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MEETING REPORT

RESPIRATORY DRUG DISCOVERY, CURRENT DEVELOPMENTS AND FUTURE CHALLENGES

HIGHLIGHTS FROM THE SOCIETY OF MEDICINES RESEARCH SYMPOSIUM, HELD ON JUNE 14TH, 2012 – HORSHAM, UK

S.P. Collingwood[1], D. Coe[2], D. Pryde[3] and R. Lock[1]

[1]Novartis Institutes for Biomedical Research, UK; [2]GlaxoSmithKline, UK; [3]Pfizer, UK

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SUMMARY

Chronic respiratory diseases such as asthma, chronic obstructive pulmonary disease (COPD), allergic rhinitis and cystic fibrosis remain a considerable healthcare burden with a significant unmet medical need. Acute lower respiratory tract infections are the leading cause of death among all infectious diseases. Asthma and COPD in particular are becoming more common, and thus the demand for continued advancements in respiratory drug discovery has grown significantly in recent years. It is estimated that the commercial respiratory pharmaceutical market will grow to over USD 24 billion by 2015. A previous SMR meeting in 2007 included the current thinking, at the time, in the area of airways drug delivery, and the challenges and developments in the classic drugs for respiratory diseases which form the mainstay of inhaled treatments, including β_2-adrenoceptor agonists, glucocorticoids and muscarinic receptor antagonists. This symposium brought together international experts from the pharmaceutical industry to review the latest therapeutic developments and to discuss the challenges in both the discovery and clinical development of novel therapeutics for lung diseases. Topics included the translational medicine development challenges for novel respiratory therapies, assessing the pharmacokinetics and local toxicity of inhaled therapies, strategies to enhance duration of action in the lung, and novel approaches to treat respiratory tract infections. This symposium also featured a selection of case histories for both small-molecule and biological approaches.

Key words: Respiratory disorders – Translational medicine – Inhaled therapies – Nanobodies – Histamine receptor antagonists – PI3Kdelta inhibitors

Correspondence: Society for Medicines Research - Secretariat, 840 Melton Rd., Thurmaston, Leicester LE4 8BN, UK. E-mail: secretariat@smr.org.uk.

TRANSLATIONAL MEDICINE CHALLENGES IN THE DEVELOPMENT OF NOVEL THERAPEUTICS FOR THE TREATMENT OF LUNG DISEASES

Professor Robert Strieter (Novartis Institutes for Biomedical Research, U.S.) opened the meeting with a discussion of the challenges that translational medicine faces in the design of proof-of-concept studies in respiratory diseases. Currently, the assessment of clinical severity of respiratory diseases is often used as the major inclusion criteria for any respiratory study. This clinical status is often defined by physiological measures or patient-reported outcomes. There is a risk that neither of these approaches adequately represents "activity of disease" on a cellular or molecular pathway level. An understanding of such pathways is critical when considering a specific molecular target for a novel therapeutic intervention. The

situation is further complicated by the lack of understanding of the natural history (i.e., early, intermediate or end stage of the disease) and the magnitude of patient heterogeneity related to "activity of disease". An additional issue is the sometimes poor predictivity of animal models to adequately recapitulate human lung disease. To reduce the level of attrition of novel drugs that could otherwise have a positive impact in a high unmet need respiratory disease, Professor Strieter proposed the development of better clinical study strategies to reduce pitfalls related to these issues.

Airways remodeling is a characteristic feature of asthma, COPD and cystic fibrosis (CF); however, the extent of remodeling is not easily captured by conventional lung function measurements such as forced expiratory volume in one second (FEV1) (1). Remodeling has been shown to be a process that leads to a decrease in the number of small airways in COPD, and this loss of airways is underestimated by changes in FEV1 (2). An example of a patient with severe, persistent asthma defined by the asthma control questionnaire (ACQ) showed essentially normal lung function results: predicted FEV1 81%, forced vital capacity (FVC) 106%, FEV1/FVC 65% and peak expiratory flow (PEF) 95%. However, hyperpolarized He3 magnetic resonance imaging (MRI) could readily identify ventilation heterogeneity. Alternative measures of small airways function, for example, ventilation heterogeneity in convection-dependent airways and ventilation heterogeneity in diffusion-dependent airways determined via the multiple-breath nitrogen washout test, have correlated much more effectively with ACQ (3). Professor Strieter also described work from his own laboratory, where in the rare fibrotic lung disease Hermansky-Pudlak Syndrome 1 (HPS1), fibrocyte levels vary with the presence or absence of pulmonary fibrosis. Such fibrocyte levels may thus be a useful biomarker for pharmacological treatment.

In conclusion, pathway markers, lung imaging and unique pulmonary function test strategies are believed to be needed to better discern "disease activity" and to assess heterogeneity of patient populations. To reduce drug attrition in the clinic, better stratification and enrichment of the patient population on the basis of disease and target activity at the time of screening for a proof-of-concept (POC) trial are required. To determine a true responder from a non-responder in a POC trial requires biomarker and imaging strategies to enhance, supplement or replace conventional endpoints. Furthermore, improvements in our understanding of disease pathogenesis (i.e., remodeling of the small airways or lung parenchyma) should enhance opportunities to recognize common targets that can have parallel indications for existing compounds.

INHALATION OF DRUGS – LUNG PHARMACOKINETICS AND CONSEQUENCES FOR LOCAL TOXICITY

Rhys Jones from Pfizer (U.S.) discussed the value of understanding inhaled pharmacokinetics (PK) and the challenges and utility of predicting lung concentrations from inhaled systemic PK in order to allow an early assessment of both the safety and efficacy (PK/PD) of new inhaled therapies. Inhaled plasma PK is more routinely assessed in the drug discovery process because of its ease of collection, processing and requirements for fewer animals relative to the determination of lung PK. Rhys described how inhaled plasma PK can underwrite the systemic safety of a drug from a toxicity perspective, can aid in the understanding of general absorption, distribution, metabolism, and excretion (ADME) principles (when combined with reference i.v. and p.o. PK), and allows a measure of lung absorption rate following drug administration to the lung. In isolation, however, it is typically not informative of lung PK and lung concentrations of a drug. Without an understanding of the principles that drive an inhaled plasma profile, Rhys described how it can be difficult to differentiate between compounds that show intrinsically slow lung absorption and those that show slow dissolution in lung lining fluid. Accumulation of undissolved solid material in the lung through slow dissolution has been linked to lung toxicity resulting from stimulation of excessive foamy macrophage localization.

Knowledge of a drug's likely dissolution rate and lung particulate burden, as predicted from plasma PK, could enable early assessment of its potential for lung toxicity. Threshold values for lung toxicity have been published based on the lung burden of a drug (4, 5). Values in the range of 0.1-1 mg drug/g lung have been associated with non-adverse adaptive lung changes, whereas values > 1 mg drug/g lung have been associated with adverse lung changes. A recent publication co-authored by Rhys describes predicted lung burdens in the rat based on inhaled rat toxicology data for four Pfizer inhaled compounds that have reached the clinic. Findings supported the aforementioned threshold values and indicated that predicted lung burdens in excess of 1 mg drug/g lung were associated with adverse changes consistent with those described in the literature for inert insoluble particles (6).

There is also a desire to estimate lung concentrations and link these to a drug's pharmacodynamic effect (PK/PD). Whereas PK measures the fate of an inhaled drug molecule, it is PD that defines the quantitative relationship between drug concentration and pharmacological response. Estimating the (free) lung concentrations over time would be the first step to predicting PK/PD in the biophase of interest. The emergence of physiologically based pharmacokinetic modeling (PBPK) has allowed the potential to estimate lung concentrations and link these to pharmacodynamic effects to become closer to reality. Rhys described a number of commercially available tools (GastroPlus™ and Simcyp), but focused on the application of PulmoSim, an in-house PBPK modeling tool developed at Pfizer.

PulmoSim is based on a system of 15 differential equations which describe: drug dissolution, absorption across lung epithelium, lung tissue binding, and systemic distribution and elimination processes. Validation of PulmoSim as a PBPK tool is based on preclinical data from 25 compounds with varying physicochemical properties. Rhys presented its utility in predicting the human PK for inhaled drugs from orotracheal PK data in rats (7). One challenge they faced was validation of the estimated lung concentrations and how to relate them to regional lung concentrations (i.e., lower and upper airways). Estimation of rat lung concentrations following intratracheal solution administration was partially successful with PulmoSim; however, lung concentrations at later time points in a concentration–time profile were always underpredicted as compared to measured. Reasons for this were stated as due to artifacts of intratracheal instillation and/or processes such as lysosomal trapping in the lung. The apparent higher concentrations measured in the lung were not thought to be PD-relevant, as supported by in-house receptor occupancy data, which indicated that the measured lung levels were an

overestimate of drug levels likely available to engage the target. Greater success was, however, achieved in the prediction of human inhaled lung concentrations for fluticasone and budesonide, as compared to published lung data from the literature. Rhys suggested that one reason for this success could be due to the human inhaled dose of these corticosteroids (low-mg range) being much smaller relative to the size of the human lung. Rhys went on to present how PulmoSim has been used retrospectively at Pfizer to predict clinical outcome (dose and target modulation) for their muscarinic acetylcholine M_3 receptor antagonist program, as well as other inhaled programs that have shown positive and negative clinical outcomes.

Rhys closed by highlighting that inhaled drug discovery (PK/PD, PBPK and the use of novel biomarkers) remains challenging as compared to the oral route, but that the ability to predict inhaled efficacy based on lung concentrations is becoming more of a reality.

PROLONGED TARGET BINDING AND REBINDING AS MECHANISMS TO ENHANCE DURATION OF ACTION IN THE LUNG

Dr. Steven Charlton from the Novartis Institutes for Biomedical Research (U.K.) presented a discussion of some of the key processes postulated to account for the utility of inhaled drugs for respiratory diseases. It is well accepted that inhalational delivery of pharmaceuticals directly into the lung allows for the possibility of high local drug concentrations to be achieved in the target organ with relatively low doses, hence reducing the systemic burden of drug and thus minimizing systemic side effects. One disadvantage of this approach is that the drug may be absorbed rapidly from the lung, so the high local lung concentrations achieved are only transient, either negating the benefit of topical delivery or increasing the frequency of dosing. In order to overcome this, a number of strategies have been explored to enhance the local retention of drug at the site of action. Dr. Charlton discussed several processes that might influence the lung duration of action of inhaled therapeutics.

Firstly, for a drug substance formulated as a dry powder, the drug substance dissolution rate may be influenced by the particle size, the nature of crystalline form and the choice of salt/co-crystal former. Slower dissolution might be anticipated to increase the duration of action, provided this does not lead to significant accumulation of solid material in the lung, which has been linked to lung toxicity.

Secondly, optimizing the kinetics of dissociation from the target can be beneficial and is cited as the mechanism behind the once-daily duration of action of the inhaled muscarinic antagonist tiotropium (8). However, new studies of the binding kinetics of these agents, under arguably more physiological conditions , suggest that the dissociation rates of these agents from the M_3 receptor are much faster (approximately 10-fold faster dissociation) than originally thought (9). It was proposed that the once-daily clinical duration of action of tiotropium and glycopyrrolate was therefore not solely due to their slow dissociation from the M_3 receptor.

Thirdly, the long-acting β_2-adrenoceptor agonists do not exhibit slow dissociation from the receptor, but instead are believed to utilize high affinity for target tissue to provide a local depot from which the drug can bind even after the majority of the dose is washed away. The human dosing interval for these agents shows a good correlation with their affinity for artificial membrane and washout from cells. However, these studies have not readily demonstrated a difference between the twice-daily inhaled agent salmeterol and the once-daily agent indacaterol.

Dr. Charlton then discussed an extension of the latter two principles to the concept of drug "rebinding", wherein the drug can consecutively bind to the same target and/or nearby targets, even when the concentration in the bulk phase has dropped to insignificant levels (10). Experiments in CHO cells expressing the β_2-adrenoceptor with tritiated indacaterol demonstrated that, despite an 80-fold dilution in bulk phase, only between 3- and 10-fold reductions in local concentrations of indacaterol were induced by washing protocols. This phenomenon may be further enhanced by the microanatomic properties of many effect compartments (such as neuromuscular junctions) that likely hinder the diffusion of free drug molecules away from their target. One significant practical issue in performing cellular washout studies is that many compounds contain lipophilic structural components that can drive binding to the plastics used in such experiments. Dr. Charlton described an elegant protocol to help alleviate this problem. Firstly, compounds are incubated in polypropylene tubes with cells that do not express the target receptor. The wash step is then performed, followed by bulk transfer of these cells to a new tube containing cells expressing the receptor of interest, where the functional response is then measured. Clearly, for the compounds of interest to demonstrate activity, they must equilibrate between the two sources of tissue. Using such a protocol, it proved possible to monitor a prolonged duration of action of agents such as indacaterol.

Finally, Dr. Charlton discussed the potential for variable affinity for lipid rafts to be an influencing factor in drug functional duration of action. Lipid rafts have a higher concentration of receptors and often form bowl-like structures, both promoting rebinding. Additionally, lipid constituents differ between tissue type, the implication being that some ligands may preferentially bind to rafts over other types of membrane. In this instance, comparisons of indacaterol and salmeterol demonstrated a twofold higher affinity of indacaterol for lipid rafts (derived from P388 cells), an observation which might help explain its longer clinical duration of action following inhalation in the clinic (11).

ALX-0171: A HIGHLY POTENT NANOBODY AS INHALATION TREATMENT FOR RESPIRATORY SYNCYTIAL VIRUS INFECTION

Nanobodies are therapeutic proteins based on the smallest functional fragments of naturally occurring heavy chain-only antibodies, typically around 10% of the size of traditional antibodies. Due to their unique nature, they can be formatted to achieve specific features, such as high potency, while retaining characteristic physicochemical properties allowing, for example, direct delivery to the airways by nebulization.

Dr. Erik Depla (Ablynx, Belgium) discussed the Ablynx respiratory syncytial virus (RSV) neutralizing nanobodies produced against several different antigenic sites through immunization in the llama. Upon antibody selection and formatting into trivalent nanobodies, consisting of either three identical building blocks (one epitope) or two different building blocks (different epitopes), in vitro potency against both RSV serotypes increased some 2,000- to 7,000-fold

relative to the monomeric congener. Finally, ALX-0171, consisting of three identical building blocks, was selected as the lead nanobody for preclinical development. ALX-0171 is a 42-kD molecule that targets the RSV F-protein.

The antiviral activity of ALX-0171 was compared with that of palivizumab at 40 μg/mL in a plaque reduction assay with 61 recently isolated RSV clinical strains. ALX-0171 reduced viral replication below the limit of detection more efficiently than palivizumab (84% of strains versus 20%).

ALX-0171 formulated at 50 mg/mL was readily nebulized using a vibrating mesh nebulizer, without affecting potency and with only minor product quality changes. Delivered as a nebulized drug, ALX-0171 was shown to be effective in vivo in a therapeutic RSV cotton rat model. A 2-week repeated-dose toxicity study by nebulization was conducted in rats, achieving maximal feasible dose levels without dose-limiting toxicity. Immunogenicity, as measured in plasma, appeared not to be enhanced compared to intravenous administration. A phase I clinical trial commenced in December 2011 to assess tolerability and safety in man.

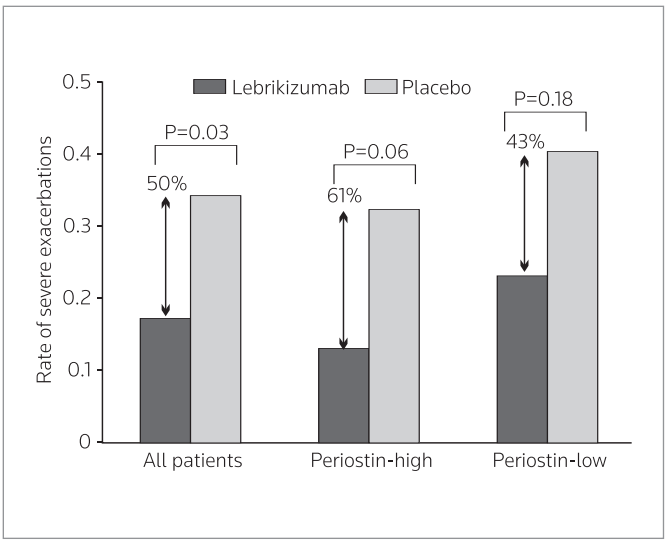


Figure 1. Severe exacerbation rates at 32 weeks – all patients and subgroups stratified by periostin serum levels.

TREATMENT OF ASTHMATIC ADULTS WITH THE IL-13 ANTIBODY LEBRIKIZUMAB

Prof. Dr. Stephan Korom (F. Hoffmann-La Roche, Switzerland) presented the results of a randomized, double-blind, placebo-controlled study of lebrikizumab, a humanized monoclonal antibody to IL-13, in adults with asthma that was not adequately controlled despite inhaled corticosteroid therapy (12). IL-13 plays a pivotal role in the pathogenesis of T-helper cell type 2 (Th2)-driven asthma by mediating: 1) B-cell-induced IgE secretion; 2) recruitment and activation of eosinophils; 3) mucus hypersecretion and goblet cell hyperplasia; 4) smooth muscle contraction; and 5) fibroblast-mediated collagen synthesis. Furthermore, IL-13 induces bronchial epithelial cells to basally secrete periostin, a matricellular protein, into the subepithelial matrix, contributing to airways remodeling (13, 14). Therefore, blockade of IL-13 may represent an advance in the treatment of severe asthmatic patients.

This study involved 219 adults over a 24-week treatment period with a subsequent 8-week follow-up phase. Lebrikizumab 250 mg was delivered subcutaneously every 4 weeks during the treatment period. The primary efficacy endpoint for the study was a change in FEV1 from baseline to week 12, with secondary and exploratory outcomes (among others) including the rate of exacerbations through 24 and 32 weeks. Prior to randomization, each patient's status with respect to an IL-13 signature surrogate (Th2 status) was characterized on the basis of a combination of the total serum IgE level and peripheral blood eosinophil count: high Th2 was defined as a total IgE level of > 100 IU/mL and an eosinophil count ≥ 0.14 x 10^9 cells/L, and low Th2 was defined as a total IgE level of ≤ 100 IU/mL and eosinophil count < 0.14 x 10^9 cells/L (15). At the end of study, prior to unblinding, a predefined assessment of outcomes on the basis of patients' periostin level was conducted (using the median value for all patients to define the cutoff point between the periostin-high vs. the -low subgroup).

The study met its endpoint, showing that lebrikizumab treatment significantly improved FEV1 by 5.5% (P = 0.02) over placebo. When the patients were grouped by periostin level, the most notable finding of the study was that periostin-high subjects displayed the greatest benefit (relative FEV1 improvements periostin-high vs. -low: 8.2% vs. 1.6%; P = 0.03 vs. 0.61). In parallel, at 24 weeks, a trend toward a greater reduction in severe exacerbation rates in the presence of lebrikizumab was noted in periostin-high vs. -low patients (67% vs. 29%), which was sustained at 32 weeks (61% vs. 43%) (Fig. 1). By 32 weeks, in all patients, lebrikizumab treatment was associated with a significant reduction in severe exacerbations versus placebo (P = 0.03). The reported adverse events were similar in both groups, with more musculoskeletal events in the lebrikizumab cohort (13.2% vs. 5.4%; P = 0.045) (16).

These findings underline the efficacy of lebrikizumab treatment in severe asthmatic patients, indicating the pivotal role of IL-13 in Th2-mediated asthma. Furthermore, periostin serum levels significantly correlated with Th2 activity and were predictive of response to lebrikizumab therapy. Ongoing phase III studies in severe asthma will further clarify the clinical value of periostin as a predictor of benefit from lebrikizumab treatment.

DISCOVERY OF POTENT AND SELECTIVE HISTAMINE H_4 RECEPTOR ANTAGONISTS FOR THE TREATMENT OF ALLERGIC DISEASES

Histamine is an important inflammatory mediator that is released in airways during an asthmatic response. The histamine H_4 receptor is an aminergic G protein-coupled receptor which is expressed on immune cells (eosinophils, neutrophils, T cells, mast cells and basophils) and is implicated in inflammatory diseases (asthma, pruritus, inflammatory skin diseases, pain, allergic rhinitis, irritable bowel disease and cancer). Histamine H_4 receptor antagonists were therefore proposed to offer therapeutic benefit in the treatment of these diseases. Dr. Nigel Swain (Pfizer, U.K.) described a medicinal chemistry program that established a tool compound, PF-3893787,

for clinical evaluation in order to build confidence in the rationale for histamine H_4 receptor antagonism for inflammatory conditions.

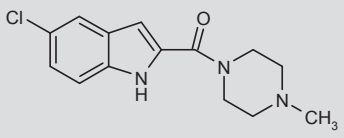
Several novel benzimidazole histamine H_4 receptor antagonists were developed starting from a competitor compound, **JNJ-7777120**, by making structural changes that improved metabolic stability and removed an indole structural alert. Toxicity findings in the rat from the initially discovered benzimidazole series, e.g., **PF-3306138**, switched attention to a series of pyrimidines derived from an in-house high-throughput screening campaign to ultimately identify a triaminopyrimidine development candidate, **PF-3893787**, which showed no adverse toxicity in rats and had a very long predicted half-life in man of approximately 40 hours, and a very low projected dose of approximately 10 mg.

Further in vitro profiling of PF-3893787 showed some interesting data in different species, in that the compound was a full antagonist at the human receptor but an agonist at the mouse receptor, complicating interpretation of animal data, including in vivo toxicity data. The compound was shown to be safe and well tolerated in man at single doses up to 48 mg. Systemic pharmacodynamics of PF-3893787 were assessed ex vivo using imetit-stimulated eosinophil shape change measured by the GAFS flow cytometric assay and demonstrated dose- and time-dependent inhibition at doses > 1 mg, with complete inhibition of the response over the 24-hour period postdose at doses > 12 mg providing validation for the histamine H_4 receptor antagonist hypothesis.

DISCOVERY OF POTENT AND SELECTIVE PI3KDELTA INHIBITORS FOR THE TREATMENT OF RESPIRATORY INDICATIONS

Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit delta isoform (PI3Kdelta) is a lipid kinase, and inhibition of PI3Kdelta should dampen down the inflammatory cascade involved in the asthmatic response through a wide breadth of pharmacology (17). Dr. Nicole Hamblin (GlaxoSmithKline, U.K.) gave a case history of the development of an indazole-containing PI3Kdelta inhibitor with properties suitable for inhaled administration, including moderate solubility.

The lead compound **1** for the medicinal chemistry program was identified by a kinase cross-screen. The initial area explored was the 4-position amide; introduction of aromatic substituents with *ortho* heteroatoms, such as the thiazole, enhanced potency and increased clearance (compound **2** rat blood clearance = 28 mL/min/kg;



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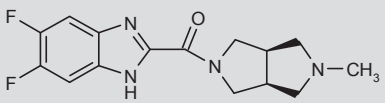

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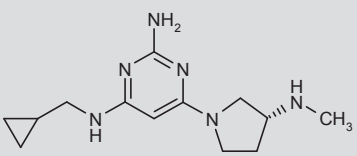

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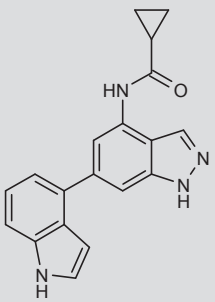

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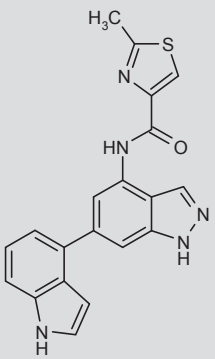

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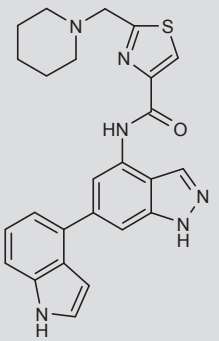
F = 2%). Incorporation of lipophilic amine substituents, e.g., compound **3,** on the thiazole increased both PI3Kdelta potency and selectivity for other PI3K isoforms. The observations were rationalized via docking in a homology model, which showed lipophilic and a cation π interaction between the piperidine and Trp760. The introduction of a basic center also had the beneficial effect of allowing salt formation to aid solubility.



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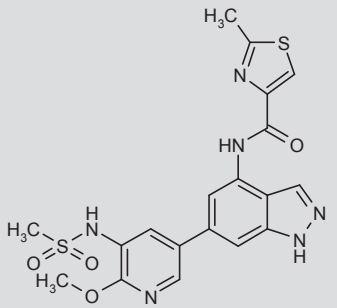
The next iteration of medicinal chemistry described by Dr. Hamblin focused on improving the physicochemical properties of the series by reducing the lipophilicity and the number of aromatic rings. Replacement of the indole motif by a pyridine sulfonamide afforded compound **4,** with comparable potency at PI3Kdelta but reduced isoform selectivity, particularly at PI3Kgamma. A systematic evaluation of the binding elements showed that the pyridyl group and secondary sulfonamide NH were key to retention of activity. The substituent adjacent to the pyridyl nitrogen also influenced potency, and the methoxy substituent, compound **5,** was selected, although being less potent than the original chlorine, and showed a reduced drop-off on progression to a cell-based assay.

The reduced isoform selectivity observed could be improved by modification of the 4-position amide group. Combining the pyridine sulfonamide with a lipophilic amine substituent on thiazole ring afforded compound **6,** with nanomolar potency and at least 500-fold selectivity for the other PI3K isoforms; the binding mode was confirmed by X-ray crystallography.



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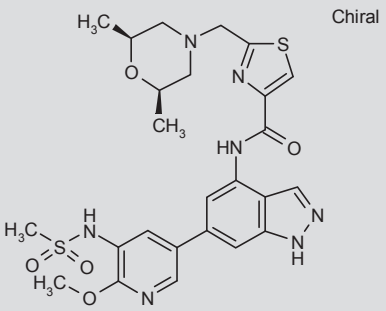

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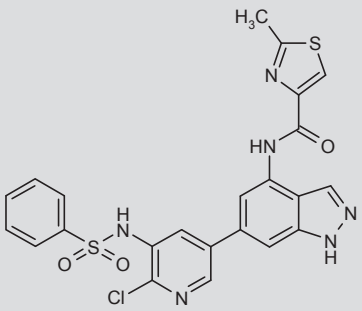

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Further optimization of the series resulted in a potent clinical candidate with 500-fold selectivity against over 250 kinases. The compound prevented T cell cytokine release in human in vitro systems, allergic airways inflammation in rats and virus-induced hypersensitization in guinea pigs when delivered inhaled. Clinical studies to evaluate human safety and tolerability have recently been completed.

DISCLOSURES

The authors are employees of Novartis, GlaxoSmithKline and Pfizer.

REFERENCES

1. Halwani, R., Al-Muhsen, S., Hamid, Q. *Airway remodeling in asthma.* Curr Opin Pharmacol 2010, 10(3): 236-45.
2. McDonough, J.E., Yuan, R., Suzuki, M. et al. *Small-airway obstruction and emphysema in chronic obstructive pulmonary disease.* N Engl J Med 2011, 365(17): 1567-75.
3. Farah, C.S., King, G.G., Brown, N.J. et al. *The role of the small airways in the clinical expression of asthma in adults.* J Allergy Clin Immunol 2012, 129(2): 381-7.
4. Warheit, D.B., Hansen, J.F., Yuen, I.S., Kelly, D.P., Snajdr, S.I., Hartsky, M.A. *Inhalation of high concentrations of low toxicity dusts in rats results in impaired pulmonary clearance mechanisms and persistent inflammation*. Toxicol Appl Pharmacol 1997, 145(1): 10-22.
5. Lee, K.P., Trochimowicz, H.J., Reinhardt, C.F. *Pulmonary response of rats exposed to titanium dioxide (TiO2) by inhalation for two years.* Toxicol Appl Pharmacol 1985, 79(2): 179-92.
6. Jones, R.M., Neef, N. *Interpretation and prediction of inhaled drug particle accumulation in the lung and its associated toxicity*. Xenobiotica 2012, 42(1): 86-93.
7. Jones, R.M., Harrison, A. *A new methodology for predicting human pharmacokinetics for inhaled drugs from orotracheal pharmacokinetic data in rats.* Xenobiotica 2012, 42(1): 75-85.
8. Disse, B., Reichl, R., Speck, G., Traunecker, W., Ludwig Rominger, K.L., Hammer, R. *Ba 679 BR,* a *novel long-acting anticholinergic bronchodilator.* Life Sci 1993, 52(5-6): 537-44.
9. Sykes, D.A., Dowling, M.R., Leighton-Davies, J.L. et al. *The influence of receptor kinetics on the onset, duration of action, and the therapeutic Index of NVA237 and tiotropium.* In press.
10. Vauquelin, G., Charlton, S.J. *Long-lasting target binding and rebinding as mechanisms to prolong* in vivo *drug action*. Br J Pharmacol 2010, 161(3): 488-508.
11. Lombardi, D., Cuenoud, B., Krämer, S.D. *Lipid membrane interactions of indacaterol and salmeterol: Do they influence their pharmacological properties?* Eur J Pharm Sci 2009, 38(5): 533-47.
12. Corren, J., Lemanske, R.F., Hanania, N.A. et al. *Lebrikizumab treatment in adults with asthma.* N Engl J Med 2011, 365(12): 1088-98.
13. Hershey, G.K.K. *IL-13 receptors and signalling pathways: An evolving web*. J Allergy Clin Immunol 2003, 111(4): 677-90.
14. Sidhu, S.S., Yuan, S., Innes, A.L. et al. *Roles of epithelial cell-derived periostin in TGF-beta activation, collagen production, and collagen gel elasticity in asthma*. Proc Natl Acad Sci U S A 2010, 107(32): 14170-5.
15. Woodruff, P.G., Modrek, B., Choy D.F. et al. *T-Helper type 2-driven inflammation defines major subphenotypes of asthma.* Am J Respir Crit Care Med 2009, 180(5): 388-95 [Erratum: Am J Respir Crit Care Med 2009, 180(8): 796].
16. McClintock, D., Corren, J., Hanania, N.A. et al. *Lebrikizumab, an anti-IL-13 monoclonal antibody, reduces severe asthma exacerbations over 32 weeks in adults with inadequately controlled asthma*. Am J Respir Crit Care Med [Int Conf Am Thorac Soc (May 18-23, San Francisco) 2012] 2012, 185: Abst 813.
17. Rowan, W.C., Smith, J.L., Affleck, K., Amour, A. *Targeting phosphoinositide 3-kinase* ä *for allergic asthma.* Biochem Soc Trans 2012, 40(1): 240-5.