanobodies[™] distinguish themselves from conventional antibodies through favorable biophysical properties and ease of manufacture. This enables reformatting of monomeric Nanobodies into multimeric constructs with tailored characteristics.

Examples of how Nanobodies can be formatted to generate clinical candidates with the desired biological activities including selectivity, high potency and appropriate half-life will be shown. These examples provide a powerful illustration of the flexibility of this technology and its potential to conquer new clinical ground.

Structural and Computational Drug Discovery I - Virtual screening applications

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Increasing hit rates by virtual screening and the use of target optimized scoring schemes*B. Kramer***4SC (Planegg-Martinsried, DE)*

Right from the start, 4SC implemented a drug-development platform, that was driven by problem-adaptable structure- or ligand-guided virtual screening processes. Many projects, in-house as well as cooperations with academic groups or pharma companies have shown evidence of the benefit of these methods and helped to gain a lot expertise. Nevertheless, we saw the need for further improvement of the scoring engine as well as the data mining techniques in order to be able to search molecular libraries of tens of millions of commercial and combinatorial compounds sufficiently fast.

The analysis of computational score and biologic activity data by means of several statistic techniques led to methods to help understand and quantify the enrichment process. The problem, however, remains the availability of a fast, universal, and accurate scoring method. A solution can be provided by using a target-specific scoring function, which improves virtual screening performance significantly and serves as a practical and easily applicable alternative.

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Lead identification by virtual screening - Chances, challenges and pitfalls*H. Köppen***Boehringer Ingelheim (Biberach, DE)*

In current pharmaceutical research, lead compounds of high quality and structural diversity are key to the successful optimization of development candidates. In pharmaceutical companies in-house compound libraries are the major source of leads for new projects. Since some years the virtual screening of compounds outside the in-house pools offers the chance to retrieve potential lead candidates from a chemical space which is orders of magnitude larger than these in-house pools. This chemical space comprises both physically existing compounds which are available from commercial vendors as well as compounds which can be synthesized on demand.

Virtual screening is a knowledge-based approach that requires information either about the 3D structure of a target or about known ligands of this target. Both approaches can be combined if the necessary information is available. The main challenge of virtual screening is the computational high-throughput assessment of the biological activity of each compound within the screened, structurally diverse library.

There is a huge number of different target-based or ligand-based virtual screening methods available. Many published examples of successful virtual screening projects demonstrate the feasibility of this approach. However practitioners often notice that virtual screening still suffers from some shortcomings which may limit its use in daily project work. Virtual screening can search huge chemical spaces but for practical reasons only a small number of compounds can be purchased or synthesized and tested. Hits which are identified within these compounds by biological testing are often only weakly active which makes them less attractive for research teams. Moreover experience has shown that the success of virtual screening can hardly be predicted and critically depends not only on the information basis and the methods which are used but also on the chemical space that is searched for hits.

Recently huge virtual combinatorial libraries could be successfully searched for bioactive compounds. The synthesis of follow-up libraries, designed according to the needs of the respective projects, is fast and straightforward in these cases. It seems that this specific approach has the potential to deliver attractive hits.

The talk will briefly summarize some technical aspects of virtual screening and highlight achievements as well as challenges. A focus will be put on the integration of virtual screening into the industrial research process and on the successful combination of virtual screening and combinatorial chemistry.

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Virtual screening: examples and applications*D.E. Clark***Argenta Discovery Ltd. (Harlow, UK)*

Over the course of the past decade, virtual screening (VS) has become a popular approach to hit finding within the pharmaceutical industry. Although the technologies underpinning VS are still being developed, the approach has already been successfully applied in many drug discovery projects. Indeed, VS has played a significant role in the discovery of several compounds that are currently in clinical trials. In the course of this presentation, the application of VS at Argenta Discovery will be described and illustrated with a number of successful examples. In closing, some of the shortcomings of current VS technologies will be discussed and some of the active areas of research in the field will be highlighted.

Chemical Space: Maximum Compound Value I - Nature of Chemical Space

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Probing the link between efficacy, ADMET and physico-chemical parameter space*M.P. Gleeson***Kasetsart University (Bangkok, TH)*

Analyses have been performed: (a) on a large corporate database from a leading pharmaceutical company to help elucidate the underlying properties fundamental to ADMET liabilities and (b) on an extensively compiled marketed drug dataset where the relationship between in-vitro potency and dose for oral drugs has been compared. From these comprehensive analyses the following issues are discussed:

1. What are physico-chemical properties influencing almost all ADMET liabilities?
2. Do we require compounds with nM in vitro activities for in vivo efficacy?
4. Are we searching in the optimal region of physical property and biological space?

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Pharmacography: the art of mapping drug-target interactions*T. Oprea***University of New Mexico School of Medicine (Albuquerque, US)*

Our understanding of drug-target interactions has been limited by the amount of experimental evidence, and by our ability to attribute drug-target interactions to clinical relevance. Dubbed polypharmacology or secondary pharmacology, this emerging area challenges our notions of drugs selectivity. Several drug-target informatics attempts focussed on mapping drug interactions to the intended drug targets, i.e., those targets claimed as being associated with relevant clinical phenotype. Antitargets, such as hERG (human Ether-?-go-go Related Gene, a potassium channel) and M1 (muscarinic M1 cholinergic receptor), or targets responsible for significant adverse drug reactions, are also receiving increased attention. Annotating drugs, and more generally ligands, to their appropriate targets and proteins, is the main focus of pharmacography.

We will place the relationship between documented (i.e., measured IC50) drug-target interactions and clinical relevance, within the context of several pharmacokinetic parameters such as MRTD (the maximum recommended therapeutic dose), plasma protein binding, and availability. Extensions to a CNS-centric model of ligand-protein interactions will be discussed. Our preliminary results suggest that most drug compounds exhibit their clinical effect (both medicinal action and adverse drug reactions) via interacting with a panel of biological targets rather than being selective to one. Indeed, the term "selective" in "SSRI" (selective serotonin re-uptake inhibitors) is a mis-nomer, since SSRI drugs are documented to interact with hERG, M1 and other targets. Though limited by data availability, a pattern is beginning to emerge: Namely, that the claimed drug selectivity towards a small number of therapeutic targets is an artefact due to lack of systematic observation against all possible targets; an additional component is localized drug penetration (e.g., aerosol, ophthalmic). More integrated ligand-protein datasets, supported by improved drug target network models, are required in order to derive a fully comprehensive picture. Such an effort may serve as the rational basis for drug repurposing, i.e., identifying novel modes of action and therapeutic applications for already-approved drugs, and may have a profound effect on the way drug design will be conducted in the future.

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DrugSpace: expanding the chemical space around known drugs*J. Mestres***Municipal Institute of Medical Research (Barcelona, ES)*

Drugs represent a particular type of molecules that have reached the market after being thoroughly characterised and passing an enormous amount of physicochemical, biological, and clinical filters. They can be regarded as tiny bright stars in the vast chemical universe and thus may argueably be the best starting points for drug discovery projects. Accordingly, expanding the chemical space around those drugs could become a good source of novel progressible hits.

The structures of 1000 drugs were taken as seeds from which the respective chemical spaces were expanded. Each drug seed is subject to an exhaustive set of structural modifications, such as atom mutation, bond saturation, branch creation, and ring generation. Then, each of those slightly-modified drug structures becomes a seed itself to which all structural modifications are applied. The process continues until the molecular weight and clogP of the molecules generated reach some predetermined values. This chemical space constructed in silico around drugs will be referred to as DrugSpace.

Drug Discovery Technologies III - Methods for Measuring Molecular Interactions

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Characterizing proteins and protein interactions by analytical ultracentrifugation

P. Schuck*

National Institutes of Health (Bethesda, US)

In the last decade, the introduction of modern computational approaches has substantially increased the potential of analytical ultracentrifugation to characterize proteins and protein interactions. In particular, sedimentation velocity analytical ultracentrifugation has emerged as a powerful tool with high sensitivity and hydrodynamic resolution, which can be applied to study reversible protein complexes in free solution with regard to their number, stoichiometry, affinity, size and shape. This presentation will provide an overview of the principles of modern analytical ultracentrifugation and give examples from a variety of areas of application, including multi-protein complexes, membrane protein interactions, nano-particles, and the quantitation of trace aggregate populations.

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Analysis of noncovalent complexes by ESI-MS

R. Zenobi*

ETH (Zurich, CH)

Soft ionization techniques used in modern mass spectrometry (such as ESI) even allow to keep noncovalent complexes intact in the gas phase. ESI is in fact believed to keep proteins in a near-native state in the gas phase. Under well controlled experimental conditions, ligands or inhibitors therefore can stay bound. Since the first reports of using ion spray MS (a variant of ESI) for assessing the binding strength of noncovalent interactions, over 15 years have passed, and one would think that K_D measurements of complexes using soft ionization MS should be routinely used by industry now, in particular in combination with autosamplers or nanospray array chips. However, with very few exceptions, this is not the case. There are a number of reasons for this, of fundamental and practical nature, which will be addressed: does the measured K_D s depend on the spray method used? Does it depend on the charge state? What is the range of binding constants that can be reliably measured by MS? What is the accuracy of K_D s that are obtained by mass spectrometry? Are solution phase affinities rather than gas-phase affinities measured? Are solution phase equilibria distorted by the ESI process? Can active protein concentration in solution be determined?

We have investigated many of these questions in detail. Native solutions buffered with volatile buffers were used to study noncovalent complexes. Measurements were performed on a QTOF instrument (QTOF ULTIMA, Waters). Normal ESI, electrosonic spray ionization (ESSI, using a home built source), and chip-based nanoelectrospray ionization (Nanomate 100, Advion) were used for measuring K_D s with the titration method, and compared in their ability to obtain accurate solution phase K_D s. Using the titration method, K_D s in the range of high μ M to low nM can be reliably measured. A novel method based on ligand competition will also be introduced. It allows K_D s down to the 10^{-15} M range to be determined. Finally, benchmarking against established solution phase methods such as ITC, SPR, or CD spectroscopy, show that MS-based methods yield accurate K_D values.

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Some like it hot: immobilization-free, contact-free molecular interaction studies using microscale thermophoresis

P. Baaske* (1), C.J. Wienken (2), S. Duhr (1)

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Microscale thermophoresis is the directed movement of molecules in optically generated microscopic temperature gradients (1, 2). The thermophoretic movement is determined by the entropy of the hydration shell around the molecules (3). As almost all interactions between molecules alter this hydration shell, molecular interactions can be determined and quantified. The readout method of the interaction studies is based on, but not limited to, fluorescence techniques and is conducted immobilization-free in solution. This approach employs an exceedingly robust and flexible optical train which can be used with a broad range of sample supply systems: microfluidic chips, capillaries, multiwell plates as well as simple droplets on a slide. Depending on the chosen sample supply, material consumption can be as low as 1nl. Microscale thermophoresis can be used to determine the affinity of interactions between proteins, nucleotides and proteins, proteins and ions and proteins and small molecules with a high dynamic range (K_D ranging from 10pM to 1M). Using microscale thermophoresis dissociation constants were quantified for GFP with a GFP-antibody, DNA-aptamer with ATP, DNA-aptamer with thrombin and several low molecular weight inhibitors (<350Da) with different kinases.

In this presentation we will describe the technical details and the benefits of the microscale thermophoresis technology platform. With the focus on small molecules we will discuss how microscale thermophoresis can be applied in different stages of the drug development process: for example for high throughput screening in early stages and later on for studies under conditions close to in-vivo. The technique is very feasible for these kinds of measurements as it can be applied in nearly all aqueous solutions, ranging from standard buffers or high content DMSO buffers to blood serum and whole blood. Thus, using the same measurement method, substances showing feasible affinities in artificial screening buffers can be tested if they maintain their binding properties in the biological liquids where they have to act as a drug.

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Early Safety Evaluation

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How can we discover new drugs to block multidrug ABC transporters?

*R. Callaghan**

University of Oxford (Oxford, UK)

Multidrug efflux pumps belonging to the ABC superfamily of membrane proteins represent a biological enigma; namely the ability to bind to, and translocate, astonishingly large numbers of drugs. Three multidrug efflux pumps from the ABC family are found in humans and include ABCB1 (P-glycoprotein), ABCC1 (Mrp1) and ABCG2 (Breast Cancer Resistance Protein). ABCB1 has been the focus of research efforts into ABC transporters and is known to contribute to the resistant phenotype in numerous cancers. It has recently been suggested that the protein is also responsible for resistance in epilepsy and the failure of several HIV/AIDS chemotherapeutic agents. ABCB1 is also expressed in healthy tissues including barrier sites and excretory or absorptive organs, which presumably reflects its role in ADME of therapeutic agents. Clearly there are a number of clinical situations that would benefit from the ability to modulate the activity of ABCB1. Considerable effort has focussed on producing a clinical inhibitor of the protein; yet despite four generations of compounds none has been successfully translated to clinical usage. We propose that a more rational approach should be used to develop potent and effective inhibitors. The strategy requires the incorporation of information on the mechanism underlying the biological enigma of multidrug recognition and/or a greater understanding of the complex mechanism of drug translocation by efflux pumps. The translocation process requires initial drug association to specific sites followed by reorientation of the site to the alternate face of the membrane and drug dissociation. These key steps in the process are "powered" by events in the nucleotide binding domains, which mediate ATP hydrolysis. The inter-domain communication is co-ordinated by several regions of ABCB1 although their precise identity remains elusive. Continuing biochemical investigations and the recent structural data for ABCB1 will facilitate identification of these key functional regions. The structural features and associated chemical interactions of ABCB1 with drugs may then be utilised to direct inhibitor design strategies.

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The blood-brain barrier and CNS penetration: a drug discovery point of view

*A. Reichel**

Bayer Schering Pharma (Berlin, DE)

The discovery of new medicines to treat diseases of the central nervous system is one of the most challenging objectives for the pharmaceutical industry with drug attrition in development being much higher than in most other therapeutic areas.

While there are many hurdles any oral drug has to overcome between administration and reaching its site of action, in addition to the barriers of and various mechanisms for absorption, metabolism, excretion and distribution, CNS drugs have to be able to penetrate the blood-brain barrier (BBB). This is a highly selective barrier which is formed by the endothelial cells lining the cerebral microvessels, and acts as a rigorous gatekeeper of the CNS by forcing all molecular traffic to take a transcellular route across the BBB. Drug penetration into the CNS and the amount available to bind to its target is dependent on the rate of entry through the BBB, the distribution within the CNS and the rate of removal from the CNS. Each of these processes may be passive or driven by active uptake, protein binding, drug metabolism and/or efflux transporters.

CNS drug discovery groups currently utilize data from different *in vitro* assays and *in vivo* studies in order to be able to adequately evaluate and rank compounds and to generate confidence that these compounds will be sufficiently CNS-penetrant in man. Successful drug discovery depends on optimizing both the rate and the extent of CNS penetration *in vivo*. A high rate of penetration results from high permeability as well as low binding to brain tissue. Measurement of whole-brain drug concentrations alone can be misleading and the extent of brain penetration is best assessed by determining the unbound drug concentration in the brain, i.e. the extracellular fluid levels, in relation to the free plasma concentration.

In order to link the exposure of drugs to their effects in the CNS and to delineate key PK parameters, a new integrated approach to CNS drug discovery is about to emerge which more rigorously distinguishes total from unbound brain concentrations. As the complex nature of the brain requires different compartments to be considered when trying to understand and improve new compounds, several complementary parameters need to be measured *in vitro* and *in vivo* and integrated into a coherent model of CNS penetration and distribution.

The integration of pharmacokinetic and pharmacodynamic data also supports the prediction of dose to humans and facilitates, with the aid of safety assessment data, to define a safe starting dose for the first trials in man. It is important to take into consideration species differences in the pharmacokinetics and metabolism and in the CNS penetration and disposition of drug candidates, especially when interpreting toxicology findings.

A fully integrative approach between research, preclinical and clinical departments will improve the success in the translation from animals to man, leading to reduced late-stage attrition in development for drugs targeted to the CNS.

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Monitoring ATPase activity and transport by P-glycoprotein - A new approach*P. Nervi, X. Li-Blatter, P. Äänismaa*, A. Seelig
Biozentrum der Universität Basel (Basel, CH)*

P-glycoprotein (ABCB1) hampers passive drug influx into the cytosol by binding drugs in the cytosolic membrane leaflet and flipping them to the outer leaflet at the expense of ATP hydrolysis. Here, we show that it is possible to monitor P-glycoprotein transport by comparing the ATPase activity in cells and inside-out plasma membrane vesicles, thereby exploiting the different substrate access mechanisms to the P-glycoprotein binding region. In cells drugs have to cross the membrane by passive diffusion to reach the P-glycoprotein binding region, whereas in inside-out plasma membrane vesicles they can directly insert into the cytosolic membrane leaflet. Compounds diffusing rapidly across the membrane compared to transport by P-glycoprotein exhibited identical ATPase activity profiles in cells and inside-out plasma membrane vesicles because the substrate concentrations in the cytosolic plasma membrane leaflet of the two systems were similar. However, compounds diffusing slowly exhibited different ATPase activity profiles in the two systems because the compound concentration in the cytosolic membrane leaflet of cells was distinctly reduced. This shows that P-glycoprotein could cope with the influx of slowly diffusing compounds. The comparison of the ATPase activity profiles in cells and inside-out plasma membrane vesicles obtained by monitoring the extracellular acidification rate (ECAR) and the phosphate release rate, respectively, yielded direct information on substrate transport by P-glycoprotein which neither method alone could provide.

Biological Space: Targets and Tools III - Anti infectives and Malaria

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Natural products as anti-bacterials*P. Hammann***Sanofi-Aventis (Hoechst, DE)*

An analysis of the marketed antibacterial structural classes today reveals that 75% are secondary metabolites from micro-organisms. Although the rate of resistance of bacterial pathogens is constantly increasing, the number of new and improved antibacterials is dramatically decreasing. While 16 new antibiotics came to the market in the early 1980's (1983-87), there were only 4 approvals recently (2003-07). One of the reasons of this lower output of new antibacterials can be attributed to the reliance on synthetic chemical libraries used for HTS based on a large scale mining of targets and a parallel downscaling of natural product screening within of the pharmaceutical industry. Based on the present status the advantages and disadvantages of phenotypic vs. target based screening as well as extract or pure natural product screening vs. synthetic libraries will be discussed and the possible future of natural product research will be outlined.

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Revisiting non-target based screening in TB*E. Alvarez***GlaxoSmithKline (Tres Cantos, ES)*

Tuberculosis is a major cause of morbidity and mortality in humans, and is responsible of more adult deaths worldwide than any other single infectious agent. Problems associated with the disease have been exacerbated by its link with human immunodeficiency virus and emergence of isolates resistant to commonly used therapeutic agents. In addition to that, the tubercle bacterium is able to live in a persistent stage that is supposed to be the main reason for most of the problems associated with the disease. The WHO estimates that around one third of the world population is infected by the latent form of the pathogen and thus becomes the main reservoir for active tuberculosis cases. Also, drug resistance in tuberculosis is a matter of great concern for TB control programs since there is no cure for some multidrug-resistant TB (MDR-TB) strains of *M. tuberculosis*. The emergence of extensively drug resistant (XDR) strains has increased the concern that these strains could spread around the world, stressing the need for additional control measures, such as new diagnostic methods, better drugs for treatment, and a more effective vaccine. Thus, development of novel antitubercular drugs is a first priority for all the groups involved in the fight against this disease.

The GSK strategy to develop new drugs against tuberculosis is based on the study of biochemical targets considered to be essential for the tuberculosis bacteria. Recently, as a complementary approach to target-based programs, we have started a program for the discovery of inhibitors of the *M. tuberculosis* growth using a whole cell approach. For that, we have developed a miniaturised HTS assay using *M. bovis* BCG as a surrogate strain. The assay is based on the measurement of the intracellular ATP pool as an indicator of the bacterial growth, in a 5 µL assay volume in 1536 well plates. Using these assay conditions we have screened the GSK corporate collection. Primary hits were confirmed in a single shot assay and the confirmed hits were progressed to dose response. After MIC determination of the confirmed hits a more detailed hit characterisation process is currently being performed.

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New medicines to drive the eradication of malaria*D. Leroy***Medicine for Malaria Venture (Geneva, CH)*

Malaria kills up to one million people annually. The majority of malaria's victims are children under five and pregnant women. Innovative medicines are needed to cure and protect the millions of people at risk of malaria and help to ultimately eradicate this terrible disease. Medicines for Malaria Venture (MMV) is a non-profit organization created to discover, develop and deliver effective and affordable antimalarial drugs. The current presentation will describe how we, at MMV, federate public-private partnerships between multidisciplinary teams and orchestrate modern drug discovery for the eradication of malaria through three main paths:

- 1) Development of methods to determine and understand how parasite resistance to antimalarials, and especially artemisinin, may occur. We are measuring the resistance to mutants of *Plasmodium* generated in the laboratory, with a view to mapping the cross-resistance potential and also to identify its molecular basis.
- 2) Identification of high content assays to measure and predict anti-hypnozoite activity. We anticipate that in 10 years time, the overall global burden of *P. falciparum* malaria will have diminished considerably. By this stage the reservoir of *P. vivax* malaria will probably become a much more significant health question. To date, only 8-aminoquinolines are clinically active against hypnozoite, the dormant liver stage of *P. vivax* and no cellular model exists in humans. The only reliable model is in primates. Therefore, we are supporting the development of an in vitro assay for hypnozoites. In addition, we are working on the infection of liver cells by sporozoites (the liver stage infective forms of the parasite). We believe that some clues on how to handle the hypnozoites may come from these studies.
- 3) Validation of methods to look for compounds with anti-gametocyte activity and acting as Malaria transmission-blocking agents.

The role of gametocyte-killing activity in the next generation of antimalarials is going to be very important. With a research group at Imperial College London, we have set up high content assays to screen for activity against gametocytes. Indeed, it is important to enable the selection of compound series that have dual activity against both erythrocyte and gametocyte stages. Taken together, those three approaches constitute the basis of what we call 'lifecycle fingerprint', and in the near future all of our drug discovery projects will benefit from this process.

Structural and Computational Drug Discovery II - Structure-based Design of Protein Therapeutics

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Engineering insulin for better diabetes therapy

*R. Hilgenfeld***University of Lubeck (Lubeck, DE)*

Insulin is arguably the most important therapeutic protein. Over the past two decades, major efforts have been made to improve the insulin and blood glucose profiles that can be achieved in diabetes therapy. This contribution will review a few examples of engineered insulins and discuss their relative merits and problems.

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Design of super active variants of coagulation factor VIIa

O. Hvilsted Olsen, H. Ostergaard, H.R. Stennicke, E. Persson**Novo Nordisk (Måløv, DK)*

Coagulation factor VIIa (FVIIa) is an atypical member of the trypsin family of proteases. It fails to spontaneously attain its catalytically competent conformation but is dependent on its protein cofactor tissue factor (TF) to accomplish this. Hence, association of FVIIa with TF renders it biologically active and capable of inducing blood clotting. Over a number of years, the unique behaviour of FVIIa has prompted investigations of the structural basis for the TF-induced activation mechanism of FVIIa. FVIIa has gained additional interest in the past decade because of its development into a clinically useful haemostatic agent. However, the identification and development of new, superior FVIIa variants for clinical use has great potential.

FVII is secreted as a zymogen (single chain) and depends on endoproteolytic cleavage of a peptide bond to become catalytically active. In trypsin, the newly formed N terminus spontaneously inserts into a cavity, resulting in a stabilising salt bridge which leads to maturation of the catalytic apparatus including stabilisation of the so-called activation region. FVIIa has a very low catalytic activity due to incomplete N-terminal insertion. TF allosterically facilitates this process and markedly accelerates the activity of FVIIa towards physiological substrates. Various experimental observations support a TF induced allosteric activation resulting in stabilisation of S₁ and S₃ substrate pockets, a loop in the activation domain and the N-terminal insertion. These regions are distant from the region of FVIIa interacting with TF.

Here, we present two fundamentally different approaches to enhance the intrinsic activity and efficacy of FVIIa. In the first approach FVIIa's catalytic domain is mutated. A structurally well-defined activation domain and insertion of the N terminus are prerequisites for the activation of trypsin-like enzymes. Therefore, mutations aimed at stabilising the activation domain in the conformation that permits burial of the N-terminus as well as mutations that restrict the conformational freedom of the N-terminal tail have been exploited. One of the most active FVIIa variants (NN1731, in clinical development by Novo Nordisk A/S) combines these contributions and will be discussed.

In another approach, FVIIa is coupled covalently to the soluble ectodomain of TF through a buried interface-spanning disulfide. Suitable positions for the introduction of disulfide linkages were predicted by molecular modelling and tested by co-expression of the corresponding cysteine variants. The two different approaches to identifying and generating FVIIa variants which can attain a stable active conformation will be discussed in details.

Keynote Lecture III

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Drug discovery: historical and current perspectives

*J. Drews***GPC-Biotech AG/Morphosys AG (Munich, DE)*

Drug discovery is a remarkable scientific endeavor. It began its career during the second half of the 19th century. Chemistry on the one hand and biology on the other had gained a degree of maturity, which warranted the application of these sciences to complex problems outside of their original domains. In its initial stage, drug research was clearly driven by chemistry. During the first half of the 20th century, however, biology evolved from a descriptive science into an experimental discipline capable of explaining the chemical and physical aspects of life processes. A more equal balance between the two sciences was achieved. The surge of new drugs, which resulted from this newly gained equivalence was often referred to as the first drug revolution. Novel drugs in psychiatry, phenothiazines, noradrenaline uptake inhibitors and tranquilizers were discovered. Also, new cardiovascular drugs like beta-blockers and calcium antagonists entered the therapeutic arena. Antibiotics started to play a dominating role in medicine, also the first antifungals and antivirals came along. During the second half of the 20th century molecular biology explained the genetic context of cellular and organic shapes and functions in chemical detail. It also delivered the methods that could now be employed to produce pure human proteins and make them available as drugs. The molecular details of antibody formation were elucidated and methods were developed to generate monoclonal antibodies in vitro and in vivo.

Molecular biology joined synthetic chemistry in being able to provide new molecules that had the potential to become drugs. Today, recombinant proteins, peptides and monoclonal antibodies have become integral parts of our medical armamentarium. Especially the roughly 30 monoclonal antibodies that are used as therapeutics today have made significant contributions to the treatment of certain malignancies and autoimmune diseases.

But the evolution of biology did not stop there. Whole genomes were sequenced, disease genes could be identified and pathophysiological mechanisms could be described in unprecedented detail.

However, not every change that was introduced into drug discovery can in hindsight be regarded a success. High- or ultra-high-throughput screening, a method, in which only superficially validated target molecules were exposed to millions of compounds obtained through combinatorial chemistry did not work out as expected for reasons that are now quite obvious. Chemical abundance cannot make up for the wrong biological choice. Vice versa a clever and well validated biological choice cannot compensate for the indiscriminate use of diverse chemicals that are unrelated to the target in question. These initial mistakes and exaggerations have since been understood and corrected. Given the techniques and the knowledge now available, one could expect a new surge of novel and valuable compounds, small molecules, peptides and proteins alike, similar to the one that occurred during the fifties and sixties of the past century.

A contemporary overview of drug discovery would be incomplete without a short statement regarding the position and the constitution of drug research within a company.

The secret to success in drug discovery as far as organizational matters are concerned lies in the right balance between science and medicine on the one hand and commercial interests and perspectives on the other. Scientists should be informed on commercial aspects but they should not be dominated by them.

Overall, drug research has been a tremendously successful enterprise. Therefore, the scientists engaged in drug discovery should take pride in what has been accomplished and help to educate a largely ignorant and ill informed public on the importance of their contribution to the health and well-being of millions of people all over the world.

Drug Discovery Technologies IV - Physiologically Relevant Screening with Native Cells

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Direct measurement of the QT interval in stem cell derived cardiomyocytes for the assessment of QT liability

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Drug-induced QT interval prolongation (DIQTIP) can lead to sudden cardiac death and is a major safety concern for the drug industry and regulating agencies. To reduce the risk of DIQTIP, drug candidates are routinely assayed for in vitro electrophysiological properties using cell-based assays. The patch-clamp based hERG assay is most widely used. However, the hERG assay can be inaccurate (false positives and false negatives) because it does not take account of non-hERG cardiac ion channels and does not measure the overall effect of a compound on cardiomyocyte function. Here, we report the development of QTempo (QT prolongation Examination with Myocardia derived from Pluripotent cell), a significantly improved assay that incorporates beating cardiomyocytes derived from human iPS cells (hiPSC). To validate QTempo, beating cardiomyocytes were placed on micro-electrode arrays and challenged with reference compounds known to cause clinical DIQTIP. Compounds tested included E-4031, astemizole, rofecoxib, cisapride and sotalol. All compounds could be assayed using QTempo at drug concentrations equal to, or lower than, those reported for the hERG assay. QTempo was also more accurate than the hERG assay which can yield false positives. For example, verapamil, which does not prolong QT interval in-vivo but generates a false positive in the hERG assay, was correctly scored negative for DIQTIP in the QTempo assay. The QTempo assay is more accurate because it more closely reproduces clinical mechanisms involved in cardiac action potential regulation: It is not limited to a single ion channel and is built around beating cardiomyocytes. Data output for QTempo resembles the familiar ECG and is readily understood by clinicians as well as specialist scientists. Large numbers of beating cardiomyocytes derived from hiPSC can be generated for this system allowing parallel HTS screening of drugs. QTempo is a valuable tool for the more accurate prediction of clinical cardiotoxicity.

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Cardiomyocytes generated in vitro from embryonic stem cells as a screening system to predict cardiotoxicity

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Early in vitro cardiotoxicity evaluation has been hampered by the lack of suitable cellular systems. In the present study, we analysed the applicability of commercially available, highly purified cardiomyocyte preparations that are obtained from mouse embryonic stem cells (ESCs) to classify a compound's cardiotoxic potential.

To this end, ESC-derived cardiomyocytes were employed in three assay systems. In two conventional endpoint assays troponin release and neutral red uptake (NRU) was determined after incubation with test compounds. In addition, we used an impedance-based system, the xCELLigence System, co-developed by Roche Diagnostics and ACEA Biosciences. In that system the electric impedance is generated by the interaction of adherent cells with a microelectrode biosensor, reflecting cellular parameters, such as proliferation, cell death, adhesion, spreading, and morphological alterations. Thus, real-time, label-free and continuous monitoring of cardiomyocyte cultures in the presence of test compounds is possible.

As compounds with known in vivo cardiotoxic activity we analysed amiodarone, clomipramine, doxorubicin, emetine, lidocaine, ouabain, quinidine, tetraethylammonium, theophylline, and verapamil. For comparison, also non-specific cell toxins (m-fluoro-tyrosine, mercury chloride) as well as non-cardiotoxic compounds (dexamethasone, diclofenac, kanamycin, paracetamol) were included.

Compounds were added to cardiomyocyte cultures that had been pre-cultured for either 3 oder 10 days. For troponin release assays, samples were taken from cell culture supernatants 24 h, 48 h, and 72 h after compound addition. NRU was determined 72 h after compound addition. Impedance measurements were performed continuously for 72 h following compound addition. The results demonstrate that the in vivo cardiotoxic potential of the compounds tested can be predicted correctly using the ESC-derived cardiomyocyte system. This is applicable to all three read-outs employed in the present study. The real-time measurements using the xCELLigence platform furthermore allow to follow the kinetics of compound action with high resolution. To our knowledge, the ESC-derived cardiomyocytes used in this study represent the first reliable source of purified, standardized cardiomyocytes available at large quantities for screening purposes.

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Optical measurement of action potentials in isolated adult cardiac myocytes

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Cardiac myocytes isolated from adult hearts are a widely accepted model somewhere half way between embryonic and neonatal muscle cells on one side and a working heart on the other. Thus, cardiomyocytes serve as good models for both, the investigation of cardiac cellular physiology and pathophysiology and the pharmaceutical investigation, either explorational or in safety screens.

Here we introduce a method to measure the membrane potential of adult isolated myocytes by an optical approach. In addition, we provide a concept for applying these assays in a screening context. In investigations with model substances that are well known for their ability to cause a prolongation of the QT-interval we demonstrate the applicability of single cell action potentials as a prospective parameter for QT-interval prolongation. This approach has been extended from the sole use of small molecule fluorescent dyes to the application of genetically encoded biosensors (GEB) with their potential sensitivity. Expression of the biosensor in cardiac myocytes is mediated by viral gene transfer and thus ensures a vastly increased biocompatibility and enables repetitive long-term measurement on identical myocyte populations.

Our experimental design unifies the advantages of (i) contact-free field measurements of cell clusters or tissue and (ii) single cell methods like the patch-clamp technique and thus paves the way for incorporating QT-screens on adult myocytes into screening environments.

This work was supported by the Federal Ministry for Education and Research (BMBF), Germany in the framework "Biophotonics III" and by the Federal Institute for Risk Assessment (BfR, Germany).

Chemical Space: Maximum Compound Value II - Bioisosteres

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In silico identification of bioisosteric functional groups

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Bioisosteric replacement can be defined as the replacement of a part of a bioactive molecule with another group that is similar in size and exhibits similar physicochemical properties. Bioisosteric transformations are used in the process of lead optimization to improve the properties of potential drug candidates, such as selectivity or transport characteristics, or to remove unwanted side effects such as toxicity and metabolic liabilities. This process is considered while also endeavoring to maintain the original bioactivity of the molecule. Bioisosteric replacements are also often used in situations where the optimizations are intended to improve the synthetic accessibility of the molecule, or to obviate potential issues in intellectual property rights.

Identifying bioisosteric analogues of more complex groups is not trivial. This requires a considerable amount of medicinal chemistry experience. Although even if this experience is available, the identification of a bioisosterically suitable group with an optimal balance of steric, hydrophobic, electronic and hydrogen-bonding properties, all of which influence ligand-receptor interactions, usually requires an intensive procedure of trial and error.

In silico methods have been shown to provide useful help in the navigation of the functional group space and the identification of proper bioisosteric analogs. These methods apply various cheminformatics techniques, such as bioactivity guided database mining, characterization of groups by a range of calculated descriptors and identification of bioisosteric functional pairs based on the similarity between their properties. An overview of these approaches will be the topic of this presentation.

Related Publications:

P. Ertl, In silico Identification of Bioisosteric Functional Groups, *Curr. Opin. Drug Disc. Dev.* 10, 281-288 (2007).

P. Ertl, S. Jelfs, J. Muehbachner, A. Schuffenhauer, P. Selzer, Quest for the Rings - In Silico Exploration of Ring Universe to Identify Novel Bioactive Heteroaromatic Scaffolds, *J. Med. Chem.* 49, 4568-4573 (2006).

P. Ertl, Cheminformatics Analysis of Organic Substituents, *J. Chem. Inf. Comp. Sci.* 43, 374-380 (2003).

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Design of bioisosteres in hit to lead expansion

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General definition of the term bioisostere: Over the time many wordings such as isosterism, bioisosterism, classical isosterism, ring equivalents, etc., were used in relationship to the isosterism concept, we propose a very general definition of the term bioisostere:

"A bioisostere is a compound which results from the replacement in an active molecule of an atom, a group of atoms or a scaffold by another one, with conservation of the initial biological affinity for a given target"

The different degrees of bioisosterism: When considering and comparing the most common active molecules generated by bioisosteric considerations, three categories of bioisosteres can be distinguished. (see figure 1)

The place of bioisosterism in drug design: The design of bioisosteres constitutes one of the major components of any hit optimization program. The different options relevant to bioisosteres design are: Functional exchange, Fragment exchange, Scaffold exchange, Biological accompaniment Physico-chemical accompaniment, acquisition of intellectual property. (see figure 2)

Case histories in bioisosterism-based drug design: Presentation of some case histories related to bioisostere design.

Figure 1

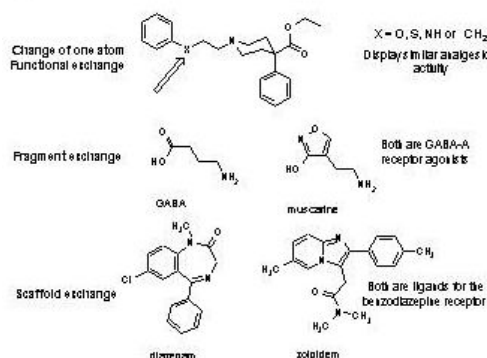
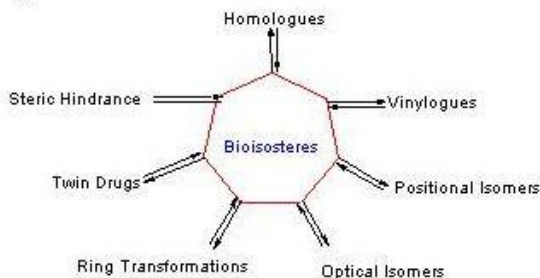


Figure 2



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Mining chemical databases for bioisosteres and compound optimisation

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The link between the biological and chemical worlds is of central importance in many fields, not least that of healthcare. For example, a major focus in systems biology research is the signalling networks and pathways describing the interactions and functions of large numbers of genes and proteins. Similarly, within healthcare-related chemistry research there is much interest in efficiently identifying drug-like compounds that specifically interact with these proteins/genes. Key to our work in this area has been the construction of a large and general structure activity relationship SAR database, linking pharmacological activities of compounds through to their targets, and understanding how particular compounds recognise their cognate receptors.

Application of rules derived from these databases leads to rapid, economic, and effective identification of hypotheses and evidential examples for compound optimisation, specific examples in the area of automated bioisostere identification and development candidate optimisation and selection will be presented. Current status and future challenges for these informatics resources will be discussed.

Biological Space: Targets and Tools IV - Novel Approaches in Drug Discovery for Old Targets

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Screening multiple signalling pathways in GPCR's*M. de los Frailes***GlaxoSmithKline (Tres Cantos, ES)*

Although the molecular events which orchestrate receptor desensitization and internalization are complex and not yet fully elucidated, it is clear that β -arrestins 1 and 2 play a critical role. In spite of their function as negative regulators of GPCRs activity, new roles for β -arrestins in receptor signalling have been discovered in recent years. They can function as scaffold proteins that interact with several cytoplasmic proteins and link GPCRs to intracellular signalling pathways such as mitogen activate protein kinase (MAPK) cascades, ERK 1 and 2, JNK3 and p38. The "ligand bias" concept that describes the ability of ligands to selectively stabilize receptor conformations that stimulate or inhibit subsets of receptors activities has recently been demonstrated for beta-arrestin signalling. B-arrestin-biased ligands might provide opportunities for the development of novel therapies. This presentation will review the available technologies to explore G-protein independent receptor signalling and their application to drug discovery.

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GPCR allosteric modulators: from suitable screening to fast human tissue validation*T.A. Bennett***Vivia Biotech (Madrid, ES)*

Allosteric modulators of G protein-coupled receptor function have received increasing credibility and attention as candidates for drug therapy due to their unique mechanisms of action that may offer advantages at both "undrugable" and well established targets. Screening for these modulators has posed difficulties due to their subtle mechanisms of action. We have developed a cell-based screening platform that incorporates both automated sample preparation and automated evaluation by flow cytometry, that has been applied towards the discovery of allosteric modulators of GPCR targets. The use of a flow cytometric based screening system allows for single cell analysis that is inherently more sensitive than other methods. A library of 1300 known drugs has been screened against cells stably transfected with human GPCRs to test their ability to potentiate the endogenous ligand of each receptor. The results of these screens have produced several candidates that individually have no effect on the receptors, but are able to potentiate the effect of the endogenous ligands. Results will be presented which illustrate the screening technology and the successful characterization of initial hits as allosteric modulators.

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New developments in kinase drug discovery: jumping in and out of the box*D. Fabbro***Novartis Pharma AG (Basel, CH)*

Kinases fulfill essential role in many signaling pathways that regulate normal cell functions and their dysregulation leads to a variety of pathologies ranging from cancer and inflammatory diseases to diabetes, infectious diseases, cardiovascular disorders, cell growth, survival and many other biological functions (1-6).

About 30% of the protein targets under investigation by pharmaceutical companies are protein or lipid kinases. To date 10 kinase inhibitors, mainly targeting the ATP binding site have been launched (mainly in oncological indications) and more than 60 kinase targeted drugs are in clinical development with many more in various stages of pre-clinical development (4). These kinase inhibitors can be viewed as first generation inhibitors.

Although we have a good knowledge on the structural determinants of kinase inhibition by small molecules binding to the ATP pocket (4, 7- 9), selectivity and the limited set of chemotypes targeting the ATP binding site - a highly crowded area- have become one of the major issues in kinase drug discovery (4-9).

This review will highlight the different inhibitor approaches in the context of mechanism of efficiency and compound novelty including target selectivity, binding kinetics, and resistance formation with particular emphasis on non-ATP competitive inhibitors. In particular the non-ATP competitive inhibitors of the Abl kinase activity will be discussed in more details.

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Pharmacodynamics/Biomarkers II

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Marker endpoints in early phase CNS clinical trials

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Trials designed to evaluate clinical outcomes based upon conventional testing are of long duration, highly subjective and variable and, therefore, expensive. This is particularly true for neurodegenerative and 'orphan' diseases such as Alzheimer's disease and Amyotrophic Lateral Sclerosis (Motor Neuron Disease).

Proposed novel treatments and predicted therapeutic doses are often developed in animal models of diseases, but extrapolation of these findings to human pathology are frequently invalid, therefore the majority of compounds that reach the clinical stage fail to meet their primary endpoints. The reasons underlying this high failure rate are often not fully understood, however insufficient target validation across species, poor CNS penetration, inappropriate patient selection, or inadequate exposure at the site of action (i.e. poor dose selection) have all been implicated in historical cases. Likewise, in the absence of reliable surrogate markers, it is often difficult to perform exploratory studies to build up confidence on whether novel therapies are sufficiently promising to justify the conduct of larger-scale, and longer-term clinical trials. This is particularly true for chronic neurodegenerative diseases requiring large number of patients to demonstrate efficacy in randomized, placebo-controlled clinical trials (due to the high variability of responses) and/or diseases with low incidence/prevalence, which makes patient recruitment very challenging.

Advancements in the identification of surrogate markers of disease progression and treatment efficacy, as well as enhanced interactions between Academia, Industry, Research Organizations and grant Authorities, will accelerate efforts toward the identification and testing of future effective and safe medicines.

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Phenotyping cells without antibodies: mass spectrometric identification of cell surface protein markers

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Cell surface protein markers represent essential diagnostic and therapeutic markers for hematological malignancies. Diagnostic markers are frequently used for immunohistochemistry, such as CD20 for B cell neoplasms or CD13 for myeloid leukemias. Cell surface protein markers are not only suitable for diagnosis but they are also exploited for effective therapeutic intervention.

Prominent examples are targeted therapies utilizing monoclonal antibodies against cell surface proteins, such as treatment with anti-CD20 antibodies (rituximab) or anti-CD33 antibodies (gemtuzumab).

However, the number of known cell surface classification markers for hematological malignancies is limited and only few are suitable for diagnosis and therapy. Therefore, a systematic and quantitative analysis of cell surface proteins is required to identify new, reliable classification markers. The mass spectrometry-based cell surface capturing (CSC) technology enables a systematic and quantitative analysis of cell surface protein expression patterns. The CSC technology applies complementary protein tagging strategies for high affinity enrichment of peptides derived from cell surface proteins. The complementary strategies increase the number of identified cell surface proteins as well as the protein sequence coverage. Hence, the CSC technology enables reliable identification and quantification of cell surface proteins and it allows in-depth characterization and comparison of malignantly transformed cells.

Our study comprises human leukemia, non-Hodgkin's lymphoma and Hodgkin's lymphoma cell lines. The CSC technology enables a birds eye view of the cell surface proteomes through the identification of 1100 membrane proteins, including 224 CD annotated cell surface proteins. Differentially expressed cell surface proteins are characteristic for each blood cancer subtype and thus the protein expression pattern may be utilized, like a barcode (containing protein identities and quantities), for improved molecular classification of cancer subtypes. We verified a panel of new candidate classification markers on cell line microarrays and a patient tissue microarray with over 120 distinct lymphoma cases was analyzed.

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Latent variables modeling in metabolomics: a case study

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Metabolomics studies generate increasingly complex data tables, harder and harder to summarize and visualize without appropriate tools. Multivariate data analysis (MVA) methods must be applied to extract latent information from the data.

Projection methods such as Principal Components Analysis (PCA), Partial Least-Squares to latent structures [1,2] (PLS) regression and Orthogonal Projections to Latent Structures [3,4] -Discriminant Analysis (OPLS-DA) are perfectly suited to this task. Here we present an application of these approaches to analyse the outcome of the following studies: Liquid Chromatography - Mass Spectroscopy (LC-MS) data on mouse urine samples were collected for three three genetically distinct strains of mice with the aim of finding potential markers. Visualization tools[5] that are vital and useful to extract information and understand statistical models will also be thoroughly discussed.

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- [4] J. Trygg and S. Wold, O2-PLS, a two-block (X-Y) latent variable regression (LVR) method with an integral OSC filter, J. Chemom. 17 (2003) 53-64
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Structural and Computational Drug Discovery III - Advances in Kinase Inhibitor Design

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Selectivity in CDK-family inhibition: from an inhibitor's perspective, what defines the character of the ATP-binding site?*A. Echaliier-Glazer, R. Suckling, J. Endicott, M. Noble**
University of Oxford (Oxford, UK)

The character of an inhibitor binding site depends on the physicochemical properties of the amino acid side-chains that line that site, as well as the structural framework on which those side-chains are presented. The structural framework modulates the spatial disposition of side chain atoms, but also affects both the plasticity of the inhibitor binding site and the overall electrostatic environment sensed by inhibitors bound therein. In order to assess the relative contributions of these effects to inhibitor selectivity, we have collected inhibitor-binding finger prints for members of the CDK family, and chimeric forms thereof, in various states of activation. As well as illustrating important principles of kinase inhibitor recognition, this study has significant implications for the use of surrogate kinases in structure based drug design.

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Insights into kinase regulation and inhibition by large scale structural comparison*S. Knapp**
University of Oxford (Oxford, UK)

Recently protein family targeted structural genomics has significantly increased the structural coverage of the human proteome. In the kinase area, our laboratory released more than 50 novel catalytic domain structures in addition to a large number of kinase inhibitor complexes during the past four years. This effort led to a significantly improved structural coverage of the human kinome, structure determination of many disease related kinases and suggested new avenues for the development of more selective inhibitors and gave new insight into the regulation of these highly dynamic enzymes. The continuously growing knowledge led to a dense structural coverage of the kinome that allows us now to compare catalytic domain features and try to understand mechanisms that lead to inhibitor cross-reactivity. In this presentation a number of examples of current inhibitor development projects will be discussed, including targeting unique active site features, structurally diverse kinases as well as new possibilities for the development of allosteric kinase inhibitors

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Probing the ATP binding site of protein kinases by novel chemotypes*C. Kunick**
Technische Universität Braunschweig (Braunschweig, DE)

Protein kinases have emerged as a major biological target class in recent drug discovery research. A survey of the diverse protein kinase inhibitor structures published to date reveals that many of them are structurally based on distinct privileged motifs, e.g. 2-aminopyrimidines, maleinimides, indolinones, and pyrazoles. The heterocyclic parent scaffolds of the inhibitors compete with ATP in its binding pocket where they are aligned by hydrogen bonds to amino acids that are part of the hinge region connecting the N and the C terminal domains of the protein kinases. In order to identify additional general hinge binding motifs we investigated the d-annulated 1-benzazepin-2-one scaffold present in the paullones, a class of kinase inhibitors selectively inhibiting cyclin-dependent kinases (CDKs) and glycogen synthase kinase-3 (GSK-3). For this purpose we replaced the indole substructure of the paullones by other nitrogen containing heteroaromatics. The structures were then docked into the ATP binding sites of selected tumor related protein kinases and a counter kinase, the insulin receptor kinase (INS-R). While inhibition of the tumor-relevant protein kinases was intended, it was desired that the counter kinase would not be affected. The docking studies revealed that only for a minority of the designed novel heterocyclic systems a paullone-like alignment in the ATP binding site was predicted. In order to evaluate the predictive value of the docking investigations, all designed scaffolds were synthesized and tested on a variety of protein kinases. Actually, we discovered a class of VEGF-R2 inhibitors displaying selectivity against the insulin receptor kinase and activity in cellular test systems. However, the results indicated that the d-annulated 1-benzazepin-2-one scaffold is less suitable as a general hinge binding motif compared to the privileged structures mentioned above.

Chemical Space: Maximum Compound Value III - Chemistry Space and the Medicinal Chemist

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What compounds, libraries (and assays) are required to address biological networks - the reality of future drug design?*J. Harris***BioFocusDPI (Saffron Walden, UK)*

It is often said, with 20:20 hindsight, that the easy therapeutic targets ("low-hanging fruit") have been successfully dealt with. Whatever the validity of this view, the complexity of the chronic diseases that society needs us to address in the future has highlighted the weaknesses of the single-target therapeutic paradigm that has dominated medicinal chemistry for many years. How should we go about exploring the chemical space for such drugs that modulate the target network rather than simply blocking one element of it? In this presentation I shall examine, using examples, the implications for compound design of the need to address biological networks rather than single protein targets. In particular, I shall emphasise practical approaches to the discovery and design of multitargeted therapeutic candidates, from assay platforms, the key input of chemoinformatics, through to the exciting challenges that are posed to current medicinal chemistry thinking.

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Chemistry space and the medicinal chemist*J. Loesel***Pfizer (Sandwich, UK)*

Increasing the size of your screening collection is easily measured – and provided you have the budget – can be easily achieved. There is also a wealth of literature available to ensure that the increased size also correlates with an increase in diversity, chemical space coverage as well as novel compounds added. While all of the above is laudable – it doesn't address the issue of the overall quality of the screening file. How can you ensure that by changing your screening set that you will end up with a set of increased quality. The physical condition of new samples – like purity, concentration, solubility etc. can be easily measured. The other aspect is the selection of the right compounds based purely on their chemical structure. In the past some of our work has focused on the evaluation of compound collections – especially generating a single score which can be used as a measure for the quality of a complete set in regard to properties or activity space. Faced with the issue to prioritize individual compounds we have been working on a more individual score to capture the 'quality' of an individual compound – based on structure alone. To define quality we tried to generate an algorithm which mimics the decision making of a chemist faced with a decision during the HTS triage process – if he 'likes' a compound or not. Presented with the right questions and scale to evaluate a number of compounds we can show a good correlation between the opinions of individual Medicinal Chemists with the consensus opinion. In addition we can show that an algorithm based on structure alone is able to get close or even outperform individual medicinal chemists in ranking the 'beauty' or 'sexiness' as defined by the consensus opinion of the chemists. Our approach is distinct from existing approaches like filters (Ro5, lead like filters) or the concept of drug likeness which in a simple machine learning test seemed not to correlate at all with the 'beauty' as defined by the consensus opinion of our medicinal chemists. We regard the score as an important step towards quantifying the value of chemical space – complementing existing algorithms for novelty and diversity as well as filters like the Ro5.

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SubScape - A new tool to explore chemical space*Q. Yuan, T. Haubenreich***Chemical Abstracts Service (Columbus, US)*

Exploring chemical space requires that data and tools work effectively together. We have been investigating new tools and information to help scientists find the most valuable chemistry starting points for drug discovery. In a new tool called SubScape, we have correlated 150 bioactivity categories, and 30,000 protein targets with small molecules within Registry, the most authoritative and comprehensive collection of substances. Chemical diversity around bioactivity and protein targets can be analyzed by structural frameworks within SubScape's landscaping map. Specific examples will be discussed.