

# PROTEIN KINASES INHIBITORS

## ▷ USING STRUCTURES TO INFORM THE OPTIMIZATION OF PROTEIN KINASE INHIBITORS

Several approaches to inhibitor design are characterised by the experimental use of a chemically diverse set of fragments to map a target site, followed by molecular chimerization to produce selective and potent leads. Two examples of this, applied to inhibition of cyclin dependent kinases, will be described.

**Martin E. M. Noble**, *University of Oxford, United Kingdom*

## ▷ MULTITARGETED KINASE INHIBITORS: AN OLD PARADIGM?

Based on its clear disease association, the protein tyrosine kinases (PTKs) FGF-Rs, Kit, PDGFR, Bcr-Abl, c-Fms and Flt-3 represent ideal targets for validating the clinical utility of protein kinase inhibitors as therapeutic targets. Gain of function (GOF) mutations of these PTKs have been found to be involved in a variety of myeloproliferative disorders, a subset of gastrointestinal tumors (GIST) as well as in CML (chronic myelogenous leukaemia) and AML (acute myelogenous leukaemia). Midostaurin, a broad spectrum kinase inhibitor, has been used for validating the clinical utility of kinase inhibition as a therapeutic modality. For example in AML the mutated versions of Flt-3 predicts a poor clinical outcome for a subset of patients. Midostaurin has been shown to be a potent inhibitor for Flt-3 *in vitro*, *in vivo* and in the clinic indicating the utility of this agent for the treatment of AML. As midostaurin has also activity against the FGFRs, PDGFR, VEGFR-2 and Kit, this drug has been used in indications that are driven by these RTPKs. The potential and limitations of these therapeutic approaches will be discussed.

**Doriano Fabbro**, *Novartis Institutes of Biomedical Research, Basel, Switzerland*

## ▷ INHIBITORS OF NEMO, A CRUCIAL PROTEIN REGULATING THE IKK COMPLEX OF THE NF- $\kappa$ B PATHWAY

The NF- $\kappa$ B signal transduction pathway is involved in many cellular processes, including inflammation and cancer. NEMO is a non catalytic scaffolding protein regulating the activity of the IKK complex which play a crucial role in this pathway. Thus, inhibitors of NEMO targeting the IKK complex are potential pro-apoptotic agents and thus anti-cancer drugs. By a combination of *in vitro* and *in cellulo* experiments, we have established the proof of concept of this approach, through the design of peptides that alter the oligomeric structure of the NEMO and inhibits its function in the stimulus dependant activation of the pathway. The characterization of these peptidic inhibitors will be reported as well as our approach to isolate small molecules that could serve as leads for specific pro-apoptotic and anti-cancer drugs.

**Michel Véron**, *Institut Pasteur, Paris, France*

## ▷ TYROSINE KINASE INHIBITORS IN LEUKEMIA

Almost all patients with chronic myelogenous leukemia or myeloproliferative diseases have activating mutations in a tyrosine kinase, including the Bcr/Abl fusion protein in CML, FIP1L1-PDGFRalpha in hypereosinophilic syndrome, and the JAK2 V617F mutation in polycythemia vera. In CML, imatinib mesylate induces long lasting complete hematologic remissions, but not molecular remissions, leading to recent and successful efforts to develop second generation ABL inhibitors with higher potency. The high prevalence of JAK2 V617F mutations in other myeloproliferative syndromes has prompted a search for JAK2 inhibitors. Finally, more than one third of patients with acute myelogenous leukemia have activating mutations of FLT3, and several inhibitors are currently in clinical trials. Successes and barriers to success in leukemia patients will be discussed.

**James D. Griffin**, *Dana Farber Cancer Institute, Boston, USA*

## ▷ STRUCTURAL AND MECHANISTIC PROPERTIES OF ONCOGENIC PROTEIN KINASES THAT MANIFEST RESISTANCE: CHEMICAL BIOLOGY AND DRUG DESIGN STRATEGIES

Oncogenic protein kinases are key therapeutic targets for drug discovery. X-ray crystallographic, biochemical and cellular studies have revealed both structural and mechanistic properties of several oncogenic protein kinases. In an increasing number of cases, resistance to inhibition has shown to involve critical amino acid mutations in the ATP or proximate binding sites for small-molecule inhibitors. These challenges are being addressed by both chemical biology and drug design strategies. A case example is T3151 mutation of Bcr-Abl kinase.

**Tom K. Sawyer**, *Ariad Pharmaceuticals, Inc., Cambridge, USA*

## ▷ TARGETING SRC FAMILY KINASES FOR ANTICANCER THERAPY

Elevated SFK activity is thought to be implicated in several aspects of the life of cancer cells including survival, proliferation and migration. The activity of Src family kinases (SFKs) is progressively elevated in colon cancer from the localised colonic adenocarcinoma to the metastatic stage. This elevation is due to deregulation of SFKs and not commonly due to mutations or over-expression. Efforts are under way in the pharmaceutical industry to develop small molecule SFK inhibitors. Recent advances on SFK inhibitors in preclinical and clinical development will be presented. Challenges regarding *in vivo* models and identification of molecular biomarkers will be discussed.

**Francisco Cruzalegui**, *Institut de Recherches Servier, Croissy-sur-Seine, France*

## ▷ TARGETING THE SUBUNITS OF PROTEIN KINASE CK2 IN CANCER THERAPY

Protein kinase CK2 is a highly ubiquitous and multifunctional serine/threonine kinase described as a multisubunit holoenzyme generated by the tight association of two  $\alpha$  or  $\alpha'$  catalytic subunits with a dimer of  $\beta$  regulatory subunits. However, the transient nature of this complex has been recently highlighted by the elucidation of its structure and the analysis of the spatiotemporal organization of its individual subunits in living cells. CK2 has been found dysregulated in all cancers and evidence suggests that this kinase is essential not only in cell growth and proliferation, but also in suppression of apoptosis. In this context, CK2 has

entered into consideration as a suitable target for cancer therapy. Based on this evidence, we have initiated a high-throughput screening to identify and develop potent and selective small molecular mass inhibitors of CK2. I will describe the *in vitro* and *in vivo* characterization of two classes of potent CK2 inhibitors that either target its catalytic subunit or antagonize the formation of the CK2 holoenzyme complex. Validation of these inhibitory compounds as anti-cancer agents will be discussed.

**Claude Cochet**, *CEA, Grenoble, France*

## ▷ PHARMACOLOGICAL INHIBITORS OF DISEASE-RELEVANT CYCLIN-DEPENDENT KINASES

Cyclin-dependent kinases (CDKs) regulate the cell division cycle, apoptosis, transcription, differentiation and major neuronal functions. Inhibitors of these kinases have a strong potential to treat cancers, neurodegenerative diseases, diabetes, viral infections, unicellular parasites. Some of the early CDK inhibitors have reached the pre-clinical and clinical stages of pharmaceutical evaluation. For instance, roscovitine (CYC202, Seliciclib), is currently undergoing phase 2 clinical trials against leukaemia, lung and breast cancers, and phase 1 trials against various kidney diseases. It is undergoing pre-clinical animal evaluation against Alzheimer's disease, Parkinson's disease, stroke and Niemann-Pick's disease type III. The selectivity and intracellular mechanism of action of roscovitine has been extensively studied and will be presented as a representative example of the multiple effects of CDK inhibitors in cells, tissues and organisms.

**Laurent Meijer**, *Station Biologique de Roscoff, France*

## ▷ MODULAR INTERACTION DOMAINS IN PROTEIN KINASE SIGNALING

Signaling by protein kinases is commonly mediated by modular interaction domains, such as the SH2 domain, that bind specific phosphotyrosine- or phosphoserine/threonine-containing motifs. These domains direct the assembly of signaling pathways downstream of protein kinases, provide specificity in intracellular signaling networks, and directly regulate protein kinase activity. Interaction domains can also determine selectivity in the actions of protein kinase inhibitors. The ability of such interaction domains to control complex cellular properties, such as polarity or cytoskeletal architecture, and to drive the malignant behaviour of cancer cells, will be discussed.

**Tony Pawson**, *Samuel Lunenfeld Research Institute, Toronto, Canada*

## ▷ THE EFFECTS OF CDK INHIBITORS IN A MOUSE MODEL FOR ALZHEIMER'S DISEASE

Alzheimer's disease (AD) is an irreversible neurological disorder that progressively attenuates the cognitive abilities of those afflicted and ultimately leads to death. Amyloid plaques, neurofibrillary tangles and neuronal loss are the three invariant features of AD. Cdk5 is a neuron specific kinase essential for several functions of the nervous system. Cdk5 activity depends on binding to one of its two activators, p35 or p39. Hyperactivation of Cdk5 occurs when p25, the C-terminal fragment of p35, is liberated by proteolytic calpain cleavage. p25 levels accumulate during aging and in the early stages of Alzheimer's brains. A forebrain specific inducible p25 mouse model develops all the pathological features of AD including neuronal loss/brain atrophy, neurofibrillary tangles and A $\beta$  pathology. In addition, p25 mice exhibit learning deficits and an impairment in synaptic plasticity. The potential neuroprotective effects of small molecule inhibitors against the Cdk5 in the inducible p25 transgenic model will be discussed.

**Li-Huei Tsai**, *Harvard Medical School, Boston, USA*

## ▷ TYROSINE KINASES OF THE FOCAL ADHESION KINASE FAMILY

Focal adhesion kinase (FAK) and proline-rich tyrosine kinase 2 (PYK2) form a small group of related non-receptor tyrosine kinases (~45% sequence identity). FAK is activated by integrins engagement and many transmembrane receptors, whereas PYK2 is activated in response to increases in intracellular Ca<sup>2+</sup>. Comparison of FAK and PYK2 discloses similarities and differences in their regulation and cellular localization which help to understand better their mechanisms of activation and function. FAK is essential during development and is also important for the invasive and metastatic properties of tumor cells, making it a promising new target for anticancer drugs.

**Jean-Antoine Girault**, *Université Pierre et Marie Curie, Institut du Fer à Moulin, Paris, France*

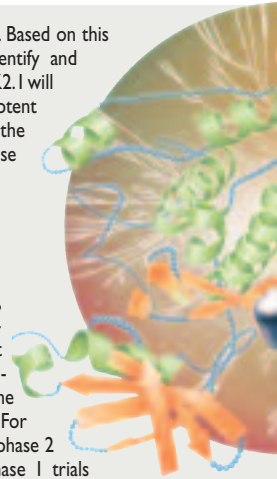
## ▷ ACTIVATION AND INHIBITION OF THE PDGF RECEPTOR FAMILY

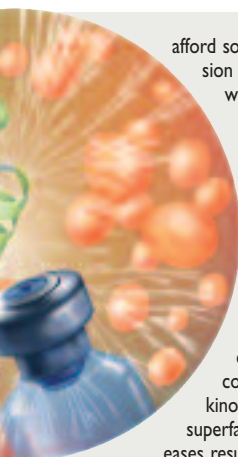
Members of the PDGF receptor family are activated in various cancers by oncogenic mutations that target the receptor kinase domain or juxtamembrane region. The molecular basis and reasons why these two regions are oncogenic hotspots will be discussed. Imatinib is known to inhibit the KIT and PDGF receptors and evidence will be presented to show that imatinib is also an effective inhibitor of FMS, the receptor for macrophage colony stimulating factor (M-CSF or CSF-1). Renal inflammatory disease is dependent upon PDGF and FMS signalling and the effect of imatinib in Rat models of this disease will also be presented.

**Nick J. Dibb**, *Imperial College London, United Kingdom*

## ▷ MLK CRYSTALLOGRAPHY AND THE DESIGN OF FAMILY SELECTIVE MLK INHIBITORS FOR THE TREATMENT OF NEURODEGENERATIVE DISEASES

Our research has focused on developing potent, selective inhibitors of mixed lineage kinases (MLKs) for the treatment of neurodegenerative diseases. The MLKs are a critical upstream activating component of the stress-activated protein kinase-signaling cascade regulating JNK activation and subsequent cJun phosphorylation leading to neuronal cell death. Several lines of evidence indicate that neuronal apoptosis may be an important mechanism contributing to the progression of disability in Parkinson's and Alzheimer's diseases. The available therapies





afford some degree of symptomatic relief; however none prevents the progression of the disease or delays the pathological neuronal cell death associated with the disease. The first generation compound from this program, CEP-1347, advanced to late clinical evaluation for Parkinson's disease. Presented will be MLK1 crystallography and SAR studies of family selective, synthetic second generation MLK inhibitors with improved properties for advancement.

**Robert L. Hudkins**, *Cephalon, Inc., West Chester, USA*

#### ▷ PROTEIN KINASES AND DISEASES IN THE POSTGENOMIC ERA

Protein kinases are key components of phosphorylation-based signaling networks. We have analyzed eukaryotic genome sequences to predict total numbers of protein kinase genes (the kinome). Protein kinases constitute ~2% of all genes in yeast, worms, flies and humans. The human kinome has 518 protein kinases - 478 are in the eukaryotic protein kinase superfamily, and the remainder are atypical protein kinases. Many human diseases result from mutations in protein kinase genes, and comparative analysis of human, chimp and mouse kinomes predicts additional disease-causing protein kinases.

The prevalence of protein kinases involved in disease has led to intensive efforts to develop specific protein kinase inhibitors for use as therapeutics.

**Tony Hunter**, *The Salk Institute, La Jolla, USA*

#### ▷ ESSENTIAL SER/THR PROTEIN KINASES AS POTENTIAL TARGETS FOR THE DEVELOPMENT OF NOVEL ANTIBIOTICS AGAINST MYCOBACTERIAL DISEASES

Microbial genomics has confirmed the widespread presence of eukaryotic-like Ser/Thr protein kinases (and phosphatases) in prokaryotes. Indeed, the number of genes coding for these enzymes in mycobacterial genomes is similar to, or greater than, those coding for classical two-component systems, emphasizing an important role of reversible Ser/Thr phosphorylation in bacterial physiology and virulence. This presentation will review recent work on the essential Ser/Thr protein kinase PknB from *Mycobacterium tuberculosis*, establishing the proof of concept that protein kinase inhibitors are promising candidates for the development of novel anti-bacterial agents.

**Pedro Alzari**, *Institut Pasteur, Paris, France*

#### ▷ EUKARYOTIC-LIKE PROTEIN KINASES AS TARGETS FOR THE DEVELOPMENT OF COMPOUNDS TO COMBAT MYCOBACTERIUM TUBERCULOSIS

*Mycobacterium tuberculosis*, the causative agent of tuberculosis is one of the most successful pathogens known today. Recent work suggest that one of the major virulence factors for pathogenic mycobacteria is an eukaryotic-like serine/threonine protein kinase, termed protein kinase G. Specific inhibitors of protein kinase G activity lead to a rapid degradation of mycobacteria inside host cells. We are currently investigating the cellular mechanisms that are targeted by protein kinase G, in order to better understand mycobacterial pathogenicity.

**Jean Pieters**, *University of Basel, Switzerland*

#### ▷ DIVERGENT PROTEIN KINASES: AN ACHILLE'S HEEL OF MALARIA PARASITES AND OTHER PROTOZOA?

Parasitic protozoa such as *Plasmodium* and trypanosomatids (responsible for malaria, and sleeping sickness/leishmaniasis, respectively) bear a tremendous impact on public health and socio-economic development in affected countries, and resistance of the organisms to existing drugs calls for new chemotherapeutic agents. Recent examination of their kinomes revealed that the vast phylogenetic distance between these parasitic eukaryotes and their mammalian hosts is reflected by profound divergences in their protein kinase complements. The presentation will describe how such divergences can be exploited towards selective inhibition, and how specific parasite protein kinases can be functionally validated as potential targets for chemotherapy.

**Christian Doerig**, *University of Glasgow, United Kingdom*

#### ▷ PROTEIN TYROSINE KINASES IN BACTERIA

Phosphorylation-dephosphorylation has long been considered a protein modification restricted to eukaryotes but it is now well established that it participates also in the regulation of bacterial physiology. Of special interest is the recent finding that a number of protein tyrosine kinases from various bacterial species share no sequence homology with their eukaryotic counterparts even though they catalyze the same overall reaction. These enzymes are involved in a diversity of biochemical pathways including antibiotic resistance, carbohydrate production, stress resistance as well as pathogenicity. The structural and functional specificity of bacterial tyrosine kinases, compared to eukaryotic kinases, therefore allows to envisage the existence of protein kinase inhibitors specific to bacterial modifying enzymes.

**Christophe Grangeasse**, *Institut de Biologie et Chimie des Protéines, Lyon, France*

#### ▷ PKA DYNAMICS: NOVEL STRATEGIES FOR INHIBITORS

cAMP-dependent Protein Kinase (PKA) serves as a prototype for the protein kinase superfamily. The structure of the catalytic subunit defines the conserved core and the conformational flexibility that is essential for substrate recognition and catalysis. Many unique features, including the C-terminal tail, are conserved throughout the AGC-sub-family. While structures of cAMP-bound regulatory subunits define the primary receptors for cAMP in mammalian cells, recent structures of holoenzyme complexes of RI and RII reveal major conformational changes in the regulatory subunits. These changes involve ordering of the flexible linker at the active site cleft as well as major reorganization of the helical subdomains in the cAMP bind-

ing domains. This major conformational reorganization generates novel sites that are excellent candidates for binding of new inhibitors.

**Susan S. Taylor**, *University of California, San Diego, USA*

#### ▷ A CHEMOGENOMIC APPROACH TO KINASE DRUG DISCOVERY

Targeted approaches to tackling cancer have become a major focus for drug discovery programs. In practice the "targeted profiles" of the majority of kinase drugs have come about more by serendipity than design. While a number of technologies have demonstrated the usefulness of retrospectively identifying the inhibitory profile of drugs, the ability to prospectively mine for and define the selectivity of a compound at the onset of a program has lacked a database with a comprehensive and precise dataset. Historically, it has been difficult to use high-throughput screening data for selectivity or structure-activity relationship analysis due to its heterogeneity in content and quality. Taking an industrialized systems approach that integrates broad biology, high precision microfluidics technologies and robust chemistry we have generated a chemogenomic database. Progress on using the database as the initiation point for drug discovery programs will be discussed.

**Kevin P. Williams**, *Amphora Discovery Corp., Durham, USA*

#### ▷ KINASE SELECTIVITY PROFILING AS A TOOL FOR DRUG DISCOVERY

Selectivity has always been an important issue for kinase inhibitors. There is ongoing debate about whether it is desirable to develop compounds that are selective, or whether promiscuous inhibitors are more likely to have therapeutic benefit. A prerequisite for answering these questions is to gain an understanding of the true inhibitory profiles of compounds. Systematic profiling of a wide variety of kinase inhibitors against large numbers of kinases has provided a broad overview of selectivity, and the ability to routinely and rapidly profile entire libraries of compounds may have a significant impact on kinase inhibitor discovery.

**Patrick Zarrinkar**, *Ambit Biosciences, San Diego, USA*

#### ▷ CHEMICAL BIOLOGY IN TARGETED DRUG DISCOVERY - THE NEXT GENERATION OF KINASE INHIBITORS

An increasing number of kinase inhibitor candidates is entering clinical development, representing an important change in the pharmaceutical industry, notably the development of small molecule kinase inhibitors for signal transduction therapies. Three distinct small-molecule kinase drugs, Gleevec, Iressa, and Tarceva act on a distinct subset of dysregulated, and often cancer-relevant kinases. The entire research field gains tremendous insights through the ongoing research and clinical trials with these three drugs and with fast following first generation kinase inhibitors, many of which are in different phases of clinical development. In addition, novel chemogenomic and chemoproteomic technologies are emanating from the current kinase research area, focussing efforts on the generation of spectrum-selective inhibitors for anticancer therapies as opposed to the mono-specific inhibitors for the remaining therapeutic areas.

**Bert Klebl**, *GPC Biotech, Martinsried, Germany*

#### ▷ HIGH THROUGHPUT CELLULAR SCREEN AND PROFILING TECHNOLOGIES AND THEIR IMPLICATION IN TARGET AND DRUG DISCOVERY

Rapid, quantitative methods for characterizing the activity of small molecules against individual kinases in broad arrays of cellular assays would allow one to discover new biological activities associated with these molecules as well as provide a more comprehensive profile of drug candidates early in the drug development process. In this presentation a novel profiling tool will be described capable of both propagating a large number of cell lines in parallel, and assaying large collections of molecules simultaneously against an array of cell lines representing more than 50 individual tyrosine kinases. Implications of this system for the identification novel biological functions of known kinases will be discussed as well.

**Markus Warmuth**, *Genomics Institute of the Novartis Research Foundation, San Diego, USA*

#### ▷ PROTEIN KINASE INHIBITORS BY DESIGN

Inhibiting the catalytic activity of protein kinases has become one of the major therapeutic concepts in contemporary drug discovery. The first protein kinase inhibitors were identified by screening more than a decade ago. From that time, the intense activity of structural biologists in the field, has given us access to hundreds of crystal structures of protein kinases (apoenzymes or ligand complexes). Concomitantly, a lot of experience has been gained in the structure-activity relationships of protein kinase inhibitors. The combined information has provided us with a deep insight into the structural determinants of kinase inhibition by small molecules binding to the ATP (cofactor) pocket. We will present and illustrate how this knowledge can be exploited to design, in a very efficient manner, new kinase inhibitors.

**Pascal Furet**, *Novartis, Basel, Switzerland*

#### ▷ FRAGMENT BASED DRUG DISCOVERY FOR THE RATIONAL DESIGN OF KINASE INHIBITORS

Fragment-based discovery has recently emerged as a new approach for the generation of novel small molecule drugs. The use of high throughput X-ray crystallography and NMR in fragment-based discovery approaches will be exemplified. This approach for lead generation has distinct advantages over conventional bioassay-based screening in that very low-affinity fragments with novel structures can be identified. These "hits" can then be rapidly optimized for potency and DMPK properties using iterative cycles of medicinal chemistry and structure-based drug design. The development of novel lead compounds using this approach will be described for targets such as the cyclin-dependant kinases, key proteins involved in cancer.

**Harren Jhoti**, *Astex Therapeutics, Cambridge, United Kingdom*