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MEETING REPORT

KINASE INHIBITORS FROM EARLY RESEARCH TO CLINIC

HIGHLIGHTS OF THE SOCIETY FOR MEDICINES RESEARCH SYMPOSIUM, HELD ON JUNE 24, 2010, AT ASTRAZENECA, ALDERLEY PARK CONFERENCE CENTRE, ALDERLEY PARK, MACCLESFIELD, UK

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SUMMARY

The Society for Medicines Research symposium was held at AstraZeneca, Alderley Park Conference Centre, Alderley Park, Macclesfield, U.K. The meeting, organized by Jack Allen, Steve Collingwood and Andrew Ratcliffe, focused on kinase inhibitors from early research to clinic. Topics included kinase inhibitor design, translating kinase selectivity profiles into early safety guidance, and the discovery and early development of kinase inhibitors in the therapeutic areas of inflammation, pulmonary arterial hypertension and oncology.

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KINASE RESEARCH IN THE 21ST CENTURY—WHERE IS IT GOING?

Professor Andrew Wilks (SYN|thesis med chem, Australia) opened the symposium with a scene-setting presentation on kinase research in the 21st century, and in particular its future direction. During the last 20 years the kinome tree has grown considerably, from a sapling detailing < 20 kinases, to today's extensive network of 518 distinct family members, which forms the basis of a smorgasbord of drug discovery targets.

Testament to the interest in kinase drug discovery is the projected NZD 100,000,000,000 spent to date on research and development. The value from such investment has manifested itself in 18 marketed inhibitors (including the non-small-molecule mammalian target of rapamycin [mTOR] class of inhibitors), with over 1,000 compounds at various phases of clinical development, the majority (around 75%) targeting oncology indications. However, a startling statistic is that nearly 60% of all trials are run with compounds against only seven targets (1). Analysis of kinase inhibitor patents between 2006 and 2009 supports the view that current kinase research has a high inertia, with > 40% claiming inhibitors against only 10 targets, the majority of which are again linked to a role in oncology (1).

Professor Wilks suggested that the basis for this situation stemmed from several factors, including a willingness to work on a limited number of targets of well-defined biology in the hope of fulfilling the expectations generated from the remarkable ascendancy of imatinib

(Gleevec®), one of the early small-molecule inhibitors to transition to market and reach blockbuster status, breaking global sales of over USD 1 billion.

One clear advancement over the years has been the discovery and development of more selective inhibitors, leveraging X-ray crystallography to open up and mine new design principles (back pocket binders, allosteric type II pharmacophore, etc.). Validation of the role of new kinase targets in a disease setting using smart biological tools, such as short interfering RNA (2, 3), coupled with the development of new assay technologies to screen for new mechanisms of action, such as allosteric inhibitors (4) or activators, were suggested as potential key future drivers in shaping the field.

Given that kinases operate via signaling network environments, the opportunity to take advantage of polypharmacology to deliver enhanced efficacy through inhibition of multiple targets represents an attractive strategy, providing acceptable safety can be maintained. Indeed, through more serendipity than purpose, several of the superior marketed oncology kinase inhibitors operate in this fashion. Going forward, at the practical level, the significant challenges in following such a strategy cannot be understated, in both identification of disease-relevant target combinations, and whether single-molecule inhibitors can be efficiently crafted to inhibit multiple selected targets, aware of the optimization of physicochemical and absorption, distribution, metabolism and elimination (ADME) properties required to deliver robust drug candidates. Nevertheless, Professor Wilks suggested that part of the next generation of kinase inhibitors may well constitute an element of rationally designed nonselective inhibitors (5).

The current interest in the *Plasmodium* kinome as an opportunity to identify and develop small-molecule kinase inhibitors to treat unmet drug-resistant malaria, a disease that today still inflicts a high mortality in many less-developed countries, was also proposed as a further source of future inhibitors (6). In contrast to the human kinome, the *Plasmodium* kinome comprises between 85 and 99 kinases, depending on which study is referenced. Of considerable interest is that divergence exists between the two systems; for example, the *Plasmodium* kinome lacks any tyrosine kinase. To date, through the use of reverse genetics, potential targets have been identified that constitute strategies toward curative or transmission-blocking intervention. Efforts to develop inhibitors to these targets are ongoing through screening, complemented by rational drug design.

THE PITFALLS OF REDUCTIONISM IN TODAY'S KINASE INHIBITOR DESIGN

Dr. Gerhard Müller (Proteros Fragments GmbH, Germany) opened his talk "on the pitfalls of reductionism in today's kinase inhibitor design" by making reference to the typical sequential activity pathway followed in kinase drug discovery, with emphasis on the fact that IC_{50}s generated in biochemical kinase inhibitor in vitro assays often represented the leading drivers in compound selection and initial optimization work. However, Dr. Müller challenged the physiological nature of such in vitro assays, citing that the target kinase under prosecution was often recombinant and comprised of only the catalytic core, substrates were typically of an artificial peptidic nature, adenosine-5′-triphosphate (ATP) concentrations were at a much reduced level tuned to the K_m of the kinase, and high concentrations of $MgCl_2$, plus other additives (DTT, Mops), were required to fuel phosphor transfer.

It was also highlighted that misconceptions existed over linking kinase selectivity to the degree of 3D complexity engrained in a kinase inhibitor. Comparison of staurosporine (**1**) (inhibition of 104/113 kinases at 0.7-7 μM) with vatalanib (**2**) (inhibition of 5/113 kinases at < 3 μM) nicely serves to illustrate the case in point (Fig. 1).

Enhanced kinase selectivity for some inhibitors is gained by accessing a back pocket, primarily hydrophobic in nature, but of low sequence conservation and high plasticity, not utilized by the catalytic machinery (7, 8). Entrance to the back pocket is controlled by a gatekeeper residue, which also serves to help modulate the shape and size of the cavity. Of the human kinome, approximately 25% project a small amino acid gatekeeper, such as Gly, Ala, Ser, Cys, Thr and Val, that is more programmed to allow access of inhibitors to this potential selectivity binding pocket. Examples of kinase targets where this strategy has been successfully utilized to generate selective inhibitors include proto-oncogene tyrosine-protein kinase Src, vascular endothelial growth factor receptor 2, Aurora kinase A, tyrosine-protein kinase Kit, epidermal growth factor receptor, mitogen-activated protein kinase p38α, Polo-like kinase 1, serine/threonine-protein kinase Chk1 and serine/threonine-protein kinase B-raf. From a survey of published inhibitors, the 4-anilinoquinazoline core appears to be a common scaffold, in

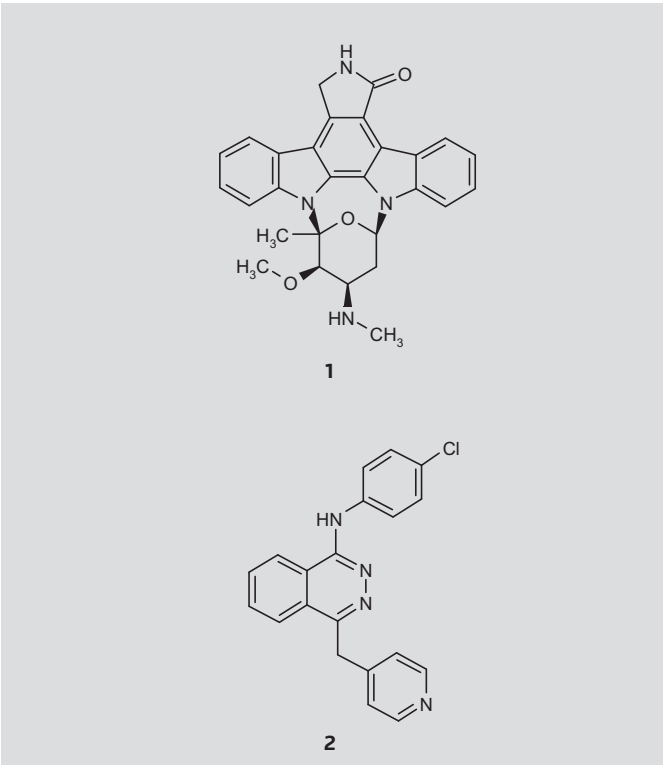

Figure 1. Structures of staurosporine (**1**) and vatalanib (**2**).

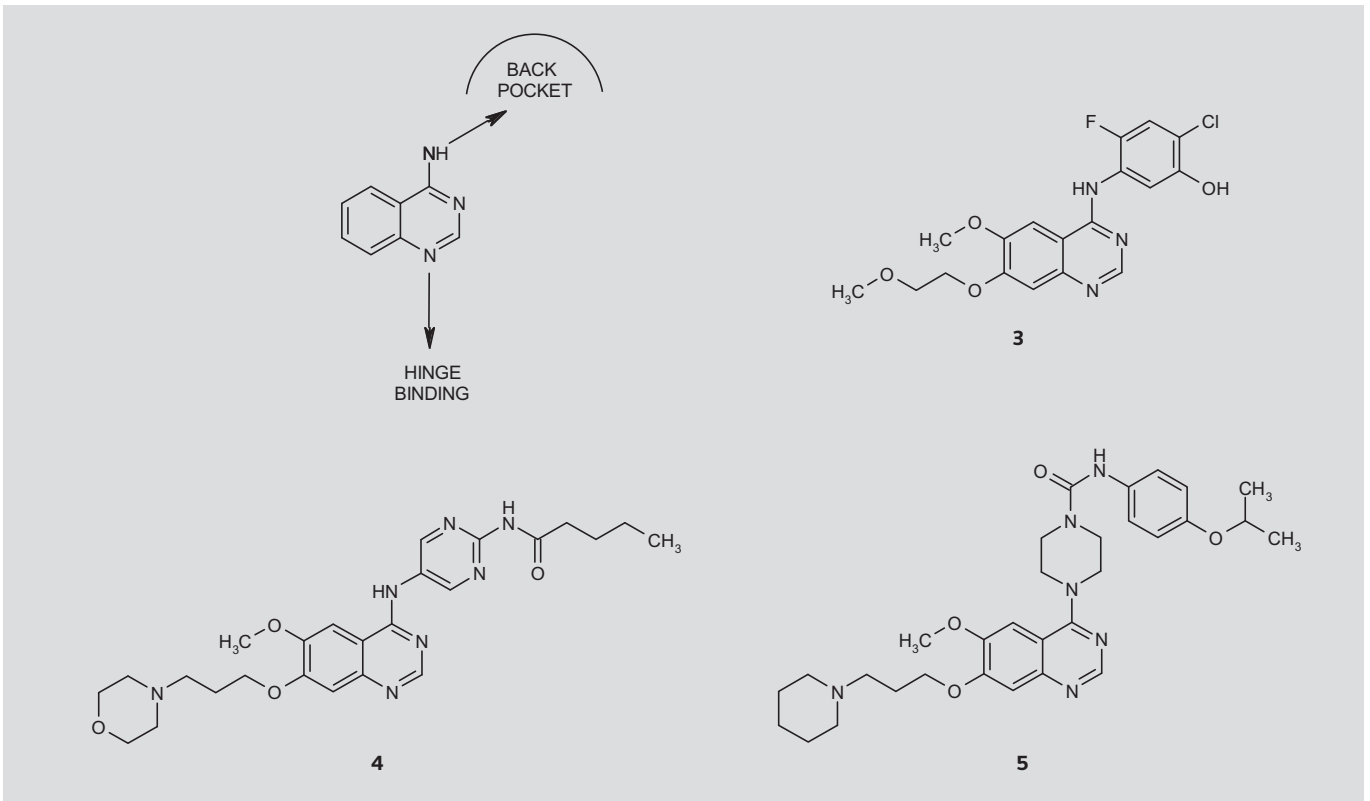

Figure 2. Back pocket 4-anilinoquinazoline kinase inhibitors **3**-**5**.

which the 4-anilino can be effectively decorated and optimized to take advantage of back pocket molecular recognition (Fig. 2, **3**-**5**).

X-ray crystallography has been an invaluable tool in mapping key interactions and aiding the rational design process. The power of this biochemical technique was used to solve the binding mode of the 4-anilinoquinazoline **6**, a submicromolar cyclin-dependent kinase 2 inhibitor (9). Rather than follow the scaffold binding interactions of **3**-**5**, with back pocket interactions stemming from the 4-anilino substituent, the presence of the 2-CF3 group causes a significant realignment of the quinazoline scaffold, such that H-bonding interactions with the hinge region are achieved through a bridging water molecule, with the 2-CF3 group directed towards the entrance of the back pocket (Fig. 3).

There is a further subset of kinases where inhibitors, classified as type II inhibitors, can be designed that display enhanced selectivity profiles through binding and stabilizing the inactive form of the kinase (7, 8). The energetic drive for such binding results from exploitation of additional favorable hydrophobic and H-bonding interactions in an allosteric cavity, formed through conformational movement of a conserved Asp-Phe-Gly (DFG) motif as it switches from its active "in" position to inactive "out" position. A low allosteric sequence identity in those kinases that bind inhibitors in this mode provides further opportunity to tune and optimize selectivity. A further hallmark of type II inhibitors is a propensity to display extended residence times, characterized by low dissociation off values (k_{off}). As a representative example, the residence time ($1/k_{off}$) of sorafenib (**7**) (Fig. 4), a B-raf inhibitor, is ≈ 560 minutes, with an IC_{50} at time = 0 of 550 nM, increasing to 5

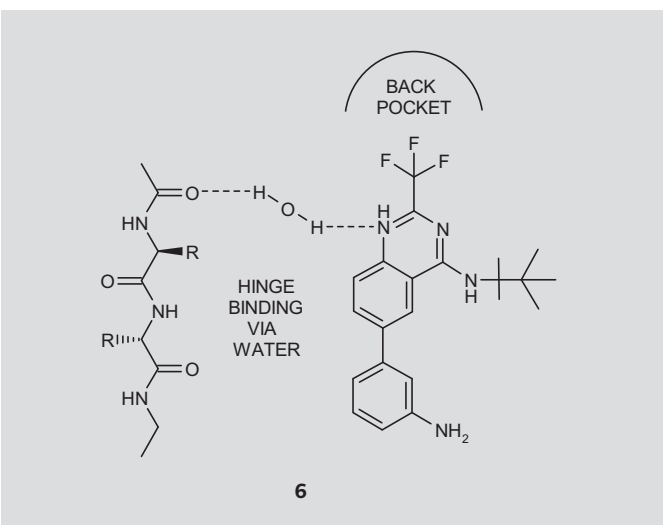

Figure 3. Structure and hinge binding interactions of 4-anilinoquinazoline inhibitor **6**.

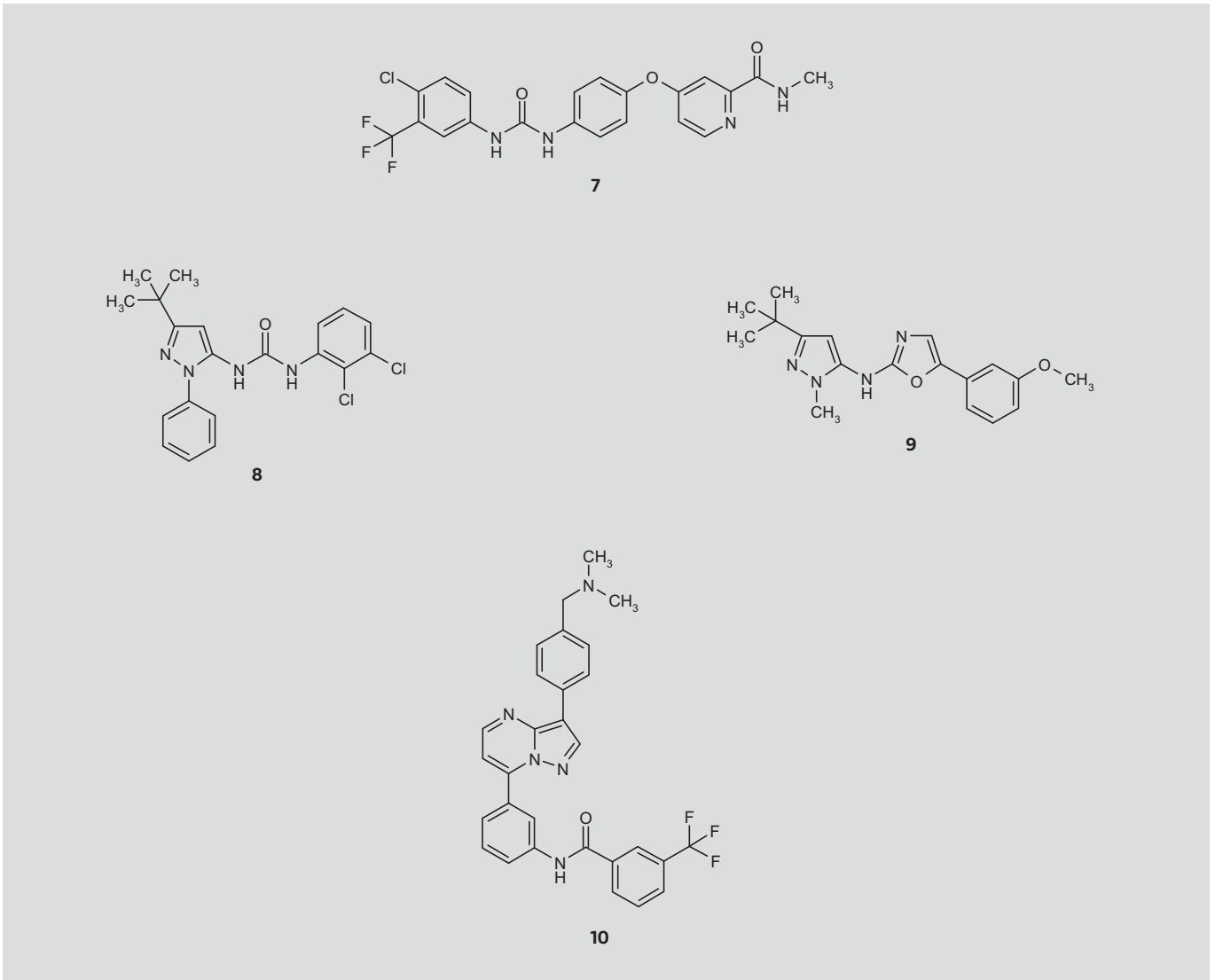

Figure 4. Structures of sorafenib (**7)** and B-raf kinase inhibitors **8**-**10**.

nM after a preincubation time of 120 minutes. The biochemical consequences of such effects are profound, with increased cellular and in vivo efficacy, given a weaker competition from cellular ATP.

The truncated urea **8** (Fig. 4) also binds to the allosteric pocket of the DFG "out" form of B-raf, without the need to make any hinge H-bond interactions. Interestingly, bioisosteric replacement of the urea with the 2-amino oxazole unit, to give **9** (Fig. 4), delivered binding to B-raf in a DFG "in" active state via formation of key H-bonds to the hinge, and clearly teaching that reality can lead to unexpected results. Recently, Pfizer researchers have published a B-raf inhibitor **10** (Fig. 4), which, based on crystallographic data, also binds to the DFG "out" form of the kinase via the allosteric pocket, without making any H-bonds to the hinge region (10). Dr. Müller suggested, given the significant structural differences between **8** and **10**, that design of further type II inhibitors not requiring hinge binding represented a novel approach worthy of further pursuit, in particular towards opening up intellectual property novelty.

From X-ray crystal structures a systematic analysis of type II inhibitors has revealed the influence of binding in the allosteric pocket. However, a striking exception to this situation appears with the sorafenib–Tie2 complex. In contrast to the binding mode of sorafenib (**7)** to B-raf, which as described above follows the typical type II binding model, with the phenyl urea occupying the allosteric pocket and the DFG in a true "out" mode, in Tie2 the phenyl urea avoids the allosteric pocket and intercalates and distorts the R spine, a hydrophobic stack that links the N and C lobes and plays a critical role in the assembly of the active state of a kinase (11). The fact that the same compound can derive significantly different binding modes dependent on the kinase in question further challenges the reductionism in kinase inhibitor design.

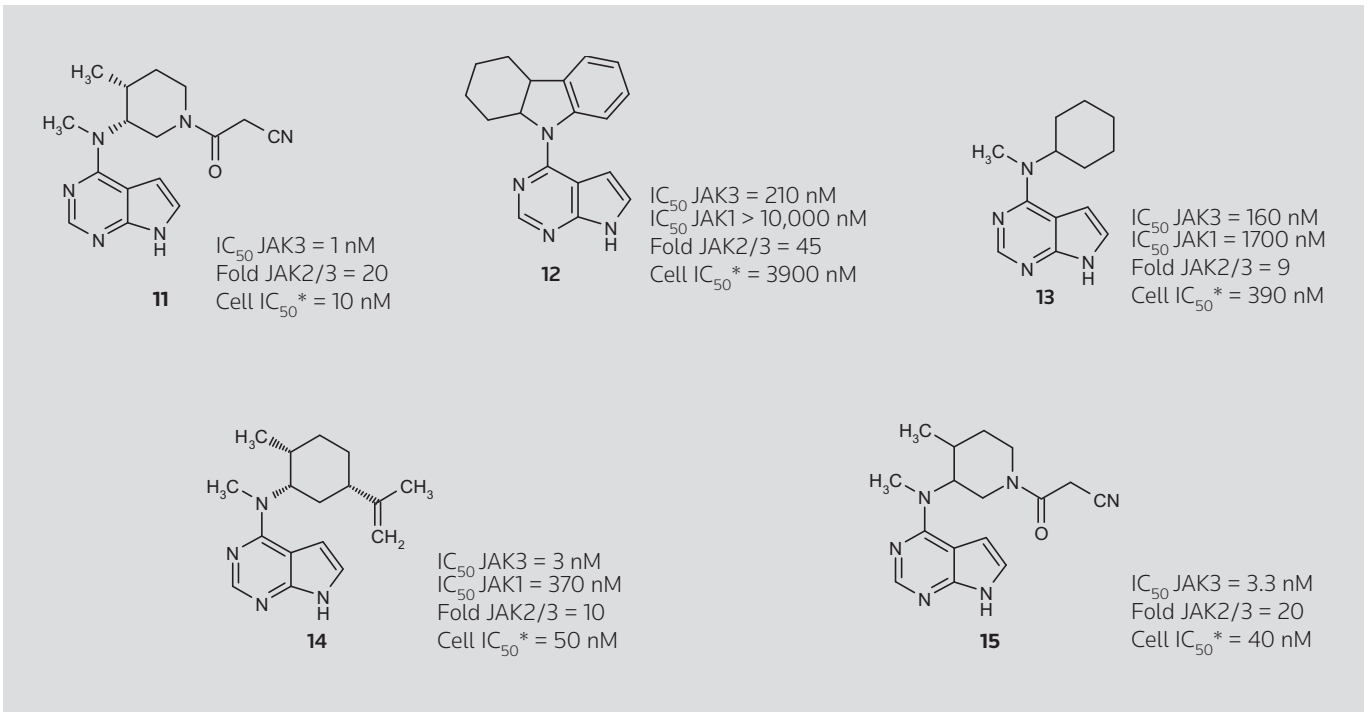

Figure 5. Structures of tasocitinib (CP-690550; **11**) and the Pfizer JAK inhibitors **12**-**15**. *IL-2-induced T-cell blast proliferation.

Evidence to support prolonged residence time without type II binding was presented with the Janus kinase (JAK) inhibitor tasocitinib (CP-690550; **11**) (Fig. 5), where the kinetic signature for residence time against JAK3 (17.1 minutes) appeared longer than that for JAK2 (3.3 minutes). It was suggested that subtle contact differences of the inhibitor with Ser936 in JAK2 and its Cys909 counterpart in JAK3 could form the basis of an explanation.

SMALL-MOLECULE KINASE INHIBITOR PROMISCUITY: TRANSLATING KINASE SELECTIVITY PROFILES INTO EARLY SAFETY GUIDANCE

The presentation from Dr. Karen Leach (Pfizer, U.S.) centered on whether kinase in vitro promiscuity was a predictor of in vivo toxicity. From analysis of current attrition factors, safety continues to be a major cause for failure in drug development. With respect to the kinase field, there has been an effort within many organizations, using computational approaches in tandem with pathway mining, towards identification of kinase inhibitor profiles that represent a high risk for exhibiting unacceptable safety margins.

As part of Pfizer's evaluation, 80 kinase inhibitors were profiled in a number of in vivo rodent models, with an emphasis on toxicity readouts, and in parallel screened against a 112-kinase panel at 10 μM. Combined analysis of both sets of data suggested a general trend of increasing kinase promiscuity with severity of organ toxicity. In addition, in terms of organ toxicity signatures, sufficient power existed in the data analysis to map cardiotoxic kinases to the tyrosine kinase branch of the kinome tree.

As part of genotoxicity testing compounds are routinely screened through an in vitro micronucleus test (IVMNT). Analysis of IVMNT historical Pfizer data revealed kinase inhibitors as the major target class eliciting a genotoxic response. From a detailed analysis of the kinase fingerprint of a subset of 44 inhibitors with associated IVMNT activity, the strong inhibition of 5 kinases, MST-2 (serine/threonine-protein kinase 3), GSK-3β (glycogen synthase kinase-3 beta), MLK1 (mixed lineage kinase 1), ROCK-1 (Rho-associated protein kinase 1) and CLK-1 (cell division cycle 2-like protein kinase 1), appears to be strongly associated with a positive outcome. However, further work evaluating GSK-3β inhibitors in detail suggests that the trigger of a micronucleus event requires the inhibition of a kinase signature containing some of the above key players in some combination rather than that of any individual kinase alone. In line with this thinking, the high positive IVMNT rate of promiscuous kinase inhibitors correlates with the more likely probability of hitting the kinase signature. It should also be highlighted that high kinase selectivity is not a prerequisite for a negative IVMNT. Several clean kinase inhibitors were described that still delivered a positive activity, reflecting the operation of non-kinase mechanisms or pathways.

In an attempt to streamline compound attrition and aid decision making, Pfizer has invested in cross-correlating in vitro cytotoxicity data with observed toxicity from early in vivo safety assessment studies (12). Measurement of ATP depletion in a transformed human liver epithelial (THLE) cell line was used as a marker for in vitro cytotoxicity. The distribution of THLE activity across a dynamic range of compounds belonging to different target classes is shown in Table I.

Table I. *Distribution of transformed human liver epithelial (THLE) activity across major protein families.*

	Kinases	GPCRs	Ion channels	Integrins	NHRs	Proteases	Transporters
Total comp. #	704	990	197	38	88	227	66
THLE $IC_{50} \leq 10\ \mu M$	90	34	1	0	11	7	7
$10\ \mu M < ... \leq 50\ \mu M$	174	146	35	3	13	35	8
$50\ \mu M < ... \leq 100\ \mu M$	117	116	20	1	23	35	6
$100\ \mu M < ... \leq 300\ \mu M$	163	286	52	6	24	66	17
$> 300\ \mu M$	160	408	89	28	17	84	28

GPCRs, G protein-coupled receptors; NHRs, nuclear hormone receptors.

A breakdown of the kinase class revealed a trend for increasing promiscuity correlating with an increased risk in THLE activity. In closing, Dr. Leach remarked that understanding kinase signatures in relation to a toxic event was still very much a work in progress.

JAK INHIBITORS AS NOVEL ANTIINFLAMMATORY AGENTS

The afternoon session focused on some specific examples of current drug discovery programs. Dr. Iain Kilty (Pfizer, U.K.) described the progress of the JAK inhibitor project leading to the discovery of tasocitinib (CP-690550; **11**) (Fig. 5). The Janus family of kinases are apparently named after Janus, the Roman god of gates and doors, which reflects their biochemical role. Interestingly, Professor Wilks, the discoverer and namer of the family, commented that he originally, flippantly, coined the term JAK from "just another kinase", and was then obliged to find a more appropriate rationale!

Pfizer's original focus in the JAK project, reflecting that of the majority of the industry, was to discover selective JAK3 inhibitors for immunomodulation (13). A high-throughput screening campaign, followed by some early structure–activity relationship (SAR) development, provided the lead structure CP-352664 (**12**) (Fig. 5). The project flowchart provided upfront screening in ELISA format against JAK1-3, followed by a functional cellular assay dependent on the JAK1 and JAK3 pathways (interleukin-2-induced T-cell blast proliferation), with a JAK2-sensitive counter screen (granulocyte–macrophage colony-stimulating factor-induced cell assay in HUO3 cells). In vitro pharmacokinetics, selected in vivo pharmacokinetics (rat, dog) and kinase selectivity screening then set the scene for the key murine heterotopic heart transplant model.

The first breakthrough in cellular potency came from replacement of the hexahydro-1*H*-carbazole group with a simple methyl cyclohexyl amine to give CP-537555 (**13**) (Fig. 5). However, it was noted that the improved cellular potency may be due to its JAK1 activity. Careful exploration of the SAR of the cyclohexyl group then led to CP-634558 (**14**) (Fig. 5).

JAK2 selectivity and poor metabolic stability were still a concern for CP-634558. The replacement of the cyclohexyl group with a piperidine scaffold was contemplated as a means of reducing lipophilicity, imparting less complex stereochemistry and facilitating analogue generation. Alkyl and sulfonamide side chains were attached to the piperidine nitrogen; however, amide groups proved most successful, allowing both improved potency and increased metabolic stability. This initial optimization was performed with mixtures of diastereomers. Resolution of the attractive advanced lead CP-681560 (**15**) led to the first synthesis of tasocitinib (CP-690550) (Fig. 5).

Tasocitinib was shown to be effective at prolonging graft survival in the murine heterotopic heart transplant mode and in a monkey kidney transplant model (14). Furthermore, it also demonstrated activity in the rat adjuvant-induced arthritis model (15). Tasocitinib entered clinical development in August 2000, despite its modest selectivity over JAK family members. A more recent kinome selectivity profile showed it to be highly selective for the JAK family (16). The clinical development program for tasocitinib is comprehensive, with six major indications: rheumatoid arthritis, acute renal allograft rejection, psoriasis, Crohn's disease, ulcerative colitis and dry eye syndrome. The results of the successful proof-of-concept study in rheumatoid arthritis were briefly reviewed. Phase IIb studies demonstrated efficacy at oral doses from 3 mg b.i.d. upwards, and it is currently in phase III studies for rheumatoid arthritis.

The absolute JAK selectivity profile of tasocitinib has been the subject of some debate. New selectivity data were presented, firstly at the enzymatic level. In a new caliper assay format, tasocitinib shows only modest (two- to threefold) JAK3 selectivity over JAK1 and JAK2. However, new cellular data suggest that it has some selectivity (15-fold) over JAK2. Interestingly, tasocitinib has also demonstrated efficacy in a mouse allergen challenge asthma model. This represents a further potential indication for this intriguing molecule.

INHIBITORS OF PDGFR KINASE AS A NOVEL THERAPY FOR PULMONARY ARTERIAL HYPERTENSION

Dr. Matthew Thomas (Novartis, U.K.) presented evidence for the therapeutic use of the clinically approved oncology kinase inhibitor imatinib (QTI-571) in pulmonary arterial hypertension (PAH). PAH is defined as a mean rise in right ventricular pressure > 25 mmHg at rest and > 30 mmHg after exercise. There are several related forms: sporadic (idiopathic PAH), familial and associated PAH. Early symptoms are mild and not specific to PAH (e.g., dyspnea, fatigue, chest pain, dizziness and fainting, peripheral edema and progressive cardiopulmonary failure). PAH is usually only diagnosed after exclusion of other disorders, and thus diagnosis is often delayed for years. The mean age at diagnosis is 36 years, when most patients (75-80%) have moderate to severe PAH. Life expectancy is dependent on the status of disease progression. For

WHO class II diagnosis this amounts to 6 years, while at WHO class IV status the time is drastically cut to 6 months.

Clinical data suggesting a benefit for imatinib in PAH was noted in a few isolated patient settings. A 61-year-old man with rapidly progressing PAH received imatinib in addition to bosentan, iloprost, sildenafil, oral anticoagulants and diuretics (17). After 3 months he had greatly improved exercise capacity and an improvement from class IV to class II disease status, with no apparent adverse effects. Similar improvements in clinical condition were documented in a 52-year-old man with refractory idiopathic PAH (18). Furthermore, 2 patients (a 34-year-old man and 65-year-old woman) with PAH who received imatinib for the treatment of leukemia showed comparable clinical improvement (19).

The rat monocrotaline model of PAH offers the opportunity to demonstrate the potential for new therapeutic agents to exhibit both symptomatic efficacy and disease-modifying behavior (20). Dr. Thomas presented the efficacy data for imatinib (QTI-571), which showed therapeutic effects on hemodynamics (right ventricular pressure) and remodeling (Fig. 6). Imatinib showed additional therapeutic effects when used in combination with the phosphodiesterase PDE5 inhibitor sildenafil.

To study the safety and efficacy of imatinib in PAH under a more formal setting, 59 patients were recruited to a randomized, double-blind, placebo-controlled 6-month trial. Although 80% of the patients were on concurrent PAH combination therapy, those receiving imatinib had a statistically significant decrease in pulmonary vascular resistance (PVR) and a significant increase in cardiac output. Post hoc analysis suggested that the more severe patients (defined as those with the highest PVR) showed more profound changes in these parameters, and that this was reflected in a significant increase in the 6-minute

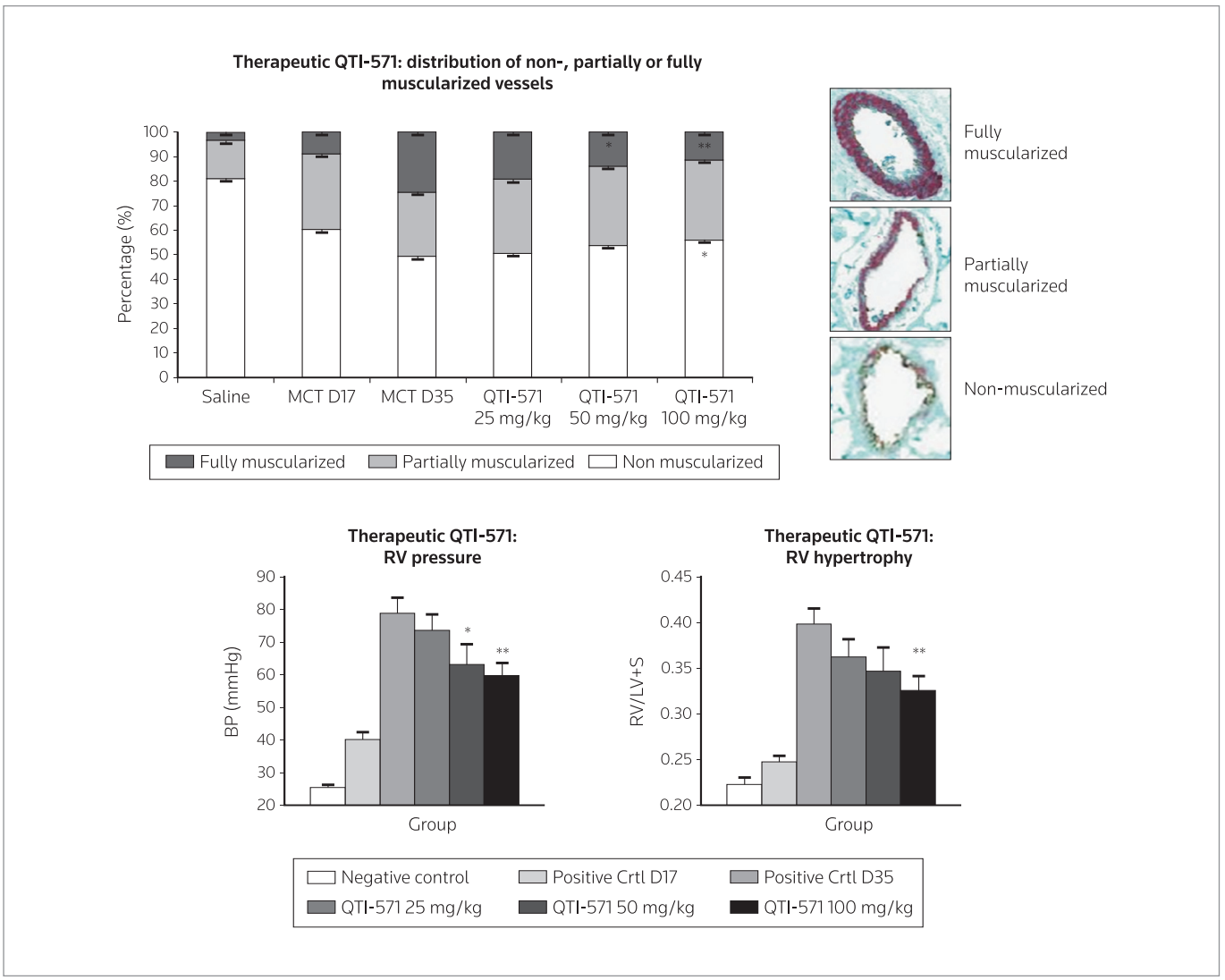

Figure 6. Efficacy data for imatinib (QTI-571) in the rat monocrotaline model of pulmonary arterial hypertension. RV, right ventricular; BP, blood pressure; LV, left ventricular; Crtl, control; S, septum.

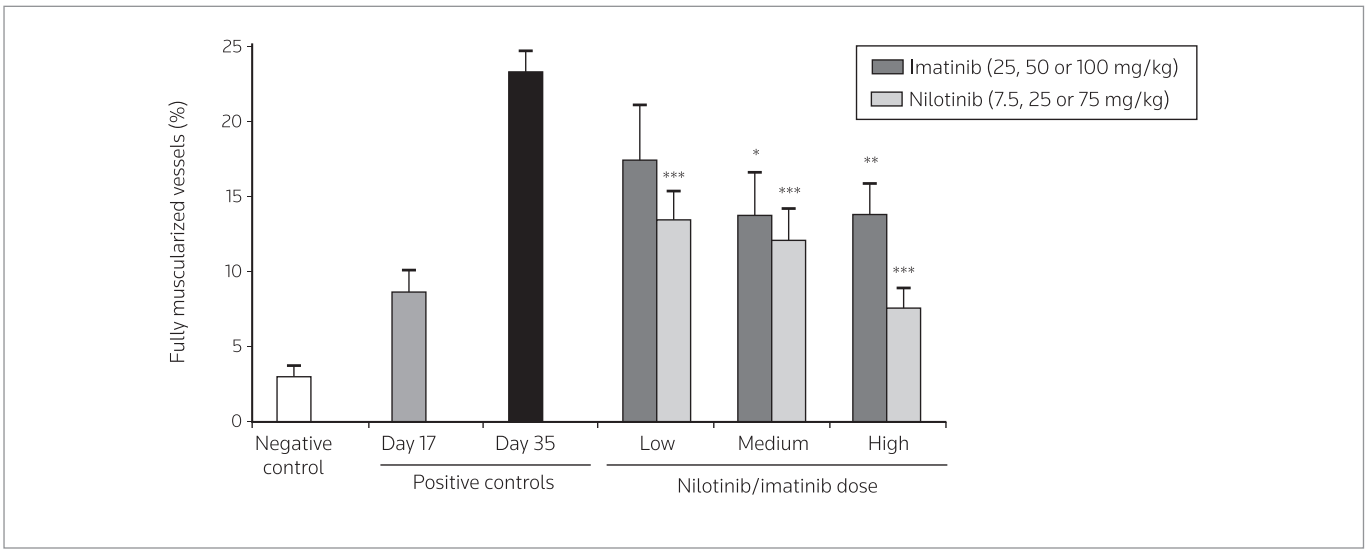

Figure 7. Comparison of efficacy data for nilotinib versus imatinib in the rat monocrotaline model of pulmonary arterial hypertension.

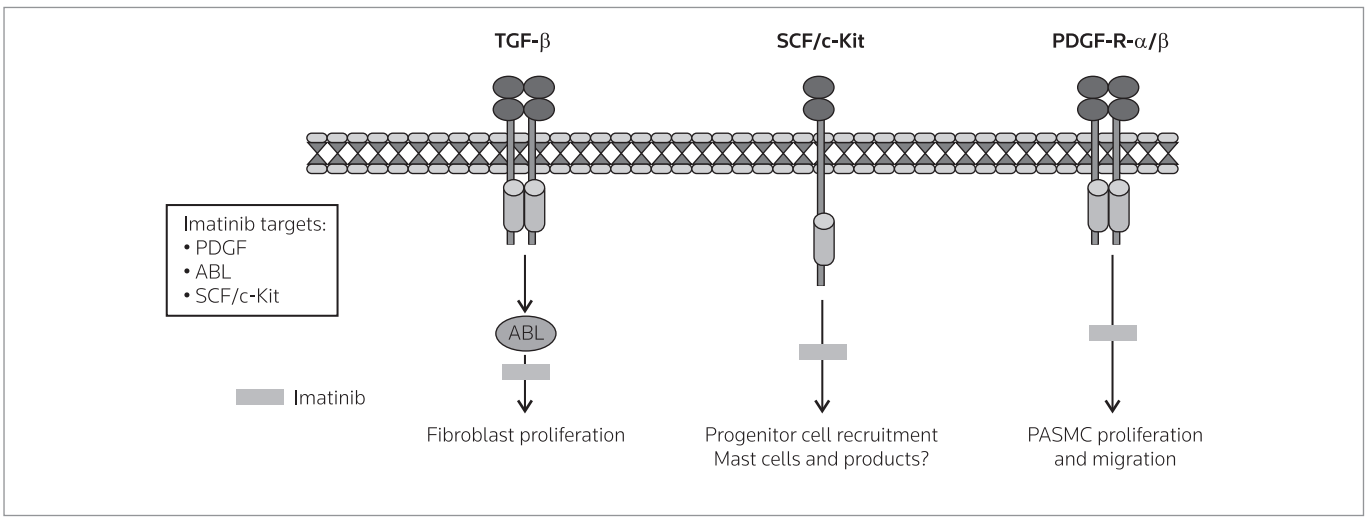

Figure 8. Potential mechanistic pathways for efficacy of imatinib and nilotinib in pulmonary arterial hypertension. SCF, stem cell factor; PASMC, pulmonary artery smooth muscle cell.

walk test. An extension study of treated patients indicates that the treatment stabilizes functional pathology for up to 2 years. Further preclinical data were presented suggesting that nilotinib (Tasigna®) may have improved efficacy over imatinib at lower doses (Fig. 7).

The mechanistic basis for the efficacy of these two kinase inhibitors was discussed, and centered on pathways involving the kinases tyrosine-protein kinase ABL1, stem cell factor/tyrosine-protein kinase Kit and platelet-derived growth factor (Fig. 8). Presently, it is not clear whether inhibition by a single pathway is key for the activity of both compounds, or inhibition of multiple kinases and exertion of polypharmacology effects are important.

UNDERSTANDING AND COMBATING RESISTANCE TO MEK INHIBITION

The next speaker was Dr. Paul Smith (AstraZeneca, U.K.), who presented "Understanding and Combating Resistance to MEK inhibition". Activation of membrane receptor tyrosine kinases (RTKs) by growth factors triggers a myriad of signaling cascades utilizing small GTPases and Raf kinases that converge on phosphorylation of mitogen-activated protein kinase (MAPK) kinase (MEK; also known as extracellular signal-regulated kinase [ERK] kinase). Activated MEK subsequently phosphorylates ERK, which duly activates pivotal transcription factors that feed into the gene tran-

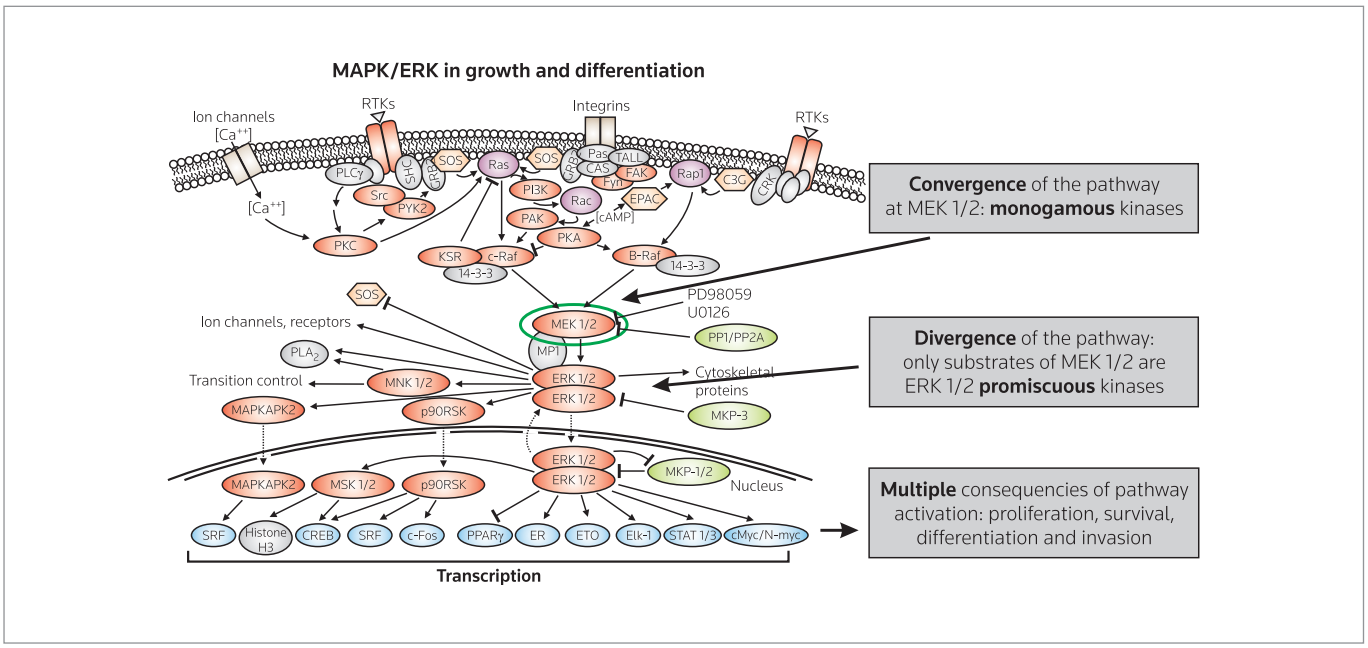

Figure 9. MAPK pathway involving MEK.

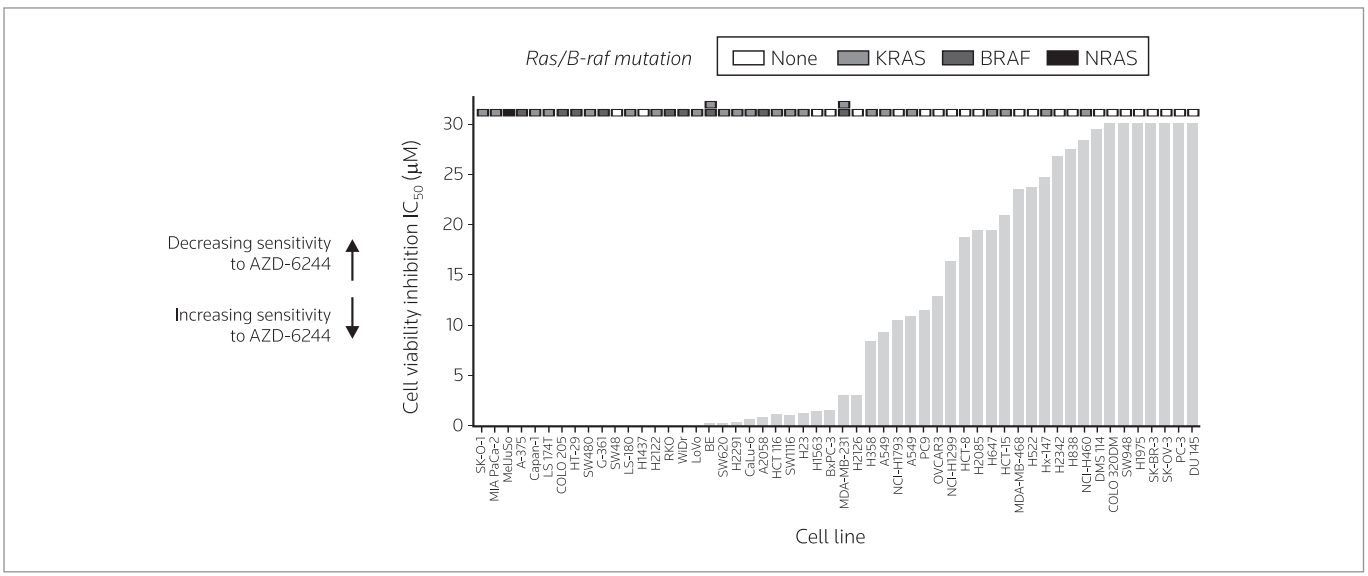

Figure 10. Cell viability inhibition (IC_{50}) after treatment with selumetinib (AZD-6244) in a pan-tumor panel of cell lines and sensitivity of individual cell lines.

scription and regulation of proteins intimately linked with control of cellular proliferation, differentiation and survival (21) (Fig. 9).

Aberrant activation of the above MAPK pathway is found in many cancers, and as a consequence, the development of potent and selective MEK inhibitors has been vigorously pursued as an opportunity to combat this disease. Tumor formation appears to be linked to genetic mutation and/or overexpression of several downstream small GTPases, such as Ras and KRas, or the protein kinase B-raf (22-25). In preclinical models there is a strong link between the presence of a *BRAF* or *KRAS* gene mutation and sensitivity to cell viability inhibition (26). Using cell line panels comprising several tumor types (including colorectal, non-small cell lung cancer, pancreatic and melanoma targets, etc.) it has been found that the combined BRAF and *KRAS* mutation status is a good predictor of sensitivity for MEK inhibitors, such as AZD-6244 (selumetinib) (Fig. 10).

Use of biomarkers (e.g., EKK, Ki-67, etc.) and a coherent strategy in order to assess target inhibition in phase I clinical studies and beyond are essential for successful drug development. The response in terms of functional activation of the target pathway or process, i.e., functional activation of the MEK pathway in terms of RTK signaling and mutational activation, and an absence of resistance factors (baseline or adaptive), compensatory RTK signaling (e.g., insulin-like growth factor receptor, other Ras effectors) and phosphatidylinositol 3-kinase (PI3K)/Akt pathway activation was discussed. It was emphasized that protein markers do not give an absolute prediction of response, since levels of activated MEK and ERK protein vary widely in patients, and therefore may not be obviously related to potency of the drug. In addition, biological markers are also difficult to measure in clinical samples due to their instability. The pharmacodynamics/efficacy relationship may be predictive of resistance, but is not absolute and is difficult to quantify.

Two basic routes to pathway activation (output) signatures were highlighted, one of which involved measuring messenger RNA (mRNA) profiles in the presence and absence of drug, and examination of drug-dependent gene expression changes in sensitive, but not resistant, cell lines (27, 28). This is probably better adapted to identification of drug target output, although it could identify adaptive activation of resistance mechanisms. The other route involved measuring baseline mRNA profiles, which is linked to drug sensitivity, and identification of correlated gene networks that show predicted expression with response or overlay to known biology (Fig. 11). It was concluded that sensitive cell lines exhibited a higher MEK output and lower Ras resistance expression signatures, and that clinical translation is enhanced if gene expression signatures are weighted relative to tumor type preferences seen in vitro.

In terms of identifying combination options and model selection, gene signatures can be applied to generate hypotheses for drug combinations. The use of MEK markers as a target to enhance pathway block (break feedback) and resistance markers as inhibitory compensatory signals, or to enable selection of appropriate cell lines, was recommended. Examples for MEK inhibitor combinations for dual targeting of the Raf/MEK/ERK pathway (B-raf inhibitors, heat shock protein 90 [HSP90] antagonists and RTK inhibitors) or for compensatory pathway inhibition (mTOR, Akt, PI3K inhibitors, RTK inhibitors [e.g., IGF-I receptor antagonists], HSP90 or relevant clients [e.g., cyclin-dependent kinase 4]) were presented. Although dual pathway inhibition can be effective, it does depend upon genetic context and inhibitor characteristics. The current generation of B-raf inhibitors binds to Raf and inhibits the pathway in wild-type B-raf cells, leading to antagonism in wild-type B-raf cells. Pan Raf inhibitors were shown to be effective across genotypes, whereas "selective" mutant B-raf pathway inhibitors were effective only in mutant B-raf cells (Fig. 12).

MEK inhibitors were also shown to enhance other therapeutic agents, including antiangiogenics, anti-invasives antimitotics/cell cycle-targeted and DNA-damaging agents. For instance, the mTOR inhibitor AZD-8055 was shown to enhance the antitumor effects of selumetinib in a number of in vivo cancer models, which was supported by pharmacodynamic effects, including suppression of compensatory signaling of p-Akt, induction of apoptosis and modulation of proapoptotic proteins.

In summary: 1) biological response is determined by drug target/pathway activation and the absence of resistance factors; 2) transcriptome networks can measure pathway activation/output and may have advantages over genetic or protein markers in predicting drug response; 3) pathway addiction can condition resistance mechanisms to target the drug target/pathway and 4) the efficacy of MEK inhibitors can potentially be enhanced in combination with numerous agents with differing mechanisms of action.

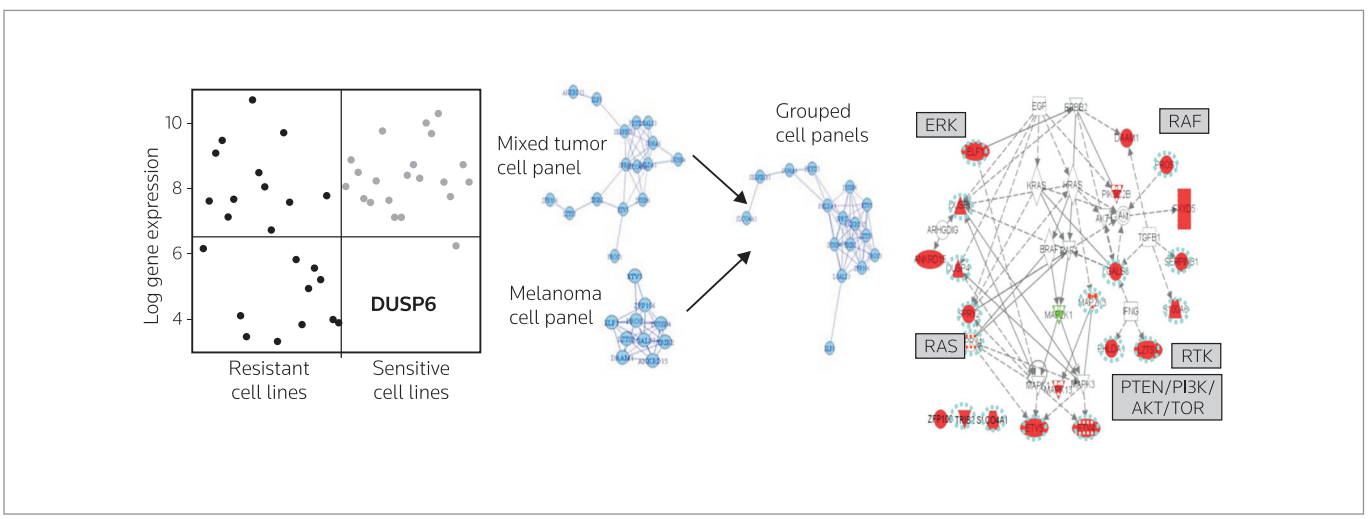

Figure 11. Genes representative of the same biological mechanism show a similar coordinated pattern of expression.

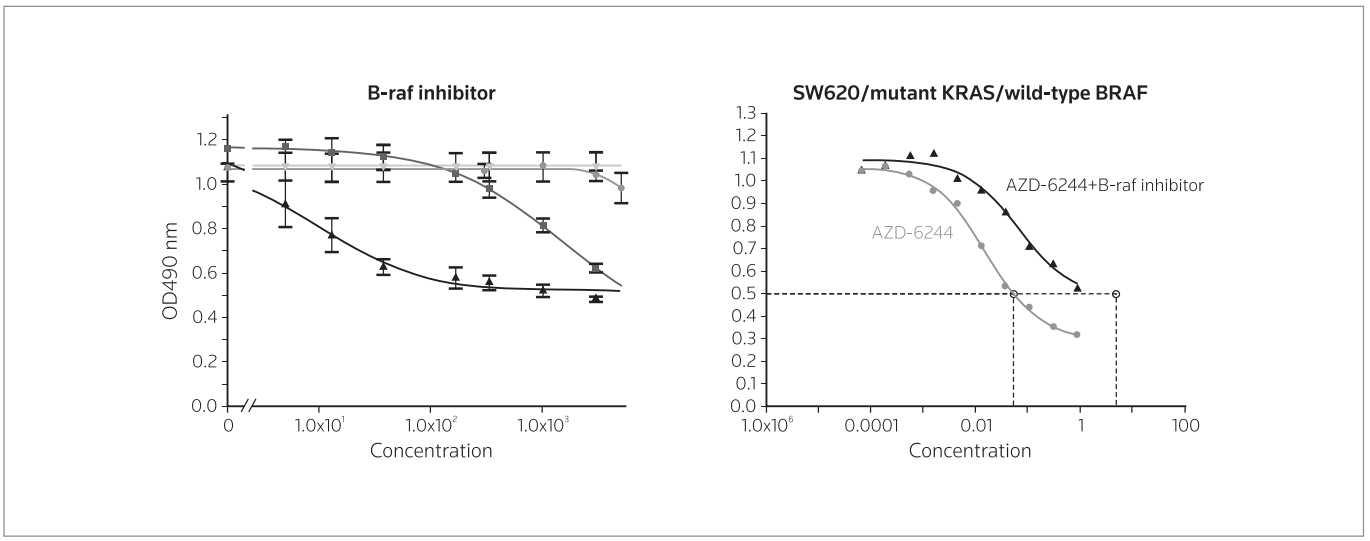

Figure 12. Combinations of a MEK inhibitor plus a Raf inhibitor (potential added benefits of normal tissue antagonism).

RAPID GENERATION OF ALK-5 INHIBITORS FOR ONCOLOGY VIA HYBRIDIZATION

The final speaker was Dr. Frederick Goldberg (AstraZeneca, U.K.). Initially, the LEGO® building approach to mapping the activin receptor-like kinase ALK-5 binding site was presented. In the search for quality lead compounds, a strategy focused on target- or pharmacophore-based approaches ("franchises"). To this end, potential pharmacophores, constructed from analysis of competitor ALK-5 patents and prioritized through docking scores, were identified as the basis for chemical synthesis (Fig. 13).

A number of ALK-5 competitor scaffolds share similar pharmacophores and were used to identify hit compounds (Fig. 14). This resulted in two main chemical series being chosen for progression, and only "hits" with < 1 μM activity were progressed further for profiling/SAR (Fig. 15).

Further hit-to-lead progression resulted in identification of a lead compound with good physicochemical and drug metabolism/pharmacokinetic properties (Fig. 16).

In summary: 1) designed hybrids and directed screen approaches were used to generate hits; 2) two hits were selected for optimiza-

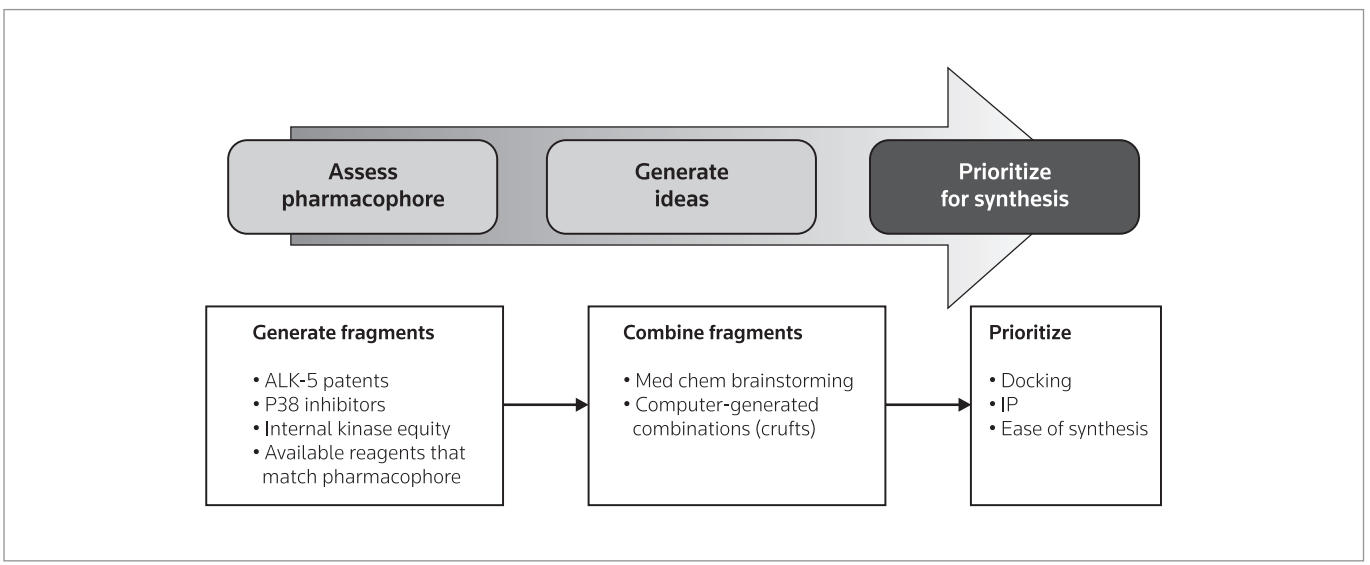

Figure 13. Strategy for rapid generation of lead compounds for ALK-5 inhibitors.

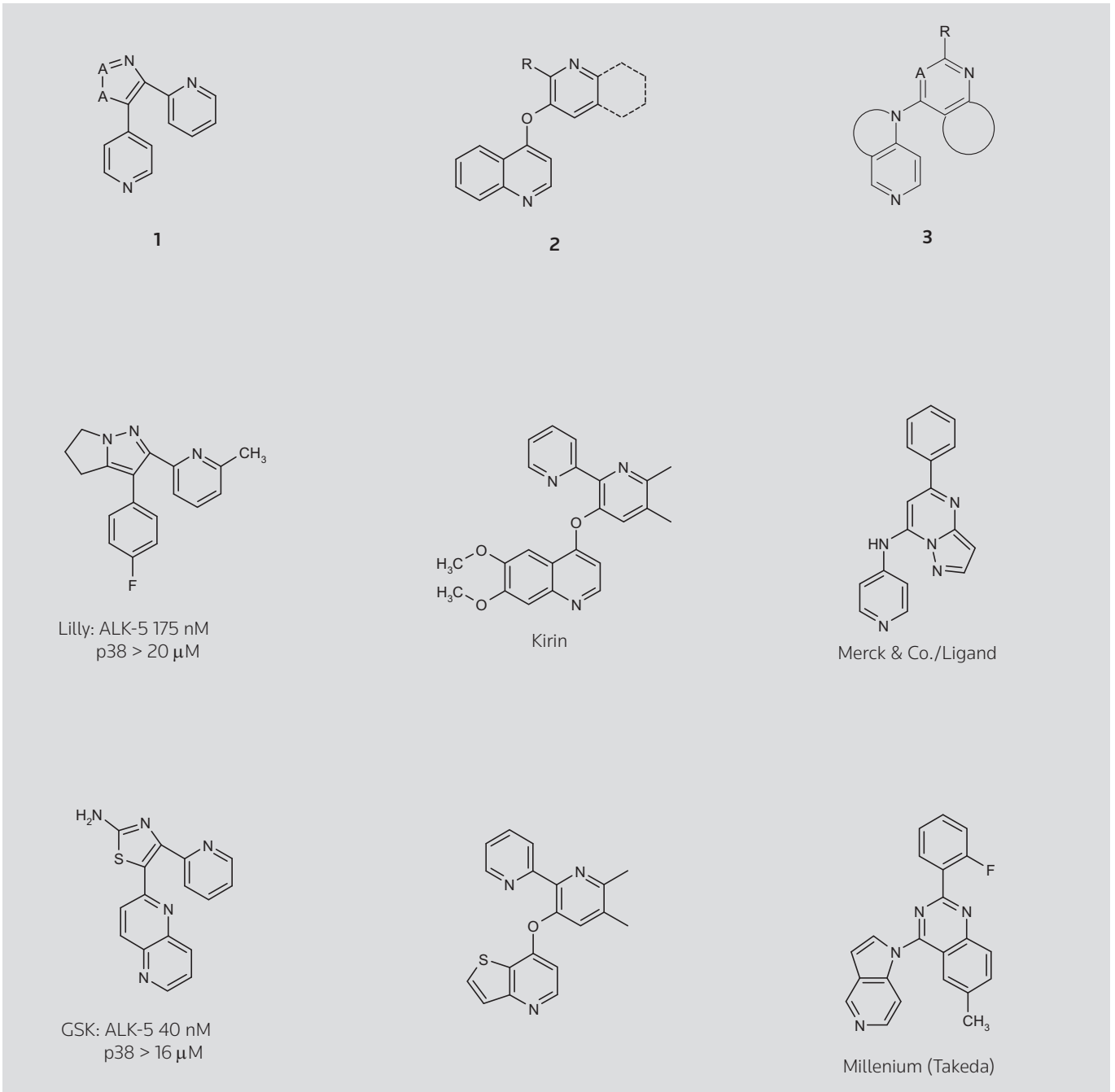

Figure 14. Competitor scaffolds used for ALK-5 inhibitors.

tion from designed hybrid approach; 3) both series provided simple tool compounds to explore disease linkage; 4) series 1 hit-to-lead resulted in two rounds of optimization; e) series 2 initial hit resulted in a suitable probe compound, with no optimization required (structure not disclosed).

Dr. Goldberg also described a franchise for "pit-binding" kinase inhibitors, where a lead chemical series can be developed using an underexplored pharmacophore, as shown in Figure 17.

Frequency-of-group analysis on all patents with appropriate substructures and assessment of the likely binding mode by eye was conducted, generating 896 library ideas (Fig. 19). Only "pit-binding" structures were selected (66 scaffolds), based on synthetic tractability related to known intermediates or having synthetic precedent in-house, in addition to portfolio prioritization. The resulting franchise has benefited up to six active projects within the AstraZeneca portfolio. Going forward, a focus on solving synthetic challenges, so

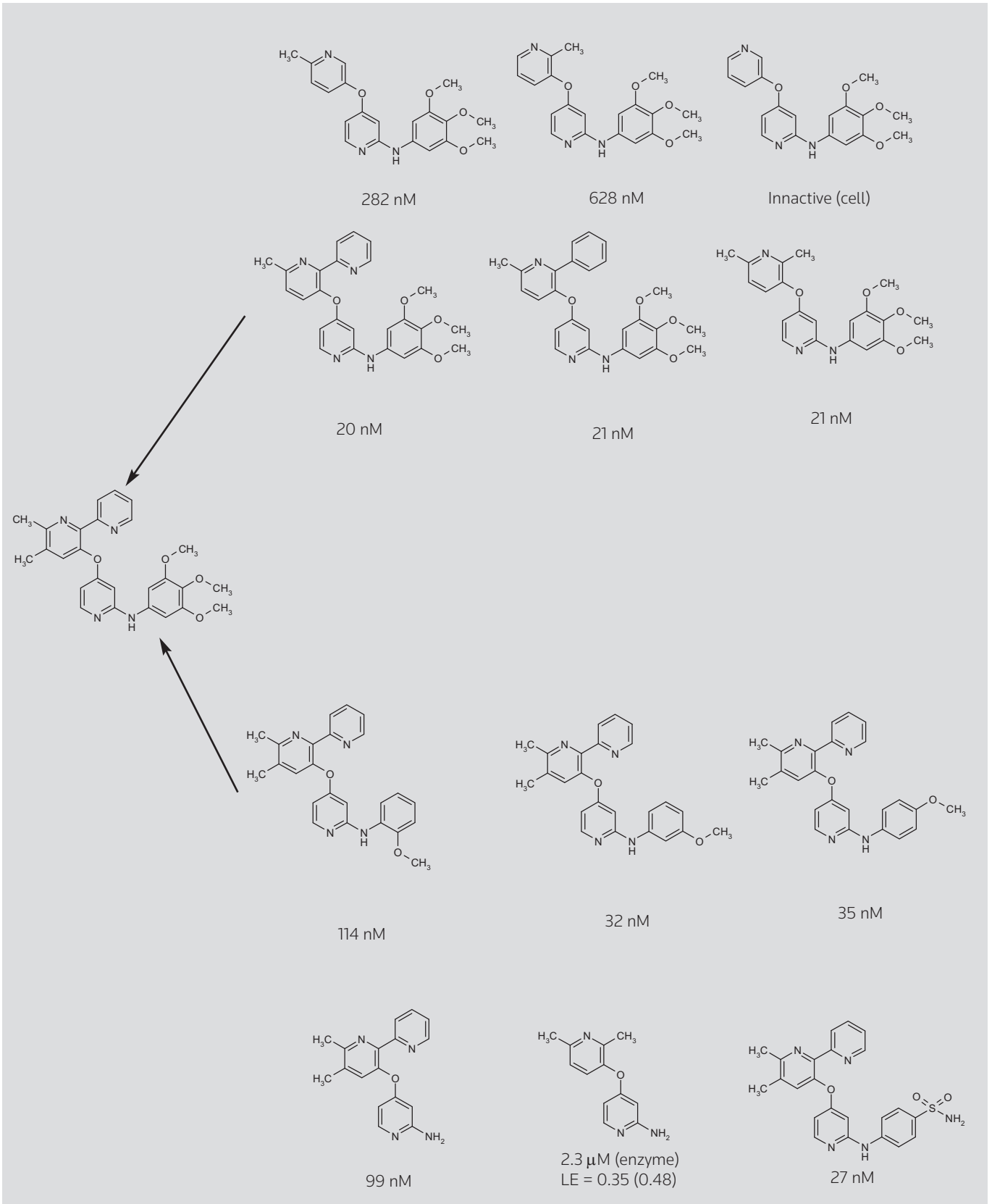

Figure 15. Hit ALK-5 inhibitors identified.

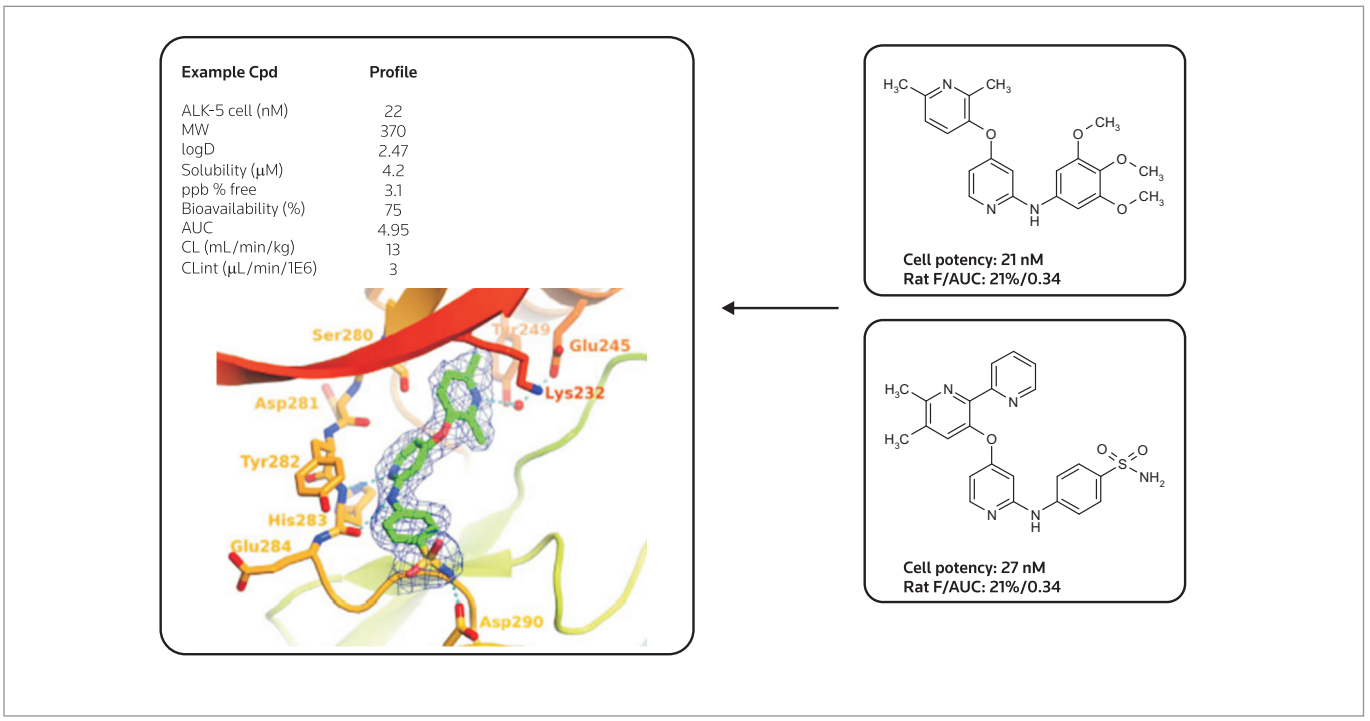

Figure 16. ALK-5 inhibitor lead compound.

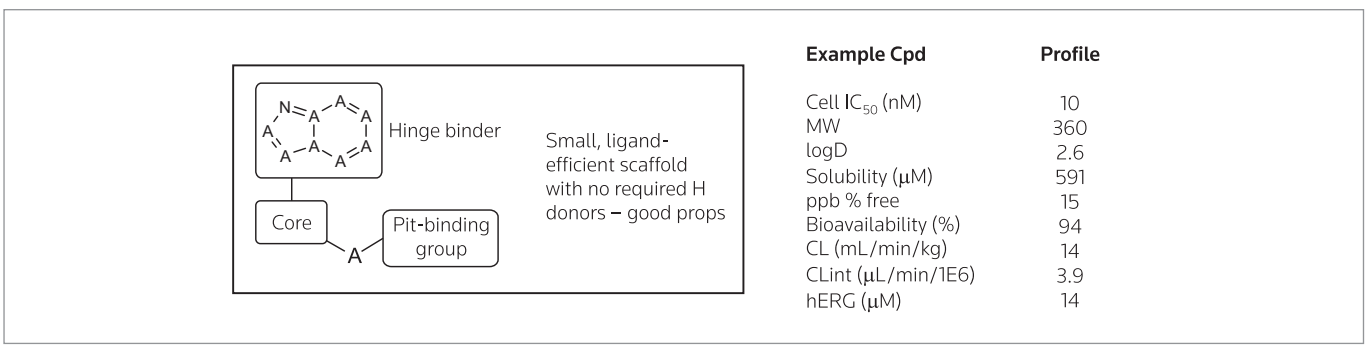

Figure 17. Creating a "franchise" for "pit-binding" kinase inhibitors.

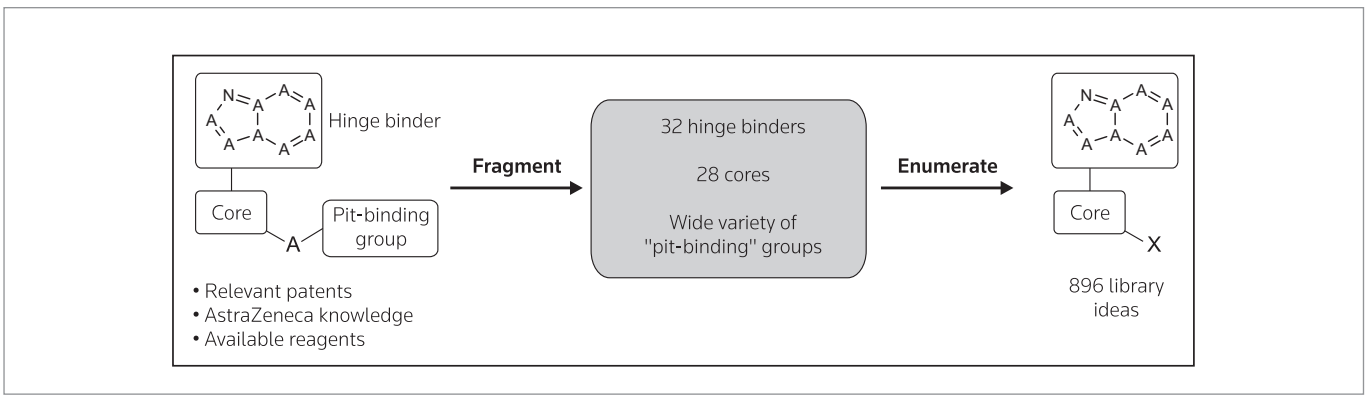

Figure 18. Idea generation for "pit-binding franchise."

allowing an increased reduction of ideas to practice, will further enhance the methodology.

DISCLOSURES

J. Allen is an employee of AstraZeneca. S. Collingwood is an employee of Novartis Institutes for Biomedical Research. A.J. Ratcliffe is an employee of Cellzome Ltd.

REFERENCES

1. Fedorov, O., Muller, S., Knapp, S. *The (un)targeted cancer kinome.* Nat Chem Biol 2010, 6(3): 166-9.
2. Barbie, D.A., Tamayo, P., Boehm, J.S. et al. *Systematic RNA interference reveals that oncogenic KRAS-driven cancers require TBK1.* Nature 2009, 462(7269): 108-12.
3. Sheng, Z., Li, L., Zhu, L.J. et al. *A genome-wide RNA interference screen reveals an essential CREB3L2-ATF5-MCL1 survival pathway in malignant glioma with therapeutic implications.* Nat Med 2010, 16(6): 671-7.
4. Simard, J.R., Kluter, S., Grutter, C., Getlik, M., Rabiller, M., Rode, H.B., Rauh, D. *A new screening assay for allosteric inhibitors of cSrc.* Nat Chem Biol 2009, 5(6): 394-6.
5. Morphy, R. *Selectively nonselective kinase inhibition: Striking the right balance.* J Med Chem 2010, 53(4): 1413-37.
6. Leroy, D., Doerig, C. *Drugging the Plasmodium kinome: The benefits of academia-industry synergy.* Trends Pharmacol Sci 2008, 29(5): 241-9.
7. Zuccotto, F., Ardini, E., Casale, E., Angiolini, M. *Through the "gatekeeper door": Exploiting the active kinase conformation.* J Med Chem 2010, 53(7): 2681-94.
8. Liao, J.J. *Molecular recognition of protein kinase binding pockets for design of potent and selective kinase inhibitors.* J Med Chem 2007, 50(3): 409-24.
9. Sielecki, T.M., Johnson, T.L., Liu, J. et al. *Quinazolines as cyclin dependent kinase inhibitors.* Bioorg Med Chem Lett 2001, 11(9): 1157-60.
10. Berger, D.M., Torres, N., Dutia, M. et al. *Non-hinge-binding pyrazolo[1,5-a]pyrimidines as potent B-Raf kinase inhibitors.* Bioorg Med Chem Lett 2009, 19(23): 6519-23.
11. Kornev, A.P., Taylor, S.S. *Defining the conserved internal architecture of a protein kinase.* Biochim Biophys Acta 2010, 1804(3): 440-4.
12. Greene, N., Aleo, M.D., Louise-May, S., Price, D.A., Will, Y. *Using an in vitro cytotoxicity assay to aid in compound selection for in vivo safety studies.* Bioorg Med Chem Lett 2010, 20(17): 5308-12.
13. Pesu, M., Candotti, F., Husa, M., Hofmann, S.R., Notarangelo, L.D., O'Shea, J.J. *Jak3, severe combined immunodeficiency, and a new class of immunosuppressive drugs.* Immunol Rev 2005, 203: 127-42.
14. Changelian, P.S., Flanagan, M.E., Ball, D.J. et al. *Prevention of organ allograft rejection by a specific Janus kinase 3 inhibitor.* Science 2003, 302(5646): 875-8.
15. Milici, A.J., Kudlacz, E.M., Audoly, L., Zwillich, S., Changelian, P. *Cartilage preservation by inhibition of Janus kinase 3 in two rodent models of rheumatoid arthritis.* Arthritis Res Ther 2008, 10(1): R14.
16. Karaman, M.W., Herrgard, S., Treiber, D.K. et al. *A quantitative analysis of kinase inhibitor selectivity.* Nat Biotechnol 2008, 26(1): 127-32.
17. Ghofrani, H.A., Seeger, W., Grimminger, F. *Imatinib for the treatment of pulmonary arterial hypertension.* N Engl J Med 2005, 353(13): 1412-3.
18. Patterson, K.C., Weissmann, A., Ahmadi, T., Farber, H.W. *Imatinib mesylate in the treatment of refractory idiopathic pulmonary arterial hypertension.* Ann Intern Med 2006, 145(2): 152-3.
19. Souza, R., Sitbon, O., Parent, F., Simonneau, G., Humbert, M. *Long term imatinib treatment in pulmonary arterial hypertension.* Thorax 2006, 61(8): 736.
20. Stenmark, K.R., Meyrick, B., Galie, N., Mooi, W.J., McMurtry, I.F. *Animal models of pulmonary arterial hypertension: The hope for etiological discovery and pharmacological cure.* Am J Physiol Lung Cell Mol Physiol 2009, 297(6): L1013-32.
21. Crews, C.M., Alessandrini, A., Erikson, R.L. *The primary structure of MEK, a protein kinase that phosphorylates the ERK gene product.* Science 1992, 258(5081): 478-80.
22. Deramaudt, T., Rustgi, A.K. *Mutant KRAS in the initiation of pancreatic cancer.* Biochim Biophys Acta 2005, 1756(2): 97-101.
23. Kohl, N.E., Mosser, S.D., deSolms, S.J. et al. *Selective inhibition of ras-dependent transformation by a farnesyltransferase inhibitor.* Science 1993, 260(5116): 1934-7.
24. Eberhard, D.A., Johnson, B.E., Amler, L.C. et al. *Mutations in the epidermal growth factor receptor and in KRAS are predictive and prognostic indicators in patients with non-small-cell lung cancer treated with chemotherapy alone and in combination with erlotinib.* J Clin Oncol 2005, 23(25): 5900-9.
25. Davies, H., Bignell, G.R., Cox, C. et al. *Mutations of the BRAF gene in human cancer.* Nature 2002, 417(6892): 949-54.
26. Solit, D.B., Garraway, L.A., Pratilas, C.A. et al. *BRAF mutation predicts sensitivity to MEK inhibition.* Nature 2006, 439(7074): 358-62.
27. Pratilas, C.A., Taylor, B.S., Ye, Q., Viale, A., Sander, C., Solit, D.B., Rosen, N. *(V600E)BRAF is associated with disabled feedback inhibition of RAF-MEK signaling and elevated transcriptional output of the pathway.* Proc Natl Acad Sci U S A 2009, 106(11): 4519-24.
28. Dry, J.R., Pavey, S., Pratilas, C.A. et al. *Transcriptional pathway signatures predict MEK addiction and response to selumetinib (AZD6244).* Cancer Res 2010, 70(6): 2264-73.