Drugs of the Future 2013, 38(11): 789-794

CCC: 0377-8282/2013
DOI: 10.1358/dof.2013.38.11.2072090

MEETING REPORT

KINASES: NEW HORIZONS

HIGHLIGHTS FROM THE SOCIETY OF MEDICINES RESEARCH SYMPOSIUM, HELD ON OCTOBER 3RD, 2013 – NATIONAL HEART AND LUNG INSTITUTE, KENSINGTON, LONDON, UK

M.S. Alavijeh[1], R.J. Davenport[2], K.A. Brown[3] and S.E. Ward[4]

[1]Pharmidex, 14 Hanover St., Mayfair, London W1S 1YH, U.K; [2]Takeda UK Ltd.; [3]University of Cambridge, Trinity Lane, Cambridge CB2 1TN, U.K. and University of Texas U.S.; [4]University of Sussex, Brighton BN1 9QJ, U.K.

CONTENTS

SUMMARY

Of all the protein classes under investigation in the pharmaceutical industry, kinases represent one of the most well-worked (at least 20% of drug discovery research has been focused on kinase inhibitors for oncology) and successful (currently 13 small-molecule inhibitors have been approved by the FDA). Despite this, significant challenges and opportunities exist for both the exploitation of kinase inhibitors in new therapeutic areas and the development of novel technologies and methodologies to support their discovery and development. This meeting brought together experts in the field to review both areas. The first part of the meeting focuses on new technologies and strategies for the discovery of kinase inhibitors, including novel technologies and the exploitation of covalent and allosteric ligands. The second part of the meeting is dedicated to the discovery and development of kinase inhibitors for new and emerging therapeutic areas, in particular in CNS and tropical diseases.

Key words: Allosteric – Covalent binder – CNS – Alzheimer's disease – Malaria – Neglected tropical disease

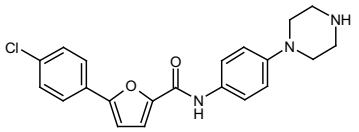
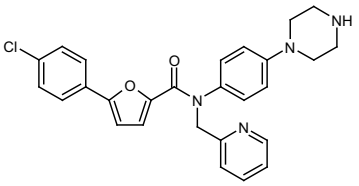
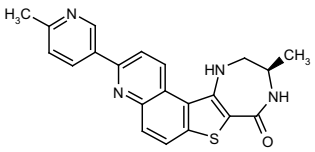
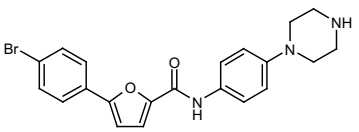
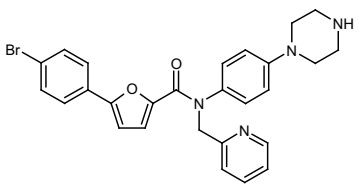
Correspondence: secretariat@smr.org.uk.

APPROACHES TO THE IDENTIFICATION AND CHARACTERIZATION OF NON-ATP-COMPETITIVE KINASE INHIBITORS – MK2, A CASE STUDY

Dr. Heather Tye from Evotek outlined the reason for moving away from ATP-competitive kinase inhibitors, such as challenging cross kinase selectivity (1), limited efficacy from competing with physiological ATP concentrations and often crowded intellectual property space, before discussing the way forward with allosteric inhibition (2). Traditional screening methodologies, such as SPR and NMR, can be used to find allosteric hits; however, in the MK2 case study described, a cascade assay system was utilized. The cascade assay involved active MAP kinase p38α (which is involved in activating MK2), inactive MK2, ATP and the MK2 substrate, and enabled hits that inhibited either the active or inactive form of MK2 to be identified. A series of tool compounds were assessed in this assay, with results presented in the following table.

Compounds **1-5** were assessed in a variety of orthogonal assays, such as an ATP-competitive assay, a substrate peptide competitive assay, and SPR and NMR binding assays. Compound 4, which was characterized as a non-ATP-site binder was crystallized with a truncated MK2 construct. A 3.25 Å structure was obtained, demonstrating that the binding site was not in the ATP pocket and actually was consistent with the predicted binding site from Reconnaissance Meta Dynamics

Table I. *Tool compounds assessed in biochemical assays.*

Compound	IC_{50} µM active (MK2 full)	IC_{50} µM cascade (MK2 full)	IC_{50} µM active (MK2 41-338)	IC_{50} µM cascade (MK2 41-338)
 **1**	> 250	> 250	116	106
 **2**	0.8	8.7	7.4	4.6
 **3**	< 0.01	< 0.01	0.019	0.031
 **4**	> 250	> 250	114	162
 **5**	6.4	76	30	18

Shift in IC_{50} observed in cascade assay (full-length MK2). Truncated MK2 aa41-338 constitutively active (no shift). Br analogues 4 and 5 similar to Cl analogues. All compounds active in the p38α assay.

(RMD) (3). The talk concluded with the following learning points: understand your kinase mechanism; choose any screening constructs carefully; and appreciate that different screening formats (biochemical or biophysical) will make a difference to what hits you identify.

COVALENT KINASE INHIBITORS: TOX OR TREATMENT?

Dr. Tjeerd Barf from Acerta Pharma presented the concept that a covalent drug could provide significant efficacy with only minimal exposure, as its pharmacodynamic effect is driven by the biological turnover of the target and not the turnover of the drug. This in turn could lead to a reduced toxicity profile, as only a small dose may need to be given when compared to noncovalent drugs, which require reasonable exposure over a 24-hour period. Whereas historically covalent drugs have been viewed quite negatively by the pharmaceutical industry, in 2010, 10% of the approved drugs were covalent binders, with a couple of recent kinase successes being afatinib, the first irreversible epidermal growth factor receptor (EGFR) kinase inhibitor, and ibrutinib, an irreversible inhibitor of the tyrosine-protein kinase BTK as a breakthrough treatment in different B cell lymphoma subtypes. Covalent inhibitors can target either catalytically active amino acid residues or specific non-catalytically active cysteines in the ATP pocket of a kinase. These mechanisms of inhibition can lead to increased potency, selectivity (only 150 kinases have available cysteines for example) (4) and prolonged in vivo efficacy, with irreversible kinase inhibitors behaving in a non-ATP-competitive manner. A major upside of these inhibitors is that they do not suffer from reduced efficacy due to high intracellular ATP levels, which render conventional reversible kinase inhibitors less effective in a cellular context. To help discover novel covalent binders, a structure–activity relationship was developed, which defines the activity window between a covalent binder and its noncovalent partner compound. The greater the window, the more selective a compound, which will ultimately lead to a larger therapeutic window.

NOVEL TRIAZOLOPYRIDINE COMPOUNDS AS SELECTIVE JAK1 INHIBITORS: FROM TARGET DISCOVERY TO GLPG-0634

Dr. Christel Menet presented Galapagos efforts towards the identification of a novel tyrosine-protein kinase JAK1 selective inhibitor. Small-molecule JAK1/2, JAK1/3 and JAK3 inhibitors have demonstrated an improvement in the symptoms of rheumatoid arthritis (RA) in several clinical trials. A selective JAK1 inhibitor may show advantages over earlier compounds by preserving the antiinflammatory activity required for efficacy in the absence of side effects such as anemia (associated with JAK2 inhibition). Initial screening of the BioFocus SoftFocus® kinase library compounds against JAK1 led to the identification of one main chemical series, showing promising activity and selectivity in a JAK1 biochemical assay based on the triazolopyridine scaffold. The initial hit, primary data and SAR summary is shown below (Fig. 1).

This hit was developed further by optimization of ADME and pharmacokinetic properties that led to the identification of GLPG-0634 (structure not disclosed), which had excellent in vivo activity in a rodent collagen-induced arthritis (CIA) model, with comparable efficacy to that shown by the TNF-α blocker etanercept (Enbrel®). In these in vivo studies, a significant reduction in inflammation was observed, as well as bone protection, even at low doses. However, in biochemical assays GLPG-0634 inhibited JAK1 and had only modest selectivity against JAK2. In human whole blood assays, GLPG-0634 inhibited JAK1 30-fold over JAK2. In phase I studies, at ascending and multiple doses (25-450 mg), once- and twice-daily GLPG-0634 showed good safety and tolerability. At the dose of 300 mg once daily, GLPG-0634 showed a dose-dependent reduction in $CD4^+$ T cells, and phase IIa trials in RA patients dosed at 200 mg once daily were initiated.

This study demonstrated significant clinical improvement in RA symptoms within four weeks, with 83% of patients attaining an ACR20 score. A unique safety profile was observed, with an absence of anemia and no increase in low-density (LDL) cholesterol was observed. An expanded phase II trial was performed, with GLPG-0634 being administered in a wide range of once-daily dose levels, including placebo, 30, 75, 150 or 300 mg. The results confirmed the phase IIa data, with the compound found to be safe, well tolerated and efficacious in this study. GLPG-0634 is the first selective JAK1 compound that has demonstrated clinical efficacy and a favorable safety profile in two phase IIa clinical studies in patients with RA.

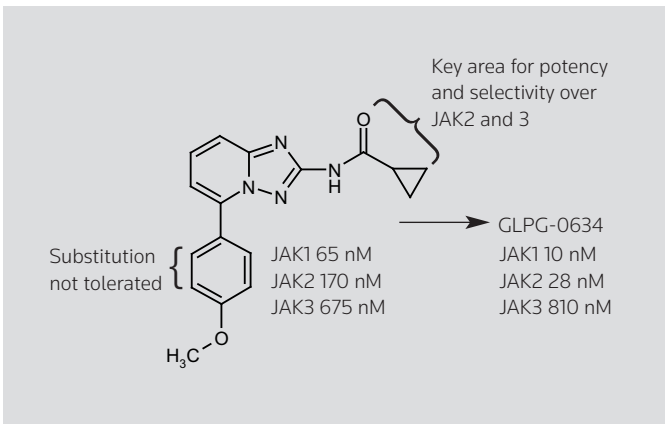
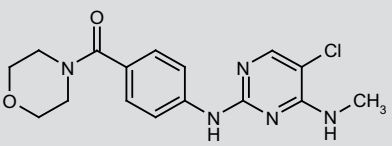


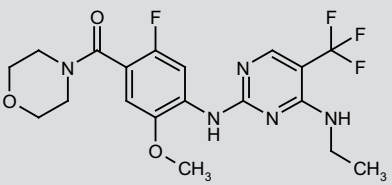
Figure 1. Good ADME properties of GLPG-0634.

GNE-7915: A POTENT, SELECTIVE AND BRAIN-PENETRANT LRRK2 INHIBITOR

Dr. Mark Chambers of BioFocus gave an account of the design of inhibitors of leucine-rich repeat serine/threonine protein kinase 2 (LRRK2), which has been a popular target for potential Parkinson's disease therapy due to its clear genetic association. This has driven the need for potent, brain-penetrant, selective inhibitors; however, early attempts in this field yielded molecules with either low CNS penetration or poor selectivity. A high-throughput screening (HTS) campaign at BioFocus afforded a potent diaminopyrimidine hit, **1** (5). Optimization of this hit towards the clinical candidate focused on the introduction of selectivity, informed by computational studies of the kinase binding site. Introduction of a substituent *ortho* to the amino (later to become the methoxy [OMe] in GNE-7915) gave a significantly improved selectivity profile, and a lipophilic substituent on the pyrimidine (later to become the CF_3 group) gave increased potency through a favorable interaction with the Met gatekeeper. An observed liability in the micronucleus assay was presumed to be due to dual specificity protein kinase TTK inhibition, and although this was not confirmed, introduction of another substituent (the F in GNE-7915) gave a wider separation of LRRK2-TTK inhibition and a clean profile in the micronucleus assays. The optimized molecule, **GNE-7915**, showed good preclinical pharmacokinetics (rat and cynomolgus monkey), with an acceptable ratio of unbound brain/plasma concentrations. Importantly, onward investigation demonstrated robust knockdown of phosphorylated LRRK2 in the brain after oral and i.p. dosing with the compound.



Hit **1**



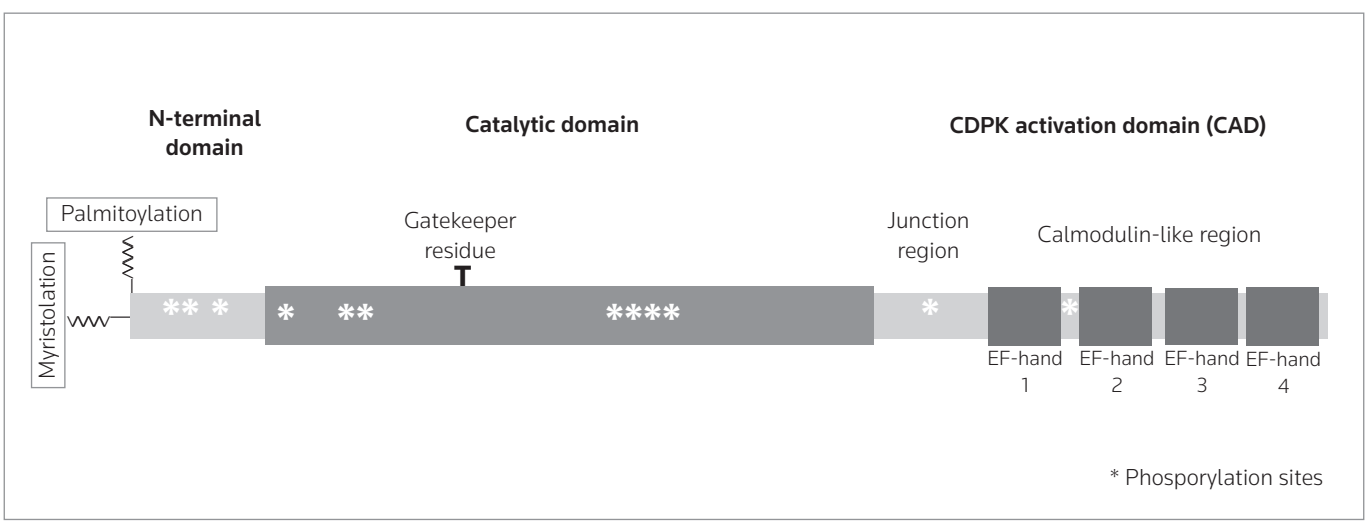
GNE-7915

NOVEL SIGNAL TRANSDUCTION PATHWAYS AND DRUG TARGETS FOR ALZHEIMER'S DISEASE

Professor Chris Miller of the Institute of Psychiatry at King's College in London demonstrated that glycogen synthase kinase-3 beta (GSK-3 beta) has been a target of many drug discovery projects but has not yielded any advanced clinical molecules. This, along with cyclin-dependent kinase 5/p35 (CDK5/p35) and protein phosphatise 1 (PP1) is strongly associated with tau phosphorylation and production of β-amyloid, and as such, are interesting targets for Alzheimer's disease. Furthermore, these proteins are implicated in axonal transport, which is disrupted in Alzheimer's disease, leading to synaptic dysfunction and neuronal loss. This has led to a number of promising preclinical reports of inhibitors of GSK-3 beta in animal studies, but its wider roles (e.g., in insulin signaling) have frustrated clinical progression. However, studies investigating the roles of both GSK-3 beta and CDK5/p35 have identified the involvement of another kinase, serine/threonine-protein kinase LMTK2 (6). Despite its name, LMTK2 is a kinase from a family of similar kinases with a long (lemur-like) *C*-terminal tail. LMTK2 also binds to the catalytic subunit of PP1C, and as such, the hypothesis is that CDK5/p35, LMTK2 and PP1C form a novel signaling pathway that regulates GSK-3 beta-Ser 9 phosphorylation to control GSK-3 beta activity. This hypothesis was supported by confirming that modulating LMTK2 expression affects GSK-3 beta-Ser 9 phosphorylation, CDK5/p35 phosphorylates LMTK2 and phosphorylation of LMTK2-Ser 1418 by CDK5/p35 regulates LMTK2 activity and phosphorylation of PP1C-Thr 320. Furthermore, it was demonstrated that LMTK2 binds to kinesin light chains and so ties the hypothesis back together with the underpinning axonal transport observations, and as such, LMTK2 may also play a role in the facilitation of axonal transport of bound CDK5/p35, PP1C and possibly GSK-3 beta.

TARGETING A PLANT-LIKE PROTEIN KINASE IN THE MALARIA PARASITE

Dr. Tony Holder from the MRC National Institute for Medical Research presented a talk about developing drugs to target an unusual calcium-dependent protein kinase (CDPK) family from the malarial parasite *Plasmodium falciparum*. He reminded the audience that malaria continues to be a major public health problem in > 90 countries, estimating the combined population at > 2.5 billion people at risk (approx. 35% of the world's population) from this disease. Malaria is responsible for approximately 300 million clinical infections and a million deaths per year, the majority of which occur in children under 5 years old. Emerging resistance to existing drugs drives the need for new therapeutics to treat this illness. His research has been focused upon identifying drug targets that would disrupt the asexual stage of the parasite life cycle. This is the disease stage where the parasite invades and then multiplies within human red blood cells. If this process of invading red blood cells could be prevented, the parasite's life cycle would be broken, leading to clearance of the infection. Dr. Holder introduced the CDPKs, an unusual serine/threonine-protein kinase family that contains both catalytic and regulatory domains and are regulated directly by Ca^{2+}. CDPKs are found only in plants and in the aveolates, protists that include the malarial parasites. The *P. falciparum* genome contains seven related genes from this kinase family. His laboratory has demonstrated that *P. falciparum* CDPK1 is essential to the survival of the parasite. In addition, its localization and its ability to phosphorylate motor proteins suggests that CDPK1 plays a role in the invasion of parasites from the bloodstream into red blood cells (7). These observations formed the basis for the selection of CDPK1 for an antimalarial drug development project with MRC Technology and the Medicines for Malaria Venture. Dr. Holder then briefly described the drug development pipeline. The pipeline started with a high-throughput screen of 35,422 compounds. The screen resulted in the identification of a series of CDPK1 inhibitors containing a 3,6-disubstituted imidazopyridazine core for further development. Compounds in this series were potent, having submicromolar 100 nM IC_{50} values, but showed poor selectivity against a human kinase panel (8). He described a multifaceted approach, using SAR with homology modeling, to improve: 1) selectivity against human kinases; 2) in vitro inhibition of recombinant *P. falciparum* CDPK1; and 3) killing of parasites in culture. Lead candidates were then assessed for in vivo efficacy



in a mouse model of infection. The outcome of these studies was that compounds could be optimized for good in vitro activity against enzyme and parasite culture, but only a reduction of parasites in the mouse infection model of 50% was achieved. In addition, the in vivo activity in mice did not appear to correlate with the antiparasitic activity seen in in vitro cultures (8). These results suggested that further optimization of the drug-like properties of these molecules is needed, as well as a better understanding of their mode of action. He concluded by pointing out that these studies highlighted the importance of carrying out target validation (e.g., by genetic attenuation) and in vivo studies as early as possible.

HARNESSING KINASE INHIBITORS AS THERAPEUTICS FOR NEGLECTED TROPICAL DISEASES

Dr. John Overington from the EMBL-European Bioinformatics Institute (EBI) in Hinxton, U.K., presented an overview of the EBI's ChEMBL resource (https://www.ebi.ac.uk/chembl/). His talk focused on how its many features can be used to aid drug discovery efforts targeted at protein kinases. ChEMBL is an open data database of bioactive data for drug discovery. It contains binding, functional and ADMET information for a large number of drug-like bioactive compounds. Dr. Overington explained how the resource provides researchers with tools to cluster relevant information across studies (using target or compound similarities, for example). Data can be integrated across a number of therapeutic areas, enabled by the availability of bioinformatic tools with a range of user-friendly search capabilities (9). The ChEMBL resource includes an integrated chemogenomics workbench called the Kinase SARfari, which incorporates and links kinase sequence, structure, compounds and screening data. Kinase SARfari aims to provide "a unique view on kinases and kinase chemistry by leveraging both public and proprietary data", and also aims to "provide user-friendly access to the data via web-based application, utilizing familiar search tools such as chemical structure sketching and BLAST" (https://www.ebi.ac.uk/chembl/sarfari/kinasesarfari). This workbench, which links chemical and biological space, provides tools to help researchers define the characteristics of the "clinical kinome" –the druggable set of protein kinases of a genome. As shown in the accompanying figure (Fig. 2), the tyrosine-protein kinase family, a prominent oncology target, is the branch of the clinical kinome for which the majority of drugs have been developed. Other kinase families may indeed represent new and novel targets for future drug development, but await clinical drivers to exploit their potential. An example is a recently published study which used bioinformatic methods to predict drug targets using compounds that were shown to have antimicrobial activity against *Mycobacterium tuberculosis* (10). Four compounds were demonstrated to be potential targets for a serine/threonine-protein kinase. These compounds were docked into the crystal structure of an *M. tuberculosis* serine/threonine-protein kinase, *pknB*, which had been reported to be required for the viability of the organism. Docking models suggested that inhibition of *pknB* could be the mode of action for at least one of the putative kinase inhibitor compounds. Dr. Overington emphasized that the major goal of this work is to encourage other researchers to experimentally validate *pknB* and other tuberculosis targets that were identified from this study (a total of 139 target-compound links were identified). The published study also requests that data from target validation studies be made available to the public as soon as possible in order to accelerate the development of improved therapies for treating tuberculosis.

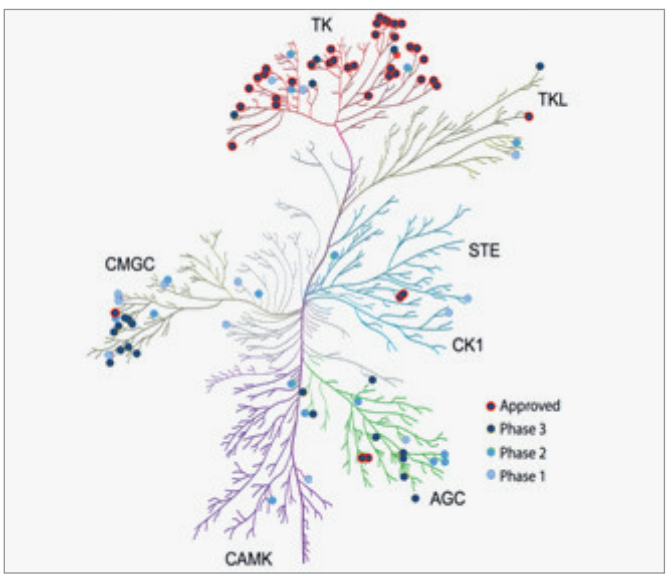


Figure 2. The clinical kinome.

DISCLOSURES

M.S. Alavijeh is an employee of Pharmidex and R.J. Davenport is an employee of Takeda UK. K.A. Brown and S.E. Ward state no conflicts of interest.

REFERENCES

1. McInnes, C. *Progress in the development of non-ATP-competitive protein kinase inhibitors for oncology*. Annu Rep Med Chem 2012, 47: 459-74.
2. Nussinov, R., Tsai, C.-J. *Allostery in disease and in drug discovery*. Cell 2013, 153(2): 293-305.
3. Soderhjelm, P., Tribello, G.A., Parrinello, M. *Locating binding poses in protein-ligand systems using reconnaissance metadynamics.* Proc Natl Acad Sci U S A 2012, 109(14): 5170-5.
4. Evans, E.K., Tester, R., Aslanian, S. et al. *Inhibition of Btk with CC-292 provides early pharmacodynamic assessment of activity in mice and humans.* J Pharmacol Exp Ther 2013, 346(1): 121-9.
5. Chen, H., Chan, B.K., Drummond, J. et al. *Discovery of selective LRRK2 inhibitors guided by computational analysis and molecular modeling.* J Med Chem 2012, 55(11): 5536-45.
6. Kesavapany, S., Lau, K.F., Ackerley, S. et al. *Identification of a novel, membrane-associated neuronal kinase, cyclin-dependent kinase 5/p35-regulated kinase*. J Neurosci 2003, 23(12): 4975-83.
7. Green, J.L., Rees-Channer, R.R., Howell, S.A. et al. *The motor complex of Plasmodium falciparum: Phosphorylation by a calcium-dependent protein kinase*. J Biol Chem 2008, 283(45): 30980-9.
8. Chapman, T.M., Osborne, S.A., Bouloc, N. et al. *Substituted imidazopyridazines are potent and selective inhibitors of Plasmodium falciparum calcium-dependent protein kinase 1 (PfCDPK1).* Bioorg Med Chem Lett 2013, 23(10): 3064-9.

9. Gaulton, A., Bellis, A.J., Bento, A.P. et al. *ChEMBL: A large-scale bioactivity database for drug discovery*. Nucleic Acids Res 2012, 40(Database issue): D1100-7.

10. Martínez-Jiménez, F., Papadatos, G., Yang, L. et al. *Target prediction for an open access set of compounds active against Mycobacterium tuberculosis*. PLoS Comput Biol 2013, 9(10): e1003253.