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MEETING REPORT

EFFECTIVE AND EMERGING STRATEGIES FOR UTILIZING STRUCTURE IN DRUG DISCOVERY

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SUMMARY

Structure-based drug design is emerging as one of the key components in drug discovery, with many approved drugs tracing, at least part of their origins, to the use of structural information from X-ray, NMR, surface plasmon resonance, differential thermal denaturation, fluorescence polarization and other techniques for analysis of protein targets and their ligand-bound complexes. Furthermore, in silico structure-based drug design approach has enabled millions of possible structures for a given protein sequence to be evaluated rapidly helping to fast forward drug discovery as well as reduce drug discovery costs. Structure-based drug design is now arguably an essential contributor to addressing the need to improve research and development productivity faced by the pharmaceutical industry. The purpose of this meeting is to highlight the impact of the intersection of structural biology with chemistry and biology particularly on how the structures of relevant drug targets can serve as a starting point for drug design and development and provide the maximal synergy between target validation, structure determination, and hit-to-lead development. Some thoughts will be proposed with regard to the future of structure-based drug design and where emphasis could be placed to further increase the utilization of this approach on drug discovery.

Key words: Fragment-based drug design – Structure-based drug discovery – Structural biology – Protein crystallography – Electron microscopy – Free energy perturbation – Membrane proteins

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DRUGGING THE UNDRUGGABLE: USING STRUCTURE-GUIDED APPROACHES TO TARGET PROTEIN–PROTEIN INTERACTIONS IN CELL REGULATORY SYSTEMS

The meeting was opened with a plenary talk from Professor Sir Tom Blundell, Cambridge University who co-founded Astex Therapeutics, now Astex Pharmaceuticals, in 1999. His talk focused on the special challenges of targeting the highly diverse interfaces of multi-component molecular assembles. Defining the three-dimensional structure of protein interfaces is experimentally achieved using structural biology methods, primarily high-resolution X-ray structures, in combination with computational biology tools. Modulation of protein interactions at these interfaces has considerable potential in the design of chemical tools and therapeutic agents. However, creating small molecules of sufficient selectivity to target protein–protein interfaces is difficult, particularly as the structural landscape is generally considered "undruggable" because of the lack of concave binding sites combined with a high lipophilic environment. Nevertheless, Prof. Blundell pointed out that multi-protein assemblies and protein–protein interfaces are of considerable interest for drug design, illustrating this with examples including kinases, insulin and nerve growth factor. He explained that interfaces formed by concerted folding and binding can result in the presence of small-

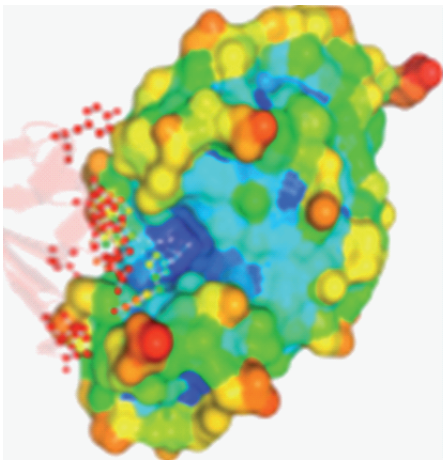


Figure 1. An impression of pocket depth at a protein–protein interface.

volume, deep pockets or grooves, shown in Figure 1, that may be exploited for the development competitive chemical modulators (1).

Prof. Blundell then described elegant fragment-based studies aimed at disrupting the interaction between the tumor suppressor BRCA2 (breast cancer type 2 susceptibility protein) and the recombination enzyme RAD51 in order to create cellular hypersensitivity to radiation and genotoxic drugs in vivo (2). He emphasized the importance of using a screening pipeline of biophysical techniques to identify a small molecule-binding site that could be targeted for disruption of this complex. The pipeline involved the combined use of binding assays and structural characterization of protein–peptide fragment complexes. This structural characterization included the use of isothermal titration calorimetry (ITC), X-ray crystallography and nuclear NMR. As a second example, he described structure-based studies of protein–protein interactions between the polypeptide growth factor, hepatocyte growth factor/scatter factor (HGF/SF), and the receptor tyrosine kinase Met (hepatocyte growth factor receptor). Modulation of interactions of this complex may have therapeutic potential in treating cancer. In these studies, characterization of the overall architecture of these large and flexible multi-domain proteins was undertaken using small-angle X-ray scattering and cryoelectron microscopy techniques (3). The structural information obtained identified key sites of protein–protein interactions that are important for Met signaling, which could be exploited for the development of agonists or antagonists.

A key message throughout this talk was the importance of using computational analyses in combination with structure-function characterization to identify "druggable" sites for selective targeting of protein–protein interactions. Several databases were described including TIMBAL, which contains a hand-curated collection of < 1200 Da molecules that modulate protein–protein interactions (4). What is clear is that different "rules" may need to be developed and applied in order to use protein–protein interfaces as sites for drug design, and to use fragment-based approaches if appropriate binding sites can be identified.

FEP/REST CALCULATIONS FOR COMPOUND LIBRARIES SUPPORTED BY MULTIPLE X-RAY STRUCTURES

Dr. Martin Packer (AstraZeneca) continued with the theme of computational prediction in his presentation by initially providing an overview about how calculations have been made to predict binding free energy (ΔG) between a protein and ligand. He explained that time and resources for carrying out calculations to yield true ΔG values, especially in solvated system, can be prohibitive in terms of resource and can also require the availability of very high-quality protein–ligand structures. Alternatively, calculation of meaningful relative protein–ligand binding affinities in solvent can be achieved using free energy perturbation (FEP), which uses molecular dynamics or Monte Carlo methods to obtain free energy differences in computational simulations. In structure-based drug design programs, FEP may be used in lead optimization to rank-order compounds for potency. In addition, appropriate potential and sampling protocols are also needed for such calculations. In the case studies he presented (described below), the calculations used REST2, Replica Exchange with Solute Tempering for sampling different ligand modes of binding with enhancements to the algorithm used for selecting ligand conformations in aqueous solvent.

Dr. Packer then went on to describe results obtained using FEP/REST2 calculations to rank the binding of a set of 38 homologous ligands to Ephrin type-B receptor 4 (EphB4). As shown in Figure 2, a good correlation was obtained between the calculated FEP values and the experimental data. Interestingly, X-ray structures of complexes of these ligands showed dual binding modes in some cases. For some dual binding-mode ligands, this behavior could be simulated using 5 ns FEP trajectories. The performance of this approach was compared with other ligand binding scoring functions. The comparison showed that MM-GBSA (which uses implicit solvent models) is of similar quality to FEP. These results suggested that further filtering and FEP could be used to enhance the quality of these computational predictions, particularly when X-ray structures are known and can be analyzed alongside the predictions. A second example was also presented showing the application of the FEP strategy to the prediction of ligand binding to the 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1), a potential target for treatment of metabolic disorders. This second example reinforced the wider applicability of this emerging computational method in fragment-based drug design.

STRUCTURAL BIOLOGY OF HUMAN INTEGRAL MEMBRANE PROTEINS

Dr. Liz Carpenter presented the final talk of the morning by introducing the Structural Genomics Consortium (SGC). SGC is a public-private partnership committed to open access research for the discovery of new medicines. It has sites in the U.K. (Oxford), Canada

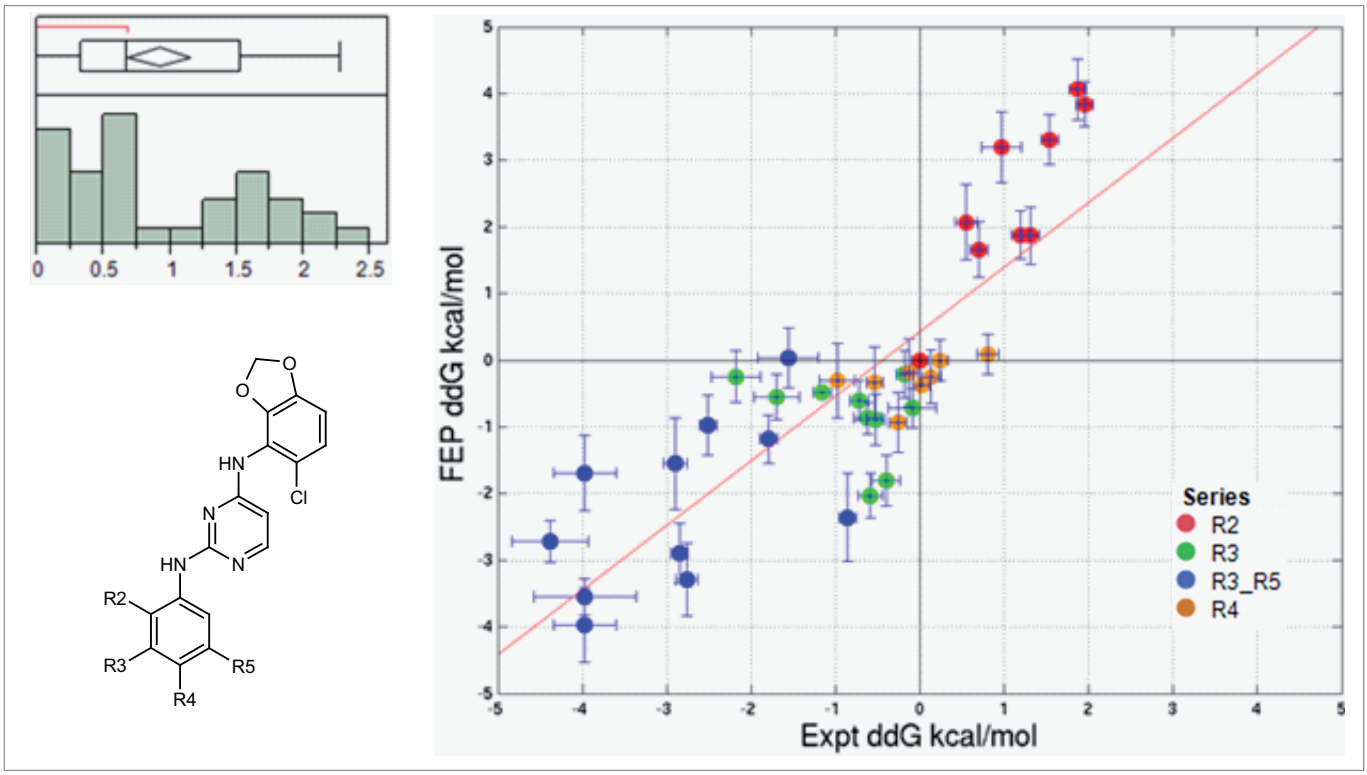


Figure 2. FEP prediction of ligand affinity for a compound library bound to the kinase EphB4. Experimental error bar is one standard deviation – error defined by quadrature – Err(A->B) = sqrt(ErrA^2 + ErrB^2); FEP errors obtained using method of Wang et al. (8).

(Toronto) and Brazil (Campinas) and a range of partners drawn primarily from the pharmaceutical industry and government and charity organizations that support fundamental and applied research. A key remit is its focus on less well-studied areas of the human genome including membrane proteins. Dr. Carpenter began by explaining that 20% of all the proteins produced by cells are membrane protein and that more than 50% of all small-molecule drugs target an integral membrane protein. Structural information for these proteins is very limited. Currently, only 50 human membrane protein structures are available out of an estimated 3,200 in the human genome. These proteins have a myriad of functions including as ion channels, solute carriers, ABC transporters and enzymes. Integral membrane proteins can also be associated with genetic diseases.

Dr. Carpenter then described the pipeline used by SGC to obtain lead crystals of membrane proteins. Their approach involves the use of multiple genetic constructs for protein expression, a variety of expression hosts such as yeast and insect cells, and purification methods that include extractions and additions of lipids and detergents to produce stable material suitable for crystallization trials. She pointed out that production of G protein-coupled receptors for structural biology studies is fairly well established but that production of other membrane protein types requires considerably more development. In addition, she introduced the concept of lipidic cubic phase (LCP) crystallization, which typically produces highly ordered but very small crystals in a membrane-mimetic medium. The utility of this technique in membrane protein structure determination is re-emerging because the crystals produced in this system have the potential to be used in serial femtosecond crystallography when injected into X-ray pulses generated by an X-ray free-electron laser (XFEL).

Dr. Carpenter then described some of the membrane protein structures solved at SGC including the first structure of a human ABC transporter, and the structure of a nuclear membrane zinc metalloprotease with an unusually stable scaffold. She also presented very recent work on the crystal structure determination of two human polymodal potassium ion channels, TREK-1 (K_{2P}2.1; KNCK2) and TREK-2 (K_{2P}10.1; KNCK10), including a complex of TREK-2 with norfluoxetine (NFX), the active metabolite of fluoxetine (Prozac®) (5). As shown in Figure 3, NFX binds within intramembrane fenestrations resulting in inhibition of channel function. The structure of this complex provides a context for understanding the mechanosensitivity of the TREK channels, and it may explain possible off-target effects of fluoxetine. Dr. Carpenter also emphasized the importance of the complementary biophysical techniques ITC, surface plasmon resonance (SPR) and differential scannning fluorimetry (DSF), to aid in the structural interpretation of ligand binding in membrane proteins for which there is still very limited information. In summary, there are numerous technical developments that are enabling membrane proteins to be more fully utilized as drug development targets, and

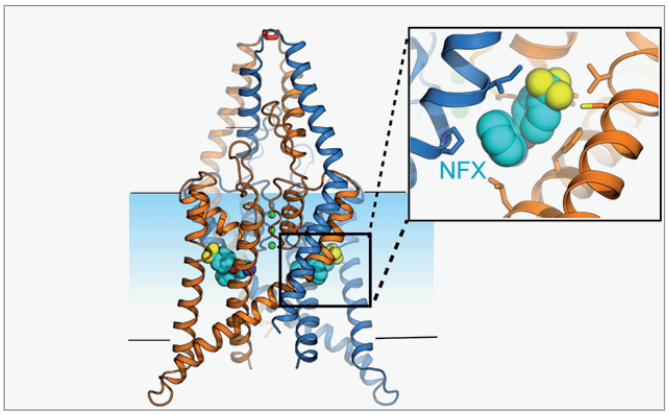


Figure 3. The TREK-2 ion channel binds norfluoxetine (NFX) in a lateral fenestration, causing channel inhibition (image produced by Liz Carpenter, SGC Oxford).

structure determination is an essential part of the drug discovery process.

UTILIZING HIGH-RESOLUTION CRYOGENIC ELECTRON MICROSCOPY FOR DRUG DISCOVERY

Dr. Alan Brown (MRC Laboratory of Molecular Biology) presented the recent advances in using high-resolution cryogenic electron microscopy, and how due to using a small amount of protein (< 0.1 mg) and not needing to grow crystals it is a technique that can be used either on its own or with other structural information to aid drug discovery. Recent advances have come about due to better microscopes and detectors with increased signal to noise ratios. Dr. Brown then presented his recent structure of the human mitochondrial ribosome (6), shown in Figure 4. He explained how, by using electron microscopy, they identified adaptations of the mitochondrial ribosome to synthesizing only membrane proteins, a GTPase on the ribosome interface, and multiple conformations that would not have been picked up by crystallography alone. Dr. Brown then presented his work around the malaria parasite ribosome for which a crystal structure does not exist. Using electron microscopy, Dr. Brown was able to solve a 3.2-Å structure with the known malaria drug emetine. It is hoped this new structural insight will enable new advances in malarial treatment options.

CASE HISTORIES OF FRAGMENT-BASED DRUG DISCOVERY

Dr. David Rees (Astex Pharmaceuticals) presented an overview of the learnings made at Astex in the field of fragment-based drug discovery (FBDD). He described the array of approaches used from X-ray, NMR, thermal shift and SPR, and how the fragment compound libraries have evolved from initially being fragments of known drugs to more diverse sets with mol. weight 120, heavy atoms count (HAC) 12-13 and cLogP 0.6-0.9. They currently still maintain a library of around 400 compounds, with very careful attention paid to QC and solubility. Dr. Rees also presented the concept of the minimal pharmacophore needed for optimal binding which they maintain through the drug optimization phase (Fig. 5).

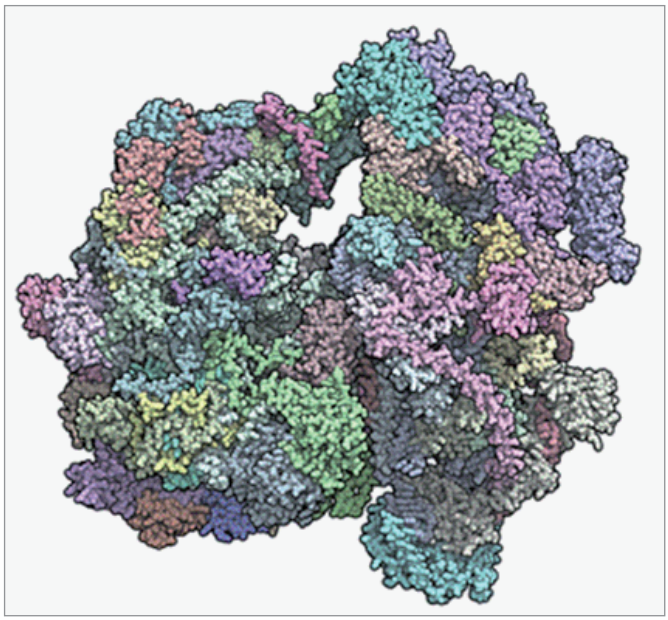


Figure 4. The structure of the human mitochondrial ribosome solved entirely by cryo-EM. Each chain in the structure, of which there are 80, is colored separately.

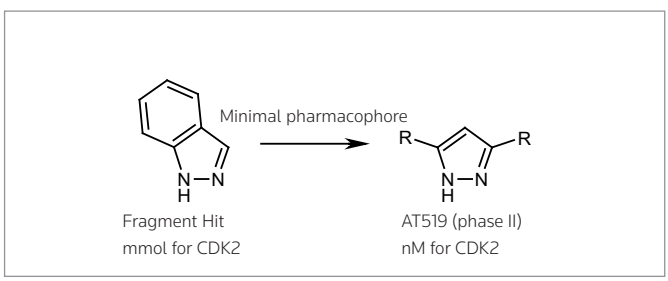


Figure 5. Minimal binding pharmacophore.

A protein–protein interaction (PPI) case study was presented around an inhibitor of apoptosis protein (IAP). An inhibitor of both xIAP (x-linked) and cIAP (cellular) was desired for the target product profile, with only inhibitors of xIAP being in the literature. A biochemical fragment screen of 1,200 fragments was performed, with only one fragment being identified that bound modestly to both xIAP and cIAP.

The fragment was successfully optimized to a nanomolar compound guided by X-ray crystallography and a small number of design cycles (Fig. 6).

USE OF FRAGMENT SCREENING AND STRUCTURE-BASED DESIGN TO IDENTIFY IN VIVO ACTIVE BCATM INHIBITORS

Mrs. Jenny Borthwick (GlaxoSmithKline) presented the work performed at GSK around branched-chain-amino-acid aminotransferase, mitochondrial (BCATm). The project performed a fragment screen using NMR, thermal melt and biochemical assays identifying

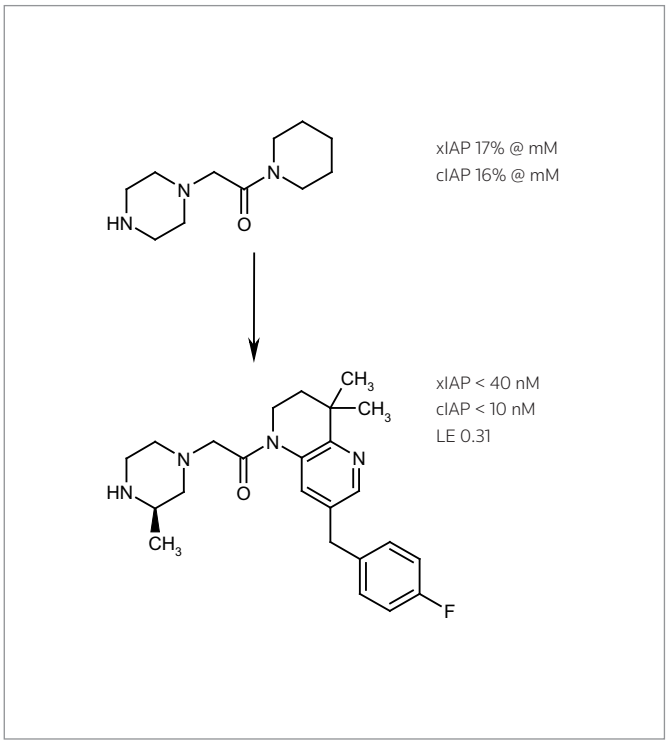


Figure 6. Optimization of IAP inhibitor.

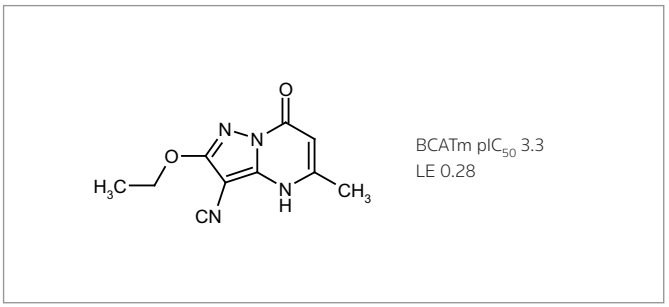


Figure 7. Starting BCATm fragment.

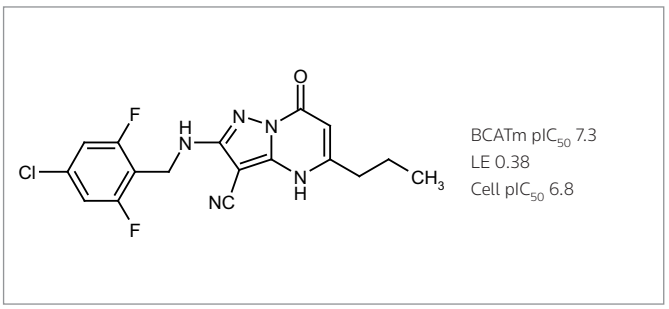


Figure 8. Final BCATm inhibitor.

in total over 100 hits. They initiated X-ray crystallography on the hits that had worked in all three methods (10-15). This identified lead [**I**](Fig. 7) which interacted differently in the active site to the other fragments enabling different vectors to be accessed in the optimization phase.

This compound was optimized using crystallography, water map analysis to identify unstable waters and information derived from HTS structures to yield a potent tool [**II**] (Fig. 8), which had excellent binding and cellular potency with good bioavailability. This compound showed a dose-dependent reduction of plasma branched amino acids in an amino acid challenge model.

THE DISCOVERY OF A POTENT AND SELECTIVE SERIES OF PAN-TRK INHIBITORS

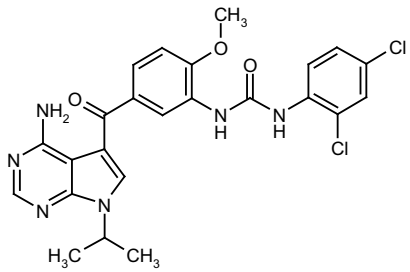
Dr. Sarah Skerratt (Pfizer) reviewed recent work at Pfizer towards the discovery of potent and kinase selective pan-Trk kinase ligands. She highlighted the widespread interest in this target stemming from the efficacy of anti-NGF monoclonal antibodies (such as tanezumab, currently in phase II and III trials) against multiple pain endpoints. NGF has very high affinity (10 pM) for the Trk-A (high affinity NGF receptor) extracellular receptor of the Trk-A/p75 protein complex, initiating Trk-A kinase autophosphorylation and activation of downstream pathways. Blocking NGF signaling through Trk-A kinase inhibition is hoped to deliver the same efficacy in pain models as the monoclonal antibody strategy. The discovery strategy focused on peripheral restriction to minimize centrally mediated side effects, and to ensure a high degree of selectivity across the kinome to maximize the clinical tolerability of a molecule which would be taken to treat a long-term, non-life-threatening condition.

An analysis of the selectivity binding determinants for the ATP-binding pocket utilizing kinase selectivity screen and kinase co-crystal structure information revealed key features required to maximize selectivity —namely increased contact area in the hydrophobic back pockets formed by the DFG-out protein conformation with minimal hinge binding interactions.

The lead optimization described started from hit shown (Fig. 9) towards the optimized lead taking advantage of the selectivity rationale and targeting PGP (P-glycoprotein) recognition to minimize CNS exposure. The optimized lead was efficacious in pain models, well tolerated in preliminary rat and dog toxicology studies and has a predicted dose in humans of 70-150 mg/day.

THE HSP90 CHAPERONE SYSTEM - AN OPEN AND SHUT CASE FOR DRUG DISCOVERY

Professor Laurence Pearl (Sussex University) gave an overview of the heat shock protein 90 (Hsp90) molecular chaperone system, which is responsible for the assembly and activation of many proteins that are involved in diverse regulatory and signaling pathways in our cells. As many of the proteins that are stabilized by Hsp90 are involved in various cancer signaling pathways, inhibition of Hsp90 represented a potential approach to target a broad-spectrum oncology agent. Indeed, encouraged by early clinical data generated using derivatives of the natural product geldanamycin, Professor Pearl and colleagues

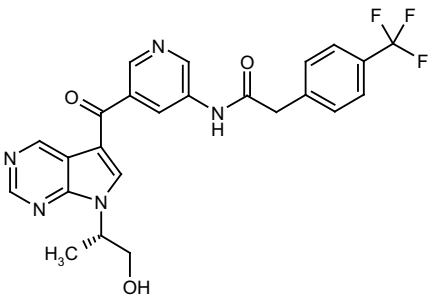


Hit CE-245677

Low solubility and poor selectivity

Non-PGP substrate

TrK-kA K_i 48 nM



Optimized lead PF-06278121

Improved solubility and selectivity

PGP substrate – peripherally restricted

Trk-A K_i 15 nM

Figure 9. Initial hit to final lead.

at the Institute of Cancer Research embarked on a structure-guided optimization of the only hit to emerge from a high-throughput screen. These efforts eventually delivered a clinical candidate that was subsequently developed with Vernalis then Novartis (luminespib, NVP-AUY922) and is currently in late-stage phase II trials.

His work has continued to define the molecular characteristics of Hsp90 and its key binding partners, which have helped to elucidate the mechanisms of Hsp90 regulation. This work has demonstrated that Hsp90 inhibitors promote their client kinase degradation, and are recruited to Hsp90 by Cdc37, which binds both Hsp90 and the kinases. The inhibition of Hsp90 then leads to degradation of a variety of kinases and clear beneficial effects in a range of tumor settings. Recently published findings (7) delivered the unexpected observations that the inhibitors of the targeted client kinase enzymes used in various cancer settings not only compete with ATP for binding at the kinase active site, but also directly antagonize the binding of Cdc37 to the protein kinase. This inhibition of Cdc37-kinase binding precludes access to the Hsp90–Cdc37 complex formation and thus leads to degradation of the kinase. This opened the intriguing conclusion that a range of clinically effective kinase inhibitors (such as vemurafenib and lapatinib) may indeed be effective agents in treating cancers due not only to their ability to directly block their target kinase action (principally B-raf and ErbB2, respectively) but also to their ability to promote degradation of these kinases by excluding chaperone stability association.

DISCLOSURES

The authors state no conflicts of interest.

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