

RECENT DISCLOSURES OF CLINICAL CANDIDATES

HIGHLIGHTS FROM THE SOCIETY FOR MEDICINES RESEARCH SYMPOSIUM, HELD ON DECEMBER 8, 2011 AT THE NATIONAL HEART AND LUNG INSTITUTE, SOUTH KENSINGTON, LONDON, UK

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SUMMARY

This symposium featured an international lineup of speakers presenting on the discovery and clinical development of novel therapeutic agents that have recently progressed through clinical trials towards the market. The program included a variety of mechanistic approaches for the therapeutics, including small molecules, macrocycles and vaccines,

and encompassed disease areas as diverse as cystic fibrosis, neurodegeneration, rheumatoid arthritis, antibacterials and oncology. The variety and quality of the subject matter, together with the uniformly excellent delivery of the presentations by the speakers, provided a memorable day for the attendees.

Key words: Tyrosine kinase inhibitors – Antibiotics – Cancer vaccines – mGlu receptor antagonist – Cystic fibrosis

OSI-906, A HIGHLY SELECTIVE, ORALLY BIOAVAILABLE INHIBITOR OF IGF-I RECEPTOR AND IR

Dr. Mark Mulvihill (OSI Pharmaceuticals) described the discovery and development of linsitinib (OSI-906), a potent and highly selective dual inhibitor of insulin-like growth factor 1 receptor (IGF-I receptor) and insulin receptor (IR) signaling, currently undergoing clinical evaluation in a number of cancers, including adrenocortical carcinoma (ACC) (1). IGF-I receptor, IR and IGF-I receptor/IR dimers are receptor tyrosine kinases (RTKs), which when activated on binding of their cognate ligands IGF-I, IGF-II and insulin, trigger critical signaling pathways involved in the etiology, progression and prognosis for a number of human cancers. Signaling through the IGF-I receptor has been linked to tumor cell proliferation and survival, as well as a key contributor toward tumor cells acquiring resistance to current anticancer therapeutics, including chemo- and radiotherapies, as well as targeted small-molecule agents or antibodies. Although the IR signaling axis is still being unraveled, evidence has accumulated supporting a role in promoting tumor cell proliferation. Clinically, in both prostate and breast cancer patients, elevated

insulin has been found to represent a poor prognostic indicator. Recent research using IR knockdown cellular systems and a specific IGF-I receptor antibody reagent has demonstrated compensatory signaling between IGF-I receptor and IR, supporting the view that co-targeting inhibition of the IGF-I receptor and IR may elicit superior antitumor efficacy in comparison to inhibition of the IGF-I receptor alone (2).

The initial hit-to-lead program at OSI centered on the identification and advancement of a privileged imidazo[1,5-*a*]pyrazine template (Fig. 1), in which optimization of structural groups R1 and R2 delivered the desired IGF-I receptor/IR potency, general kinome selectivity and drug developability properties necessary to support IND nomination.

From conventional medicinal chemistry analoging compound **1** emerged in the hit-to-lead stage, demonstrating submicromolar inhibition of pIGF-I receptor in an IGF-I-stimulated 3T3/huIGF-I receptor cell line (LISN) and encouraging mouse oral pharmacokinetics (PK) (Table I). Use of the X-ray crystal structure of compound **1** bound to IR led to the input of structural-based design and generation of lead compound **2**, in which constraining and locking the benzyloxyphenyl unit as a 2-phenylquinolinyl moiety delivered a significant increase in cellular potency, as well as significant improvements in mouse PK properties.

Lead optimization centered on tuning the R2 substituent, with generation of the advanced lead **PQIP** displaying improved inhibition of cellular pIGF-I receptor ($IC_{50} = 0.019 \mu M$). Despite exhibiting a more favorable in vitro ADME profile and lower mouse in vivo CL compared to **2**, the presence of the basic piperazine unit attached to the

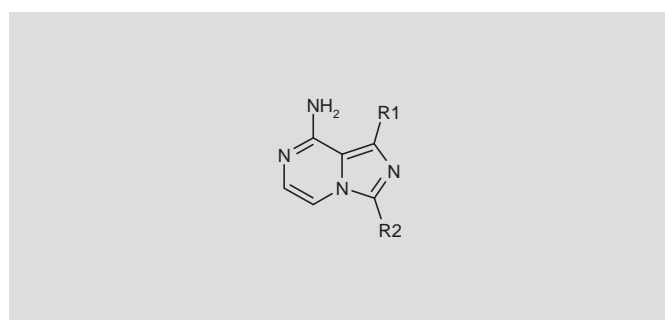


Figure 1. The imidazo[1,5-*a*]pyrazine template.

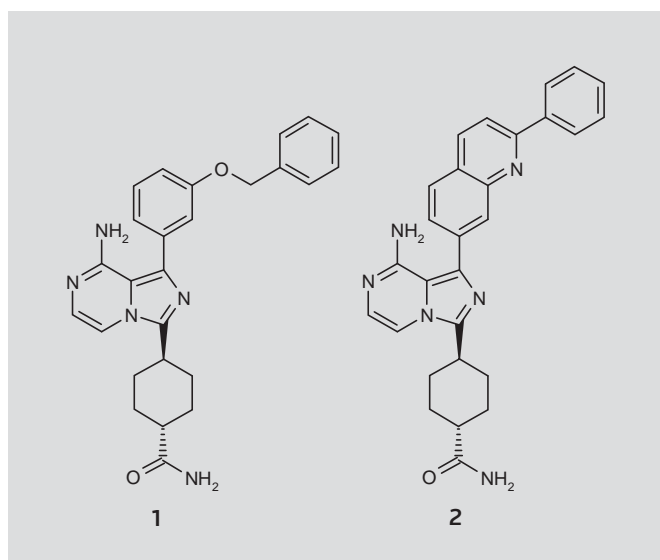
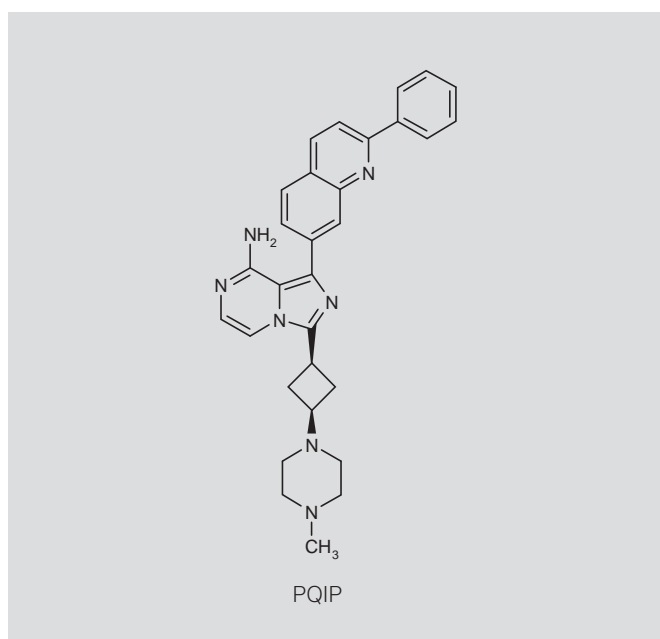


Table I. Inhibition of IGF-I receptor and pharmacokinetic parameters for **1** and **2**.

Compound	Cell (3T3/huIGF-I receptor) IC_{50}	Mouse PK at 50 mg/kg p.o. and 5 mg/kg i.v.			
		C_{max} (μM)	$AUC_{(0-24)}$ (ng·h/mL)	F%	CL (mL/min/kg)
1	0.9	2.22	1323	34	215
2	0.042	11.35	12,554	77	41

cyclobutyl ring at R2 led to the observation of a high volume of distribution and long $t_{1/2}$ approaching 24 hours. This extended half-life was further exacerbated in the rat in vivo PK, leading to concerns that multiday dosing could lead to compound accumulation in tissues and adverse events (Table II).

To address this potential liability, the piperazine unit was replaced with an alcohol, which was fixed as a tertiary alcohol to avoid metabolic oxidation and or epimerization. The resulting compound, **linsitinib** (OSI-906) (Fig. 2) fulfilled the desired target product profile as below:

- Potent cellular inhibition of pIGF-I receptor ($IC_{50} = 15$ nM) and, using a murine-derived hepatoma cell line (Hepa-1), pIR ($IC_{50} = 39$ nM)

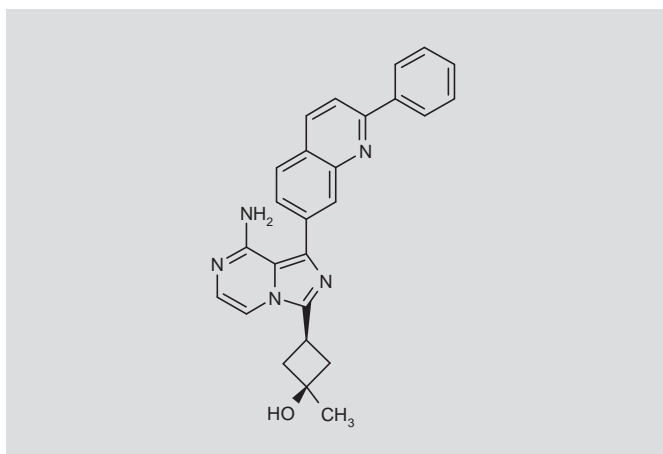


Figure 2. Structure of linsitinib.

- Strong antiproliferative effects ($EC_{50} = 20$ -300 nM) on exposure to a variety of tumor cell lines (non-small cell lung, colorectal, breast, pancreatic, rhabdomyosarcoma, ACC)
- Single-agent efficacy in a range of xenograft tumor models following once-daily dosing
- Robust PK-to-PD relationship, with maximum antitumor activity consistent with sustained inhibition of pIGF-I receptor
- Highly permeable, non-P glycoprotein substrate
- Inhibition of cytochrome P450 3A4, 1A2 and 2D6 > 10 μ M
- Good metabolic stability in the presence of microsomes from various species, with rats and rhesus monkeys sharing metabolic profiles most similar with humans, and consequently becoming the first choice for toxic species
- Excellent %F and exposure across multiple species (Table III), with linear exposure in mice at 5-250 mg/kg using a simple tartaric acid formulation

One very interesting aspect of OSI-906 is its exquisite kinase selectivity. Using caliper biochemical screening technology, OSI-906 at 0.1 μ M failed to inhibit by $> 20\%$ a panel of 166 tyrosine and serine/threonine kinases. The rationale for this specificity is thought to lie in OSI-906 inducing and locking the IGF-I receptor and IR proteins into a rare conformation, in which interaction of the compound with the C-helix causes the helix to adopt an inactive conformation, but with orientation of an activation loop more associated with the phospho protein form.

In advanced profiling, OSI-906 proved negative against a battery of genotoxicity assays and clean against a panel of 68 receptors. Although transient hyperglycemia was noted in mice during complete inhibition of pIGF-I receptor for > 24 hours, such an effect was absent in rats. More importantly, no observations from rat or rhesus monkey toxicology studies precluded OSI-906 progressing into clin-

Table II. Mouse and rat pharmacokinetic parameters for PQIP following i.v. and p.o. administration.

	CL (mL/min/kg)	V_{ss} (L/kg)	C_{max} (μ M)	AUC _(0-∞) (ng·h/mL)	$t_{1/2}$ (h)	F%
Mouse PK @ 25 mg/kg p.o. and 10 mg/kg i.v.	19	8	2.2	34,546	23.6	100
Rat PK @ 20 mg/kg p.o. and 5 mg/kg i.v.	16	21	1.35	28,705	28.7	100

Table III. Pharmacokinetic parameters for OSI-906 following i.v. and p.o. administration in different species.

	CL (mL/min/kg)	V_{ss} (L/kg)	C_{max} (μ M)	AUC _(0-last) (ng·h/mL)	F%
Mouse PK @ 25 mg/kg p.o. and 5 mg/kg i.v.	12	2.05	16.04	26,741	100
Rat PK @ 12.5 mg/kg p.o. and 5 mg/kg i.v.	4	0.79	10.01	42,832	74
Dog PK @ 5 mg/kg p.o. and 2.5 mg/kg i.v.	39	4.3	1.2	1328	64

ical trials. OSI-906 successfully completed single-agent, dose-escalation phase I trials, demonstrating linear PK and a PK-to-PD relationship in which the degree of RTK inhibition correlated with OSI-906 plasma exposure. Hyperglycemia was observed only at high plasma levels. Currently, OSI-906 is in phase II trials for ovarian cancer, hormone-sensitive metastatic breast cancer and non-small cell lung cancer in combination with Tarceva® (erlotinib). The most advanced clinical trials are in ACC, an uncommon cancer originating in the cortex of the adrenal gland. Although the incidence of ACC is low, with an annual reporting rate of 1-2 per million population, currently approved treatment is limited to either radical surgery excision or the use of mitotane. Unfortunately, mitotane, a chemical relative of the insecticide DDT, elicits limited efficacy, with a 5-30% response rate, in addition to causing significant neurological, gastrointestinal and endocrinological toxicities. ACC tumors from patients exhibit a high overexpression of IGF-II, pIGF-I receptor and pIR compared to normal adrenal, and therefore are sensitive to the mechanism of action of OSI-906, in particular, prevention of any compensatory signaling between IGF-I receptor and IR that may support tumor survival. One case study reported a 35-year-old woman who had experienced a significant reduction in the size of a primary adrenal tumor after 32 weeks of OSI-906 treatment and absence of lung metastases at 13 months following drug administration.

PRECLINICAL PROFILE OF AZD-4547, A SELECTIVE INHIBITOR OF FGF RECEPTORS

Dr. Nigel Brooks (Oncology Innovative Medicines, AstraZeneca, UK) presented on the preclinical profile of the selective fibroblast growth factor receptor FGFR-1, -2 and -3 inhibitor AZD-4547. The FGFR class of tyrosine kinases is encoded by four different genes (*FGFR1*, *FGFR2*, *FGFR3*, *FGFR4*). However, through spliced variants 7 FGFR isoforms exist, which can be activated by 22 ligands of the FGF family. Although the FGF signaling network is known to play an important physiological role in organ, vascular and skeletal development, aberrant regulation of the FGF/FGFR pathway, through point mutations, FGFR overexpression and chromosomal translocation, can be found in the pathogenesis of various types of cancer (3). Point mutations can occur either in the extracellular region of the receptor, leading to receptor dimerization, or in the intracellular kinase domain. In both cases, the net result is activation of FGFR in the absence of ligand binding. Ligand-independent signaling can also occur via FGFR overexpression, in which local crowding of cell surface individual FGFRs can result in phosphorylation of each other. FGFR overexpression may be caused by gene amplification or increased transcriptional regulation through single nucleotide polymorphisms. Chromosomal translocation can also give rise to constitutively active FGFR through the formation of fused proteins to the intracellular kinase domain. Detailed analysis of different cancers has revealed what FGFR aberrations are present and by what mechanism (Table IV).

In many cases, evidence of a functional effect of the FGFR aberration in driving tumor pathogenesis has been obtained from antiproliferative responses observed on exposure of appropriate cell lines of the cancer to specific FGFR antibody agents or small molecule-specific FGFR inhibitors. Further research in several of the cancers has revealed that only a certain percentage of tumors carry the FGFR

Table IV. FGFR aberrations identified in human cancers.

Cancer	Receptor	Mechanism
Gastric	FGFR-2	Overexpression
Endometrial	FGFR-2	Point mutations
Breast	M2	Overexpression
Non-invasive bladder	FGFR-3	Point mutations
Multiple myeloma	FGFR-3	Overexpression and point mutations
Non-small cell lung	FGFR-2 and FGFR-1	Overexpression and point mutations
Prostate	FGFR-1	Overexpression

aberration (gastric about 5%; endometrial about 15%; breast about 10%; non-invasive bladder about 70%; multiple myeloma about 20%; non-small cell lung about 10%), which in some cases is prognostic for the cancer (breast, non-invasive bladder and non-small cell lung). Although FGFR-1 is overexpressed in prostate cancer, several FGF ligands are also upregulated, some of which have been shown to contribute to the development, maintenance and progression of the cancer. In colorectal cancer, upregulation of the ligand FGF-19, which uses FGFR-4 as its cognate receptor, has been suggested as a mechanism for tumor growth (4). As a consequence, FGF-19 has become a therapeutic target for the treatment of colon cancer.

AZD-4547 (Fig. 3) is a potent and selective inhibitor of FGFR-1, -2 and -3, with enzymatic IC_{50} values of 0.2, 2.5 and 1.8 nM, respectively. Activity against FGFR-4 is noted, but much lower, with an IC_{50} of 165 nM. Selectivity against the wider "kinome" was evaluated both at the enzyme and cellular level. Screening of a panel of 70 other kinases demonstrated no significant activity at 10 μ M. With reference to cellular potency, the FGFR-2 component of AZD-4547 exhibited a > 120-fold selectivity window against KDR, despite only a 10-fold separation at the enzyme level.

Analysis of the antiproliferative responses observed on treatment of a large number of cell lines with AZD-4547 revealed those cell lines

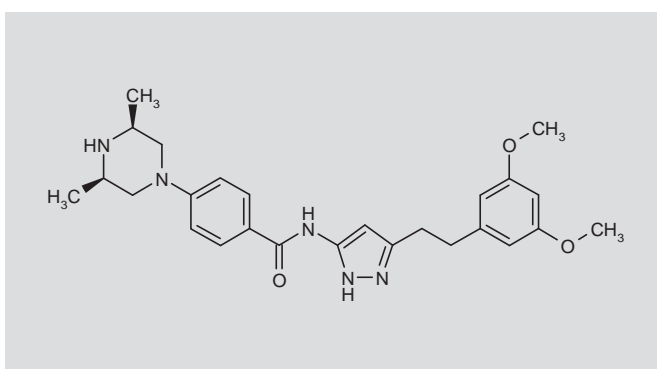


Figure 3. Structure of AZD-4547.

carrying FGFR aberrations as being most sensitive. For example, in human gastric carcinoma KATO III and SNU-16 cells carrying overexpressed FGFR-2 AZD-4547 gave GI_{50} values of < 10 nM. In contrast, gastric carcinoma NCI-N87 cells, a representative example of a gastric cancer cell line housing no overexpression of FGFR-2, required AZD-4547 at μ M levels to elicit a GI_{50} . A more detailed analysis of cellular signaling on inhibition of FGFR-2 in SNU-16 cells confirmed the potent inhibition of pFGFR and downstream substrates fibroblast growth factor receptor substrate 2 (pFGFR substrate 2) and extracellular signal-regulated kinase 1/2 (pERK-1/2).

In rodents and dogs, the blood clearance (rats: 39 mL/min/kg; dogs: 13 mL/min/kg) and oral bioavailability (rats: 42%; dogs: 100%) of AZD-4547 supported oral dosing as a means of delivering systemic exposure. Chronic oral dosing of AZD-4547 up to 12.5 mg/kg once daily in several murine xenograft models carrying FGFR aberrations resulted in dose-dependent tumor growth inhibition. Xenografts included SNU-16 (human gastric tumor model containing *FGFR2* overexpression) and KMS-11 (human multiple myeloma tumor model expressing *FGFR3* translocation). In SNU-16 xenografts, sustained tumor regression correlated with AUC exposure of AZD-4547 and inhibition of pFGFR-2 and associated downstream markers, such as pERK. Combination of AZD-4547 with cytotoxic agents currently approved for the treatment of gastric cancer led to enhanced growth inhibition in SNU-16 xenografts.

Currently, two phase II clinical trials of AZD-4547 focused on safety and efficacy endpoints are recruiting patients. NCT01457846 is directed towards comparison of AZD-4547 versus paclitaxel in patients with advanced gastric or gastroesophageal junction cancer found to be carrying FGFR-2 overexpression. NCT01202591 is centered on combination therapy of AZD-4547 with exemestane versus exemestane alone in estrogen receptor-positive (ER⁺) breast cancer patients who express a high level of FGFR-1. Recruiting is also ongoing in a phase I trial to address the safety and tolerability of AZD-4547 at increasing doses in patients with advanced solid malignancies.

POL-7080, A NARROW-SPECTRUM, PSEUDOMONAS-SPECIFIC PEM ANTIBIOTIC

The discovery and development of POL-7080, a novel *Pseudomonas*-selective inhibitor, was presented by Dr. Klaus Dembowski (Polyphor Ltd., Switzerland). POL-7080 is from a new class of protein epitope-mimetic (PEM) molecules designed using a proprietary technology. PEM molecules are fully synthetic medium-sized cyclic peptides (0.7-2 kDa) that contain key beta-hairpin and alpha-helix secondary structural motifs, which impose a well-defined conformational structure to the molecule, capable of modulating protein-protein interactions with high specificity and potency. Using multiparallel synthetic techniques, libraries of PEM molecules have been produced and used for screening against a variety of target classes.

Screening of approximately 300 PEM molecules, derived from the cyclic peptide protegrin-1, a known broad-spectrum antibacterial, against a panel of both Gram-negative and Gram-positive bacterial strains serendipitously identified several PEMs specific for the *Pseudomonas* bacterium, which is regarded as a difficult bacterium

to treat with existing antibiotics. Optimization through approximately a further 1,500 PEM molecules, in particular addressing PK/ADMET aspects, including peptide plasma stability, delivered POL-7080 as a clinical candidate. POL-7080 appeared to be very potent and specific against *Pseudomonas* strains, in particular against the *P. aeruginosa* strain, an opportunistic pathogen that can give rise to serious and often life-threatening infections in humans (Table V).

Of further interest is that the mechanism of action of POL-7080 does not involve a classical bacterial membrane lysis, but rather an interaction with a beta-barrel outer membrane protein OstA/Imp/LptD, resulting in a downregulation of lipopolysaccharide (LPS) biosynthesis (5). *Pseudomonas* LPS has been implicated as an important virulence factor, which alters host innate immune responses and promotes bacterial persistence and chronic infection.

POL-7080 has favorable ADMET properties, including:

- Low human protein binding (> 50% free)
- High stability at the microsomal level across species (humans, rats and dogs > 90% remaining after 1 hour)
- High rodent and human plasma stability (> 85% remaining after 6 hours)
- Non-cytotoxic and non-hemolytic

When tested against 400 clinical isolates of *P. aeruginosa*, POL-7080 delivered a potent and narrow MIC distribution across the range 0.06-0.25 μ g/mL. In contrast, many other known antimicrobial agents, such as colistin, polymyxin B, meropenem and ciprofloxacin, failed to deliver such broad activity, confirming POL-7080 as a superior antimicrobial. Further in vitro studies across *P. aeruginosa* strains with POL-7080 showed killing of the bacteria (MBC) at twice the MIC values, with a rapid onset of killing action (3-6 hours at 2-4 x MIC) and a low rate of spontaneous resistance development. No synergy or antagonism was displayed between POL-7080 and other known antibiotics, paving the way for combination therapy where necessary. Administration of POL-7080 via i.v. infusion in a mouse model of

Table V. *In vitro* activity of POL-7080 against bacterial strains.

Type strain	Microbial stock	MIC (μ g/mL)
<i>Pseudomonas aeruginosa</i>	ATCC 27853	0.06
<i>Pseudomonas aeruginosa</i>	ATCC 15692	0.25
<i>Pseudomonas putida</i>	DSM 291	0.06
<i>Pseudomonas fluorescens</i>	DSM 6147	0.06
<i>Pseudomonas aureofaciens</i>	ATCC 15926	0.06
<i>Pseudomonas syringae</i>	ATCC 12271	0.008
<i>Acinetobacter baumannii</i>	ATCC 19606	> 64
<i>Burkholderia cepacia</i>	ATCC 25416	> 64
<i>Stenotrophomonas maltophilia</i>	ATCC 13637	> 64

ATCC, American Type Culture Collection; DSM, Deutsche Sammlung von Mikroorganismen.

septicemia driven by different strains of *P. aeruginosa* provided protection and an increase in survival rate. ED₅₀ values ranged between 0.25 and 1 mg/kg. In a neutropenic mouse model of pneumonia and thigh *P. aeruginosa* infection, POL-7080 caused a dose-dependent killing effect of the infection. Subsequent PK/PD modeling suggested C_{max}/MIC as the principal PD driver for efficacy, with a significant contribution of AUC/MIC. Projection of the PK/PD modeling to human infections suggested a therapeutic regimen of once- or twice-daily dosing.

As part of a phase I study, infusion of POL-7080 into healthy volunteers has been completed as both a single ascending dose (SAD; 0.05 → 3.5 mg/kg) and multiple ascending dose (MAD; 1 mg/kg b.i.d and 2 mg/kg t.i.d). From SAD PK, the AUC and C_{max} of POL-7080 increased linearly with dose, delivering a t_{1/2} of 5-6 hours and a moderate volume of distribution. No accumulation of the compound was observed in the MAD arm of the trial, with blood chemistry and clinical laboratory markers being normal. Potential clinical options for a proof-of-concept phase IIa study include systemic application in complicated urinary tract infections, pneumonia, bloodstream infections (sepsis) or cystic fibrosis. Topical or local application represents an alternative delivery system, opening up clinical indications such as keratitis or burn wounds. To accelerate such studies, Polyphor is looking to out-license POL-7080.

THE DEVELOPMENT OF FOSTAMATINIB, AN ORAL SYK INHIBITOR

Dr. Martin Braddock and Dr. Björn Rubin from AstraZeneca R&D gave an overview of the scientific rationale of tyrosine-protein kinase SYK (spleen tyrosine kinase) inhibition for the treatment of rheumatoid arthritis (RA) and the clinical development program for fostamatinib. Fostamatinib was in-licensed by AstraZeneca from Rigel Pharmaceuticals in 2010, and is currently being evaluated for the treatment of RA in phase III clinical trials. RA is a chronic, progressive and disabling autoimmune disease that affects approximately 1% of the population and is a major cause of disability. Fostamatinib is a

potent oral inhibitor of SYK, an intracellular signaling kinase, which is a master regulator of immune receptor signaling in RA and modulates autoimmunity, inflammation and tissue damage in the disease. SYK plays an important role in B-cell activation and controls the expression of cytokines such as IL-6, TNF- α and proinflammatory mediators such as matrix metalloproteinases. Evidence that SYK is critically involved in the development of clinically relevant aspects of autoantibody-induced arthritis has recently been shown in studies in *Syk*^{-/-} knockout mice (6). Genetic deficiency of *Syk* in an autoantibody-induced experimental arthritis model in these knockout mice blocked the development of all macroscopic and microscopic signs of arthritis, prevented periarticular bone erosion and protected against arthritis-induced loss of articular function. Furthermore, it has also been shown that the SYK signaling pathway regulates osteoclastogenesis and has a role in bone destruction, independent of inflammation, and is required for osteoclast development and function (7).

Fostamatinib is a phosphate prodrug, which is rapidly cleaved through the action of gut phosphatases to release the bioactive metabolite **R-406** (Fig. 4).

The functional selectivity of R-406 in a diverse panel of cellular assays has been assessed in primary T cells, mast cells, macrophages and relevant cell lines (8). In rats with collagen-induced arthritis (CIA) administration of fostamatinib at 15 and 30 mg/kg b.i.d. suppressed clinical arthritis, bone erosions, pannus formation and synovitis for up to 20 days post-induction of arthritis (9). SYK inhibition in this model suppressed synovial cytokines and biomarkers of bone resorption, such as cartilage oligomeric matrix protein (COMP), in serum. Furthermore, MicroCT analysis of the hindlimbs from rats with CIA showed that treatment with fostamatinib for 18 days maintained bone integrity and reduced bone loss. Additionally, there is emerging evidence that SYK plays a role in atherosclerosis and vascular disease (10).

Fostamatinib has now been evaluated for > 1,000 patient years in several phase II clinical studies, including an ongoing open-label

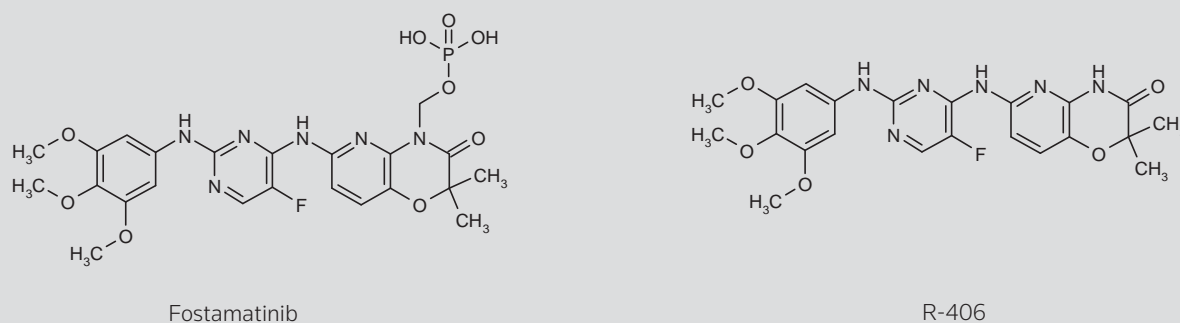


Figure 4. The structures of fostamatinib (R-788) and its active metabolite R-406.

extension study (11-13). Decreases in serum MMP-3 and IL-6 biomarkers have been observed in groups treated with fostamatinib 100 and 150 mg twice daily within 1 week of starting treatment and were sustained through treatment course (11). Clinical outcomes (ACR20, 50 and 70 scores) were improved with fostamatinib treatment, with a manageable safety profile (12, 13). Phase III OSKIRA studies are ongoing to more fully evaluate fostamatinib as a potential oral treatment for RA.

BIOMARKER-GUIDED DEVELOPMENT OF NOVEL PEPTIDE-BASED CANCER VACCINES

Dr. Harpreet Singh described the portfolio of novel, peptide-based cancer vaccines currently in development at Immantics Biotechnologies GmbH (Tuebingen, Germany), as shown below in Table VI.

The field of cancer vaccines is currently undergoing a renaissance since the 2011 FDA approvals of Provenge® (sipuleucel-T) in prostate cancer, ipilimumab in melanoma and the reporting of a number of further positive phase III studies. However, the first generation of cancer vaccines, by the nature of their discovery, are often not fully molecularly defined, are complicated to manufacture and distribute, and are therefore limited in application as continuous doses. The second generation of “drug-like” cancer vaccines is required to be molecularly defined and easy to manufacture to ensure success. They will need to be rationally designed to target the “right” antigens, using the correct immunomodulators in the best combinations in appropriate patient populations. Immantics’ cancer vaccines consist of rationally designed synthetic HLA-class I and II tumor-associated peptides (TUMAPs), confirmed to be present in human tumor tissue by a discovery platform combining mass spectrometry, immunology, differential transcriptomics and bioinformatics. This approach ensures the election of strongly immunogenic TUMAPs from relevant tumor antigens and each cancer vaccine product is typically a mixture of 10-18 synthetic peptides.

The rationale for the mechanism of action of such cancer vaccines is:

- Firstly, priming occurs whereby TUMAPs injected into the skin bind to dermal dendritic cells, which then migrate into the lymph nodes, where they encounter and prime naive T cells specifically recognizing the TUMAPs used in the vaccine

- Once T cells are primed, their number increases rapidly by clonal proliferation. They leave the lymph nodes and begin searching for tumor cells displaying the same TUMAP by which they were activated
- Finally, once cytotoxic T cells recognize a TUMAP on the tumor cells, they then mount a cytolytic/apoptotic attack against the tumor cells

Immantics, through its clinical programs, to date has shown association of multipetide immune responses, with increases in overall survival for IMA-901 in renal cell cancer and for IMA-910 in colorectal cancer. During phase I trials, T-cell response markers and cellular biomarkers, such as IL-17 and IL-1-secreting T cells, are measured and are used to guide further clinical development to optimize the treatment regimen from phase I to II and to select the best combination partner for phase III. More than 300 serum biomarkers, including cytokines, chemokines, amino acids, biogenic amines, carnitines and phosphatidylcholines, are measured as a secondary endpoint in the clinical trials and are used to select TUMAP responders in phase III trials. The vaccines are administered as a fixed dose of ~400 µg per peptide intradermally with the immunomodulator granulocyte-macrophage colony-stimulating factor (GM-CSF; 75 µg) intradermally with every vaccination and low-dose cyclophosphamide before the first vaccination. Clinical trials last up to 4 years. IMA-901 is composed of 10 peptides associated with renal cell cancer (RCC) and is currently recruiting for a phase III study. The current standard of care in advanced RCC is first-line therapy with sunitinib (Sutent®) and second-line therapy with sorafenib (Nexavar®). Preclinical evidence suggests that sunitinib is compatible and possibly synergistic with peptide vaccinations, since it has been shown to inhibit immunosuppressive cell populations in RCC patients. Therefore, the phase III trial design is testing a combination of IMA-901 and sunitinib in 300 end-stage RCC patients for 4 months of vaccinations followed by monitoring overall survival for up to 8 years.

IMA-910 is composed of 13 tumor-associated peptides and has completed a phase I/II trial in 92 advanced metastatic colorectal cancer patients in combination with GM-CSF, in which it was shown to be safe and immunogenic. A trend for increased overall survivors in multi-TUMAP responders was observed.

Finally, IMA-950 is composed of 11 peptides associated with glioma and a phase I trial with Cancer Research UK is currently recruiting 45 glioblastoma patients to be treated in combination with radiotherapy and chemotherapy. A second single-agent phase I trial is being performed by the U.S. National Cancer Institute and is also recruiting.

Table VI. The development pipeline of cancer vaccines at Immantics.

Cancer vaccine product	Indication and status
IMA-901	Renal cell cancer: completed phase I and II study in 98 patients; phase III study recruiting
IMA-910	Colorectal cancer: completed phase I/II study in 92 patients
IMA-950	Glioma: two phase I studies recruiting
IMA-942	Gastric cancer: discovery/preclinical
IMA-930	Non-small cell lung cancer: discovery/preclinical

DEVELOPMENT OF AFQ-056, A SELECTIVE mGLU₅ RECEPTOR ANTAGONIST

Dr. Fabrizio Gasparini provided a fine example of the typical twists and turns of a drug discovery project. In this case, eventually leading to the discovery of the selective, noncompetitive mGlu₅ receptor antagonist AFQ-056 (mavoglurant) for the treatment of L-Dopa-induced dyskinesia in Parkinson’s disease.

The metabotropic glutamate (mGlu) receptors are members of family 3 G protein-coupled receptors (GPCRs) and are structurally relat-

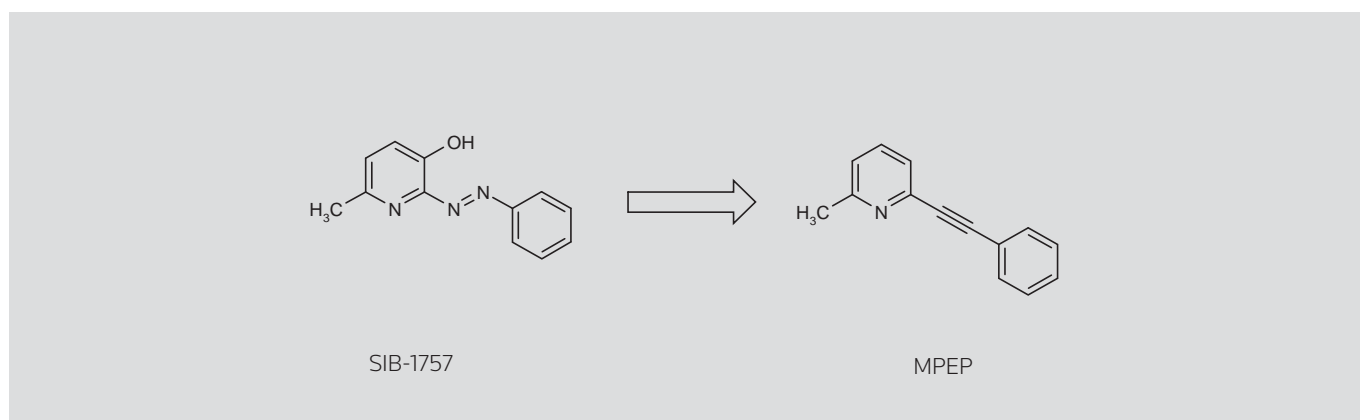


Figure 5. Early mGlu₅ receptor antagonists.

ed to the GABA_B receptor. Modulation of their signaling was postulated to impact on a variety of CNS indications due to their specific CNS localization, initially established by rat immunohistochemistry (14). As part of a research collaboration between SIBIA Neurosciences and Novartis, a small-molecule library of 960 compounds was screened against cell lines expressing the human mGlu₅ receptor. This functional screen was used to identify the mGlu₅ receptor-selective antagonist, **SIB-1757** (Fig. 5), which inhibited the glutamate-induced [Ca²⁺]_i responses at the human mGlu₅ receptor with an IC₅₀ of 0.37 μM compared with an IC₅₀ of 100 μM for the human mGlu₁ receptor (15). Schild analysis demonstrated a non-competitive mode of action. Lead optimization then led to the key pharmacological tool **MPEP** (Fig. 5), with an IC₅₀ of 0.036 μM.

Over the next few years, several studies advanced the pharmacology of MPEP, including the allosteric nature of its interaction with the mGlu₅ receptor and hence potential therapeutic applications for such antagonists in anxiety (16), inflammatory pain (17), drug addiction (cocaine, alcohol, morphine) (18), Parkinson's disease (PD) (19), depression (20) and smoking cessation (21). Despite significant efforts, it did not prove possible to optimize MPEP to a clinical candidate. A new high-throughput screening campaign was then performed with a Ca²⁺ mobilization assay using a FLIPR-based assay for human mGlu₅ receptors expressed in Ltk⁻ cells. Twenty-three validated hits were then the subject of hit-to-lead medicinal chemistry exploration, leading to the discovery of compound **3** (IC₅₀ = 0.17 μM) (Fig. 6). Further optimization focused on three main areas of the molecule, namely the carbamate group, the acetylene "spacer" and aromatic substituents. The acetylene spacer proved optimal, leading to the clinical candidate **mavoglurant** (AFQ-056) (Fig. 6).

Mavoglurant was shown to be a non-competitive antagonist at the mGlu₅ receptor, with excellent selectivity over the mGlu₁ receptor and endogenous P2Y receptors. Next came the vexed choice of appropriate CNS indication. At this point, preclinical evidence pointed to the aforementioned indications plus fragile X syndrome (FXS) and gastroesophageal reflux disease (GERD). Proof-of-concept studies were then agreed in four indications: PD, pain, GERD and

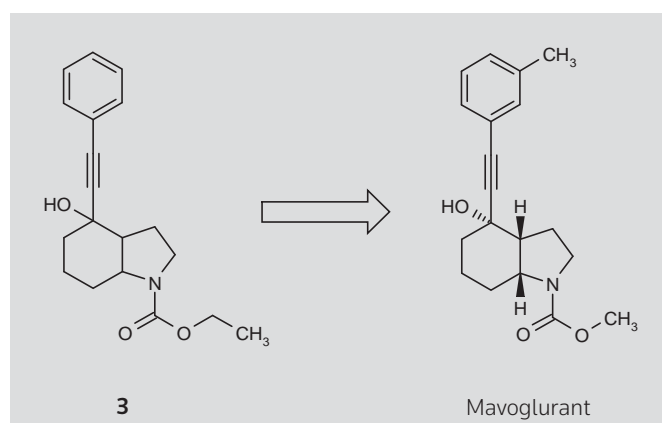


Figure 6. Structures of compound **3** and mavoglurant.

FXS. The results of the clinical studies in PD and FXS were discussed in the presentation.

A significant problem in PD is L-Dopa-induced dyskinesia (LID). Chronic L-Dopa treatment induces permanent dyskinesias in approximately 40% of PD patients in 3-4 years and is more common in younger patients. It is partly managed by reducing L-Dopa dose, but at the expense of a concomitant reduction in its antiparkinsonian effect. Current pharmacological treatments (including amantadine/neuroleptics) are unsatisfactory and invasive surgical treatments, such as deep brain stimulation and pallidotomy, are only used in severe cases. Based on preclinical studies and mGlu₅ receptor expression in human postmortem brain tissues, it was postulated that mGlu₅ receptor antagonists would be useful adjuncts to dopamine replacement therapy by increasing the L-Dopa therapeutic window, thus alleviating or preventing the expression of dyskinesia and perhaps leading to decreased L-Dopa dosing frequency. A proof-of-concept study was designed to assess the role of mavoglurant in L-Dopa-induced dyskinesia. This randomized, double-blind,

placebo-controlled, parallel-group study was performed in PD patients with moderate to severe LIDs. Mavoglurant induced a clinically relevant and statistically significant antidyskinetic effect using three different scales (LFADLDS, AIMS and UPDRS-32/33) in comparison to placebo. The magnitude of the effect was greater than that seen with current medications. Mavoglurant did not reduce the antiparkinsonian effect of concomitantly administered L-Dopa in comparison to placebo (22).

FXS is a monogenetic syndrome that is characterized by a low IQ of < 70, and increased incidences of epilepsy, autism, attention deficit hyperactivity disorder (ADHD) and anxiety. The syndrome is associated with high levels of repetition of a single trinucleotide gene sequence (CGG) on the X-chromosome, which, when accompanied by a high methylation status (23), results in a failure to express the fragile X mental retardation protein 1 (FMRP). A rationale for the involvement of the mGlu₅ receptor in FXS was described by Bear et al. (24). In synapses, FMRP acts as a translational repressor of synaptic proteins. The absence of FMRP leads to an increase of group I mGlu (mGlu₁ and mGlu₅)-mediated protein synthesis and enhanced long-term depression (LTD). Negative modulation of the mGlu₅ receptor is proposed to restore normal synaptic activity and correct the FXS phenotype. When *Fmr1* knockout mice are treated with MPEP, an mGlu₅ receptor antagonist, phenotypic deficits (seizures, locomotor activity) are restored. A randomized, double-blind, two-treatment, two-period crossover, proof-of-concept study was completed in 30 male FXS patients (23). No significant effect of the treatment on the primary outcome measure (Aberrant Behavior Checklist-Community Edition [ABC-C] score) at day 19 or 20 of treatment was observed. Interestingly, however, an exploratory analysis showed that seven patients with full *FMR1* promoter methylation and no detectable *FMR1* messenger RNA had improved, as measured with the ABC-C score, significantly more after mavoglurant treatment than with placebo ($P < 0.001$). No response was detected in patients with partial promoter methylation. If confirmed in phase III studies, these results suggest a potential benefit for mavoglurant in FXS patients with full methylation at the *FMR1* promoter, who may thus show improvement in the behavioral attributes of FXS.

Mavoglurant is currently being tested in three phase IIb clinical trials: one PD-LID trial and two FXS trials.

DISCOVERY AND DEVELOPMENT OF CFTR MODULATORS TO TREAT THE UNDERLYING CAUSE OF CYSTIC FIBROSIS

Cystic fibrosis (CF) is caused by the loss of epithelial chloride ion transport due to mutations in the gene that encodes the CF transmembrane conductance regulator (CFTR), a protein kinase A-activated chloride ion channel. Despite the clear genetic link to CF disease, there are no currently approved therapies that target the underlying defects in CFTR. Dr. Fred Van Goor presented an exciting review of the latest data on two clinical projects at Vertex Pharmaceuticals targeting the CFTR protein itself. The most advanced project, recently having completed phase III trials, has targeted molecules that potentiate the amount of chloride ion passing through the PKA-activated CFTR at the cell surface by increasing its open probability. These agents are called potentiators. The second clinical project, currently in phase II, has focused on molecules that

improve the cellular processing and delivery of the mutant CFTR to the cell surface. Such molecules should improve chloride ion flow by increasing the density of CFTR at the apical membrane. These agents have been termed correctors.

$$\text{Total CFTR current} = \text{density} \times \text{open probability} \times \text{conductance}$$

CF is characterized by the large number of mutations (> 1,500) of the *CFTR* gene which can lead to CF. Interestingly, the severity of CF varies greatly in patients and this has been related to the relative degrees of chloride ion flux. Indeed, patients with as little as 10–25% of normal function have a significantly better prognosis than those with < 10%. These data led to the hypothesis that even modest improvements in chloride ion flux could lead to significant clinical benefit.

A high-throughput screening campaign of 228,000 compounds was performed using a cell-based fluorescence membrane potential assay designed to identify CFTR potentiators (25). This led to the identification of the sulfamoyl-4-oxoquinoline-3-carboxamides. In particular, compound **4** (Fig. 7) was shown to selectively potentiate the gating of the most common mutant channel, F508del-CFTR. This was subject to a medicinal chemistry optimization program, resulting in the identification of the clinical candidate **ivacaftor** (VX-770) (Fig. 7).

VX-770 demonstrated potent in vitro effects on CFTR-mediated chloride ion secretion in both recombinant cell lines and primary cultures of human bronchial epithelium (HBE) isolated from the bronchi of CF and non-CF donor lungs. This included the predominant mutant F508del-CFTR, but also a number of mutants where the functional defect impairs the ability of CFTR at the cell surface to open correctly, in particular G551D-CFTR. This latter mutant is characterized by normal levels of expression at the epithelial surface, so its potentiation was anticipated to have a particularly favorable clinical outcome. Accordingly, clinical trials were designed to evaluate the potential of VX-770 in patients expressing at least one copy of

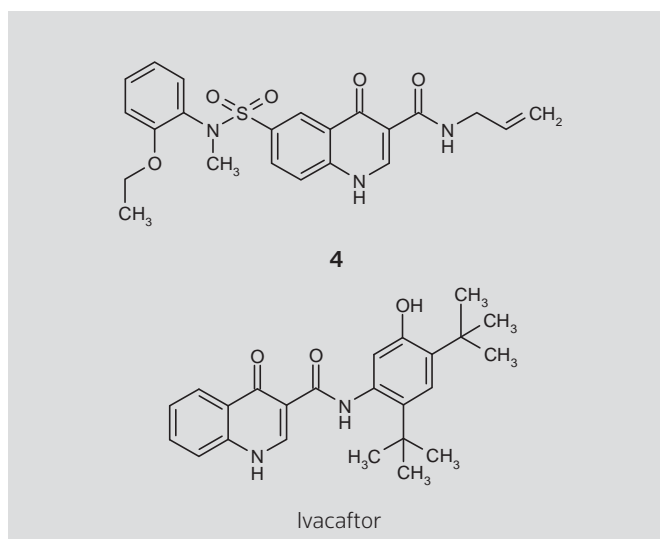


Figure 7. Structures of compound **4** and ivacaftor.

the G551D mutation. In terms of incidence, this reflects around 3-5% of the global CF population. The pivotal phase III study evaluated VX-770 in 161 people 12 years or older with the G551D mutation. Data from the study showed rapid improvements in lung function (FEV1) that were sustained through 48 weeks among those who received VX-770 compared to those treated with a placebo. Subjects taking VX-770 gained weight, on average 2.7 kg, more than those taking placebo at week 48 and had a 55% risk reduction for pulmonary exacerbation compared to placebo through week 48. Concomitant reductions in sweat chloride were observed, providing evidence that VX-770 improves CFTR function, thereby addressing the fundamental defect that leads to CF.

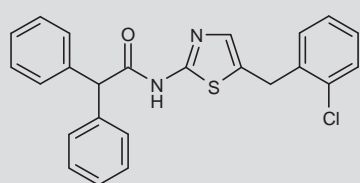
In the search for CFTR correctors, the focus was directed to F508del-CFTR, due to its high prevalence and folding defect. Vertex screened 164,000 small molecules looking for compounds that increased F508del-CFTR-mediated chloride ion transport in a recombinant cell-based assay (25). Active compounds were prioritized based on evidence of improved F508del-CFTR processing in the endoplasmic reticulum and increased functional F508del-CFTR at the cell surface. To allow sufficient time for de novo synthesis, ER processing and cellular trafficking of F508del-CFTR to reach steady state, cells were incubated with compounds for 48 hours before measurement. One active compound, **VRT-768** (Fig. 8), increased F508del-CFTR maturation by 2.5 ± 0.1 -fold ($EC_{50} = 16 \pm 6 \mu\text{M}$; $n = 4$) and enhanced chloride ion transport ($EC_{50} = 7.9 \pm 1.1 \mu\text{M}$; $n = 4$) compared with vehicle-treated controls in Fischer rat thyroid (FRT) cells expressing F508del-CFTR. An extensive medicinal chemistry program was initiated to improve the in vitro potency, efficacy and other physicochemical properties of VRT-768, eventually leading to **VX-809** (Fig. 8).

In FRT cells, VX-809 improved F508del-CFTR maturation by 7.1 ± 0.3 -fold ($n = 3$) compared to vehicle-treated cells ($EC_{50} = 0.1 \pm 0.1 \mu\text{M}$; $n = 3$), and it enhanced F508del-CFTR-mediated chloride ion transport by approximately fivefold ($EC_{50} = 0.5 \pm 0.1 \mu\text{M}$). VX-809 was orally bioavailable in rats and achieved in vivo plasma levels significantly above concentrations required for in vitro efficacy (26). The pharmacology of VX-809 was assessed in cultured HBE cells isolated from the lungs of patients with CF homozygous for the F508del-

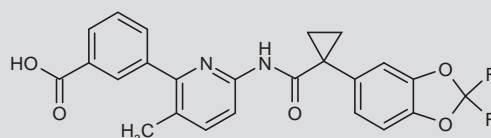
CFTR mutation (F508del-HBE). Incubation of F508del-HBE with VX-809 for 48 hours enhanced chloride ion transport by approximately fourfold. This corresponded to an increase in chloride ion transport from $3.4 \pm 0.7\%$ to $13.9 \pm 2.3\%$ of that measured in HBE isolated from four non-CF donor lungs. Following VX-809 washout, chloride ion transport returned to uncorrected levels. To further enhance chloride ion transport through F508del-CFTR corrected by VX-809, the CFTR potentiator VX-770 was added to maximize the open probability of the CFTR channel. Acute application of $1 \mu\text{M}$ VX-770 increased forskolin-stimulated chloride ion transport in cultured F508del-HBEs pretreated with VX-809 for 48 hours. At the maximally effective concentrations of both compounds, F508del-CFTR-mediated chloride transport reached levels equivalent to approximately 25% of that measured in non-CF HBEs. As noted earlier, such levels of function, if replicable in the clinic, could significantly improve the prognosis for patients carrying F508del-CFTR.

Dr. Van Goor then discussed the results from a randomized, double-blind, placebo-controlled, multicenter phase II cohort study. Subjects in the first cohort received VX-809 200 mg once daily alone or placebo for 14 days, followed by VX-809 200 mg once daily together with VX-770 at either 150 or 250 mg b.i.d. or placebo for 7 days. The effects seen here were of significantly smaller magnitude than those seen with VX-770 alone in patients with the G551D mutation. Nevertheless, VX-809 coadministered with the 250-mg dose of VX-770 led to a greater than twofold reduction in sweat chloride compared to VX-809 alone. A slight increase in lung function was evident in the VX-809 plus 150 mg VX-770 group, but not for 250 mg VX-770, although the small sample size challenges interpretation. Results from subjects in the second cohort of this study, exploring 28 days of treatment with the combination of VX-770 and VX-809, are eagerly awaited in 2012.

Vertex submitted a New Drug Application for VX-770 to the U.S. Food and Drug Administration (FDA) in October 2011 and is seeking approval of VX-770 for use in people 6 years of age and older who have at least one copy of the G551D mutation in the *CFTR* gene. Vertex has also started the registration process for VX-770 with the European Medicines Agency (EMA).



VRT-768



VX-809

Figure 8. Structures of VRT-768 and VX-809.

CONCLUSIONS

This was the fourth Recent Disclosures of Clinical Candidates meeting organized by the Society for Medicines Research. The meeting yet again proved to be popular, with many different R&D organizations represented in the audience. The range of therapeutic areas and molecular approaches gave an interesting perspective on the diversity of research and on the research strategies adopted within the various biopharmaceutical companies represented. The encouraging clinical results described during the day hold promise for a new phalanx of drugs and vaccines targeting oncology, rheumatoid arthritis, bacterial infection, CNS and cystic fibrosis moving towards registration and the market.

DISCLOSURES

Dr. Wendy Alderton is Director of Science at Abcodia Ltd., Dr. Steve Collingwood is Director in the Global Discovery Chemistry department of Novartis Institutes of Biomedical Research and Dr. A.J. Ratcliffe is Director of Chemistry at Cellzome Ltd. The SMR Committee organizes conferences on behalf of the Society for Medicines Research four times a year. These one-day symposia are multidisciplinary in nature and focus on a wide variety of aspects of medicines research. Details of forthcoming meetings can be found at <http://www.smr.org.uk> or by e-mail enquiry to secretariat@smr.org.uk.

REFERENCES

- Mulvihill, M.J., Cooke, A., Rosenfeld-Franklin, M. et al. *Discovery of OSI-906: A selective and orally efficacious dual inhibitor of the IGF-1 receptor and insulin receptor*. *Future Med Chem* 2009, 1(6), 1153-71.
- Buck, E., Gokhale, P.C., Koujak, S. et al. *Compensatory insulin receptor (IR) activation on inhibition of insulin-like growth factor-1 receptor (IGF-1R): Rationale for co-targeting IGF-1R and IR in cancer*. *Mol Cancer Ther* 2010, 9(10): 2652-64.
- Wesche, J., Haglund, K., Haugsten, E.M. *Fibroblast growth factor and their receptors in cancer*. *Biochem J* 2011, 437(2): 199-213.
- Desnoyers, L.R., Pai, R., Ferrando, R.E. et al. *Targeting FGFR1 inhibits tumor growth in colon cancer xenograft and FGFR1 transgenic hepatocellular carcinoma models*. *Oncogene* 2008, 27(1): 85-97.
- Srinivas, N., Jetter, P., Ueberbacher, B.J. et al. *Peptidomimetic antibiotics target outer-membrane biogenesis in Pseudomonas aeruginosa*. *Science* 2010, 327(5968): 1010-3.
- Jakus, Z., Simon, E., Balázs, B., Mócsai, A. *Genetic deficiency of Syk protects mice from autoantibody-induced arthritis*. *Arthritis Rheum* 2010, 62(7): 1899-910.
- Mócsai, A., Humphrey, M.B., Van Ziffle, J.A. et al. *The immunomodulatory adapter proteins DAPI2 and Fc receptor gamma-chain (FcRgamma) regulate development of functional osteoclasts through the Syk tyrosine kinase*. *Proc Natl Acad Sci U S A* 2004, 101(16): 6158-63.
- Braselmann, S., Taylor, V., Zhao, H. et al. *R406, an orally available spleen tyrosine kinase inhibitor blocks Fc receptor signaling and reduces immune complex-mediated inflammation*. *J Pharmacol Exp Ther* 2006, 319(3): 998-1008.
- Pine, P.R., Chang, B., Schoettler, N. et al. *Inflammation and bone erosion are suppressed in models of rheumatoid arthritis following treatment with a novel Syk inhibitor*. *Clin Immunol* 2007, 124(3): 244-57.
- Hilgendorf, I., Eisele, S., Remer, I. et al. *The oral spleen tyrosine kinase inhibitor fostamatinib attenuates inflammatory and atherogenesis in low-density lipoprotein receptor-deficient mice*. *Arterioscler Thromb Vasc Biol* 2011, 31(9): 1991-9.
- Weinblatt, M.E., Kavanaugh, A., Burgos-Vargas, R. et al. *Treatment of rheumatoid arthritis with a Syk inhibitor: A twelve-week, randomized, placebo-controlled trial*. *Arthritis Rheum* 2008, 58(11): 3309-18.
- Weinblatt, M.E., Kavanaugh, A., Genovese, M.C., Musser, T.K., Grossbard, E.B., Magilavy, D.B. *An oral spleen tyrosine kinase (Syk) inhibitor for rheumatoid arthritis*. *N Engl J Med* 2010, 363(14): 1303-12.
- Genovese, M.C., Kavanaugh, A., Weinblatt, M.E. et al. *An oral Syk kinase inhibitor in the treatment of rheumatoid arthritis: A three-month randomized, placebo-controlled, phase II study in patients with active rheumatoid arthritis that did not respond to biologic agents*. *Arthritis Rheum* 2011, 63(3): 337-45.
- Kinoshita, A., Shigemoto, R., Ohishi, H., van der Putten, H., Mizuno, N. *Immunohistochemical localization of metabotropic glutamate receptors, mGluR7a and mGluR7b, in the central nervous system of the adult rat and mouse: A light and electron microscopic study*. *J Comp Neurol* 1998, 393(3): 332-52.
- Varney, M.A., Cosford, N.D., Jachec, C. et al. *SIB-1757 and SIB1893: Selective, noncompetitive antagonists of metabotropic glutamate receptor type 5*. *J Pharmacol Exp Ther* 1999, 290(1): 170-81.
- Spooren, W.P., Vassout, A., Neijt, H.C. et al. *Anxiolytic effects of the prototypical metabotropic glutamate receptor 5 antagonist 2-methyl-6-(phenylethynyl)pyridine in rodents*. *J Pharmacol Exp Ther* 2000, 295(3): 1267-75.
- Walker, K., Bowes, M., Panesar, M. et al. *Metabotropic glutamate receptor subtype 5 (mGlu5) and nociceptive function. I. Selective blockade of mGlu5 receptors in models of acute, persistent and chronic pain*. *Neuropharmacology* 2001, 40(1): 1-9.
- Chiamulera, C., Epping-Jordan, M.P., Zocchi, A. et al. *Reinforcing and locomotor stimulant effects of cocaine are absent in mGluR5 null mutant mice*. *Nat Neurosci* 2001, 4(9): 873-4.
- Breyse, N., Baunez, C., Spooren, W., Gasparini, F., Amalric, M. *Chronic but not acute treatment with a metabotropic glutamate 5 receptor antagonist reverses the akinetic deficits in a rat model of parkinsonism*. *J Neurosci* 2002, 22(13): 5669-78.
- Pilc, A., K?odzi?ska, A., Bra?zski, P. et al. *Multiple MPEP administrations evoke anxiolytic- and antidepressant-like effects in rats*. *Neuropharmacology* 2002, 43(2): 181-7.
- Paterson, N.E., Semenova, S., Gasparini, F., Markou, A. *The mGluR5 antagonist MPEP decreased nicotine self-administration in rats and mice*. *Psychopharmacology (Berl)* 2003, 167(3): 257-64.
- Berg, D., Godau, J., Trenkwalder, C. et al. *AFQ056 treatment of levodopa-induced dyskinesias: Results of 2 randomized controlled trials*. *Mov Disord* 2011, 26(7): 1243-50.
- Jacquemont, S., Curie, A., des Portes, V. et al. *Epigenetic modification of the FMR1 gene in fragile X syndrome is associated with differential response to the mGluR5 antagonist AFQ056*. *Sci Transl Med* 2011, 3(64): 64ra1.
- Bear, M.F., Huber, K.M., Warren, S.T. *The mGluR theory of fragile X mental retardation*. *Trends Neurosci* 2004, 27(7): 370-7.
- Goor, F., Straley, K.S., Cao, D. et al. *Rescue of DeltaF508-CFTR trafficking and gating in human cystic fibrosis airway primary cultures by small molecules*. *Am J Physiol Lung Cell Mol Physiol* 2006, 290(6): L1117-320.
- Van Goor, F., Hadida, S., Grootenhuys, P.D. et al. *Correction of the F508del-CFTR protein processing defect in vitro by the investigational drug VX-809*. *Proc Natl Acad Sci U S A* 2011, 108(46): 18843-8.

