Drugs of the Future 2013, 38(4): 347-354

CCC: 0377-8282/2013
DOI: 10.1358/dof.2013.38.4.1968696

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MEETING REPORT

THERAPEUTIC OPPORTUNITIES IN INFECTIOUS DISEASES

HIGHLIGHTS FROM THE SOCIETY OF MEDICINES RESEARCH SYMPOSIUM, HELD ON MARCH 14TH 2013 AT THE NATIONAL HEART & LUNG INSTITUTE, LONDON, UK

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SUMMARY

Microbial resistance to chemotherapeutic agents is not a new development: the first lactam hydrolyzing enzyme was identified before penicillins were even introduced into the clinic. Extended-spectrum resistance to the major classes of chemotherapeutic agents is now common across many microorganisms, particularly pathogenic bacteria, and due in part to over- and misuse of antibiotics over the last 50 years. Global travel and greater social interaction has facilitated rapid transmission of infectious diseases such as malaria, tuberculosis (TB), human immunodeficiency virus (HIV) and hepatitis C virus (HCV), resulting in an international agenda for addressing the lack of prevention and treatment options for these diseases. This symposium brought together international experts from the pharmaceutical industry and academia to review the need for new antiinfective agents, present the latest therapeutic developments, and to discuss the challenges to be overcome in the discovery and clinical development of novel antiinfective agents and the development of new vaccines. Topics included novel approaches to small-molecule discovery and development for the treatment of TB, HCV and HIV, review of the vaccine approaches to meningitis and malaria, and presentation of the new vaccines in clinical trials for their prevention.

Key words: Antibacterial agents – Antiviral agents – Tuberculosis – HIV – Hepatitis C virus – Meningitis – Malaria

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THE NEXT GENERATION OF ANTIBACTERIAL AGENTS

Global and clinical implications of multidrug- and pandrug-resistant Gram-negative bacteria

Professor Timothy Walsh, Cardiff University, U.K., opened the meeting with a review of the discovery timeline for antibiotics in clinical use, which has seen no new classes of agents licensed since 1987, and the increasing incidence of resistance to antibacterial agents. Much recent effort has focused on meticillin-resistant *Staphylococcus aureus* (MRSA) in the U.K., resulting in a steady decrease of incidence levels, dropping from 40-50% in 2000-2005 to 14% in 2012, with projections of a steady state of 10% over the next decade, although this resistant pathogen remains at 40-50% in other countries, for example Greece and Romania (1). However, it is the Gram-negative bacteria for which resistance is predicted to rise sharply over the next 30 years; resistance to cephalosporins and other β-lactams, including carbapenems, in *Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella* species is already rising. Since the discovery of *Klebsiella pneumoniae* carbapenemases (KPCs) in 1998, this particular mode of resistance has spread across the globe, with a > 50% incidence in certain countries, e.g., Turkey and Greece. Perhaps of even greater threat, the New Delhi β-lactamase NDM-1 was first noted in clinical cases arising from the Indian subcontinent in 2002 and has since spread steadily, not only across countries and continents, but also across bacterial species. The bla_{NDM-1} gene is carried on a plasmid, along with the resistance genes for other antibacterial agents, and has been identified in many countries and species; for example, *E. coli* in Japan,

Hong Kong, Canada, Kenya and Spain, *K. pneumoniae* in Sweden and *Acinetobacter baumanii* in Germany. In the era of accessible global travel, it should not be surprising that the rising spread of bacterial resistance reflects the increased movement of travelers (2).

Despite the considerable number of articles in the press about its epidemiology, the prevalence of NDM-1, particularly in Northern India, Bangladesh and Pakistan, where it appears to have originated, is unclear. The newly initiated charity South Asian Antibiotic Resistance Research Program aims to harness collaborations between researchers in India, Bangladesh, Pakistan and the U.K. to evaluate the incidence. So far, NDM-1 has been found across a wide range of Enterobacteriaceae from hospital patient swabs, seepage water and sewage. KPC spread worldwide to an incidence of about 14% in the 15 years since it was first identified; since 2002, NDM-1 has achieved an incidence of > 40% and is still increasing in prevalence. In response to the threat posed by increasing bacterial resistance to antibacterial agents and aiming to tackle this global problem, the World Health Organization (WHO) released an updated report in March 2012 — The Evolving Threat of Antimicrobial Resistance: Options for Action (3).

However, the picture may not be as bleak as it first seems; people infected with NDM-1-producing bacteria can be asymptomatic, indicating that such bacteria are not necessarily pathogenic. In the *Galleria mellonella* (honeycomb moth) larvae model system, an inverse relationship was observed between virulence and resistance –in this species at least, NDM-1-expressing *E. coli* did not cause high mortality (4), suggesting that most people would not be at risk of mortality from infection by these resistant bacteria. If these observations were borne out clinically, the major risk from these NMD-1-expressing bacteria would be for patients in intensive care, transplant patients and other immunocompromised patients.

For the development of future antibacterial agents, a focus on *Klebsiella* and other pathogenic Gram-negative bacteria would offer a greater clinical contribution. Furthermore, *Klebsiella* species appear to acquire and spread resistance readily; tackling this species would make an important contribution to the management of clinical bacterial infections.

Development of new drugs for resistant bacteria: the challenges

The emergence of multidrug-resistant bacteria is currently a major world health problem. Hospital-acquired infections are becoming more difficult to treat with a concomitant increase in mortality and morbidity –25,000 attributable deaths in the E.U. in 2007. There is a significant risk that in the relatively near future some regular medical procedures may become impossible (e.g., hip replacement operations, care of premature babies, chemotherapy).

Dr. Seamus O'Brien, AstraZeneca, U.K., discussed recent data demonstrating the startling increase and unpredictable spread in drug-resistant bacteria. This has made it very difficult to plan and conduct clinical trials of agents targeting these bacteria using the classical disease-focused pathways. The emergence, since the turn of the 21st century, of carbapenem-resistant Enterobacteriaceae (CRE), and in particular multidrug-resistant *K. pneumoniae*, is a major concern due to the lack of effective antibacterials. Excellent data on the incidence of carbapenem-resistant *K. pneumoniae* (CRKP) are available (2011 data) for most countries in Europe, and demonstrate significantly variable rates, with the greatest incidence in South Eastern European states (up to 50%), and a relatively low incidence in the U.K. (< 1%). In recognition of this issue, the U.S. Centers for Disease Control and Prevention (CDC) recently published new guidance (March 2013) for the control of CREs.

There are three major challenges to the discovery and development of new antibacterials, which have been factors in the reduced number of major pharmaceutical corporations working on antibacterial research in recent years. Firstly, various research challenges exist. For example, antibacterials are structurally disparate to other pharmaceutical molecules and hence corporate archives used for high-throughput screening (HTS) are relatively devoid of appropriate chemotypes. Secondly, the clinical development environment, and in particular the regulatory pathway, is very challenging. Thirdly, there is currently a relatively low return on investment, as any new agents would only be used as last-resort medications and may stay so throughout their limited period of exclusivity. More recently, however, there has been a worldwide awakening to the need to tackle the issue of resistance with several high-profile U.S., E.U. and global initiatives.

A consortium of four pharmaceutical corporations has added to the debate in this area by addressing novel approaches to antibacterial clinical development and proposing the adaptation of existing regulatory frameworks to address the unmet need for new antibacterial treatments (5). Key to these proposals is that regulatory frameworks, including orphan drug and conditional approvals, recognize both the extent of unmet medical needs and the feasibility of clinical studies in the intended population to control the amount of data needed for registration. The use of a new middle way to approval that focuses on the pathogen rather than the site of infection would enable smaller clinical studies supported by extensive preclinical packages, providing a "totality of evidence" approach to registration of antibacterials for drug-resistant and rare pathogens. The aim is to avoid the paradoxical situation of being forced in the future to accept even greater degrees of therapeutic uncertainty as antimicrobial resistance progresses. Such changes to the regulatory environment are close; for example, in July 2012 the European Medicines Agency released new additional guidance for the development of antibacterials for public consultation, and in October 2012 the U.S. Congress passed the Generating Antibiotic Incentives Now (GAIN) Act, which mandates the FDA to develop guidelines for Limited Population Antibiotic Development (LPAD) programs.

There is also an accepted need for public–private collaboration, as arguably the challenges are too great for any single entity to solve. An example of such collaboration is the Innovative Medicines Initiative (IMI) – New Drugs4BadBugs (ND4BB) Programme. This has the overall vision to create an innovative public–private partnership-based approach to encompass all aspects from the discovery of new antibiotics to phase II and III clinical trials, with the aim of reinvigorating antibiotic R&D. Some topics have now been agreed, including a consortium –TRANSLOCATION– to improve the understanding of drug permeability into Gram-negative bacteria. A second consortium –COMBACTE– is tasked with improving the capability for antibiotic clinical development through the creation of a clinical investigator and laboratory networks, applying cutting-edge clinical trials and undertaking therapeutic clinical trials. Additional topics for 2013 include the discovery and early development of new drugs combating Gram-negative infections.

In summary, there are currently positive actions as government and industry realize the need to be faster and more flexible to develop new antibiotics. This is beginning, but more is needed to reinvigorate the discovery of targets and leads, improve clinical epidemiology and assess new antibiotic business models.

Lessons learned in tuberculosis drug discovery

Dr. Jan Jiricek from the Novartis Institute for Tropical Diseases in Singapore presented recent results from their tuberculosis (TB) drug discovery program. One-third of the world's population is currently infected with the TB bacillus, of which 5-10% become infectious at some time during their life. People with HIV and TB infection are much more likely to develop TB. There are 4,500 deaths/day because of TB. Combinations of drugs are the mainstay of TB treatment, which consists of 6-12 months of therapy. Multidrug-resistant (MDR) and extensively drug-resistant (XDR) cases are currently on the rise and require second-line therapy, longer duration of treatment (approximately 2 years), have greater potential side effects and are 100-fold more expensive. The cure rate for the treatment of MDR and XDR is relatively poor (60% and 30%, respectively) and it has been 50 years since the discovery of the most recent TB drug.

Dr. Jiricek gave an overview of current TB treatment options and their challenges and then discussed the Novartis Institute for Tropical Diseases (NITD) phenotypic TB drug discovery approach. Between 2006 and 2011, multiple HTS's using phenotypic cellular-based assays were performed and resulted in over 16,000 hit molecules. Applying various chemical in silico filters, and the addition of compounds from a literature review, resulted in a reduced hit list of approximately 7,000 compounds. However, few hits were in ideal "lead-like" chemical space (clogP < 4; Mol wt < 450), with a minimum inhibitory concentration (MIC) of < 1 μM. Many of these hits contained familiar functionality from the field of antibiotics and were eliminated from further follow-up if they contained such a chemotype or had an anticipated undesirable or nonspecific mechanism of action. This eventually resulted in six lead series for further consideration.

Lead series 1 illustrated Dr. Jiricek's first lesson: the importance of growth medium in the in vitro assay conditions. Compound **3** (Figure 1), along with several other close analogues in series 1, showed potent in vitro activity (MIC = 36 nM) and yet was totally inactive in an acute mouse model of TB infection. Dr. Jiricek and colleagues (6) were able to show that this was due to an in vitro assay artefact. Series 1, unlike reference antibacterials, was dependent on glycerol (the specific carbon source used in the gold standard in vitro MIC assay), whereas in vivo multiple carbon sources are available.

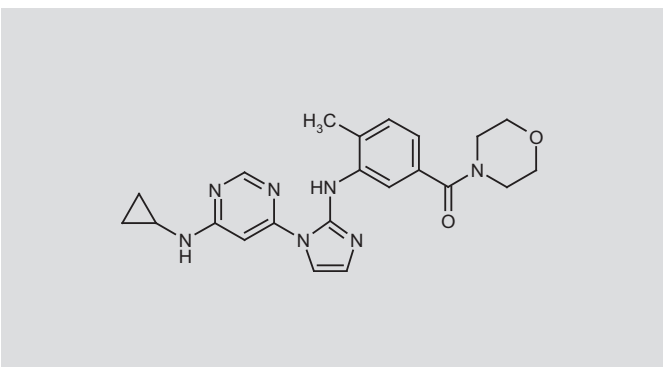

Figure 1. Compound 3 and example of lead series 1.

Dr. Jiricek then described an analysis of the physicochemical characteristics of the validated lead series 2-6 in comparison with known TB drugs. The major lessons learned here were that known TB drugs mostly occupied a different chemical space to the lead series compounds and the lead series compounds were generally of high lipophilicity (Figure 2). This latter property was anticipated to hinder the development of such molecules due to their poor aqueous solubility.

The challenges of optimizing the lead compounds towards candidate molecules were described and illustrated with the imidazopyridine series 2 compounds. In addition to the normal multiparameter optimization issues associated with drug development, in the case of such phenotypic screens, the lack of a well-defined molecular target adds additional challenges to gaining an understanding of the structure–activity relationship (SAR). In the case of series 2 compounds, minor structural modification did deliver potent in vitro compounds (MIC = 10 nM); however, in vivo assessment of bactericidal activity demonstrated that the compounds were only effective as bacteriostatic agents. The goal of this work was to develop agents which were effective bactericidal agents, and indeed, this was the final lesson from Dr. Jiricek's presentation, the need to focus quickly on the chemotypes capable of killing the bacteria.

RECENT ADVANCES IN ANTIVIRAL AGENTS

Discovery and development of simeprevir (TMC-435) and TMC-647055, two novel direct antiviral agents targeting hepatitis C virus

Dr. Pierre Raboisson, Janssen Infectious Deseases (Belgium), introduced the challenges of hepatitis C virus (HCV) as a drug target and described the discovery process leading to the development of simeprevir. HCV infection is the number one leading cause of liver transplant in the U.S. and is responsible for approximately two-thirds of all liver transplants. HCV infection is responsible for between 50 and 75% of all liver cancers, having progressed from an initial acute infection to hepatocellular carcinoma, and a health care burden that is estimated to be several billion dollars worth per year. Approximately 3% of the world's population is infected with HCV across the six major genotypes, and the current standard of care (PEG-interferon alfa-2 and ribavirin combination, and the recently approved NS3 protease inhibitors telaprevir and boceprevir) is limited due to issues of toleration, long duration of treatment (up to 48 weeks) and limited efficacy in partial and null responders. This presentation described the drug discovery program that was initiated by Tibotec (now Janssen) to identify novel direct-acting antivirals that were suitable for once-daily dosing and could be incorporated into an enhanced-efficacy, interferon-sparing regimen.

The first part of the presentation focused on the discovery and development of TMC-435, a next-generation NS3 (7, 8) protease inhibitor that has recently been submitted for approval in Japan for the treatment of HCV genotype 1 treatment-naive and nonresponder patients.

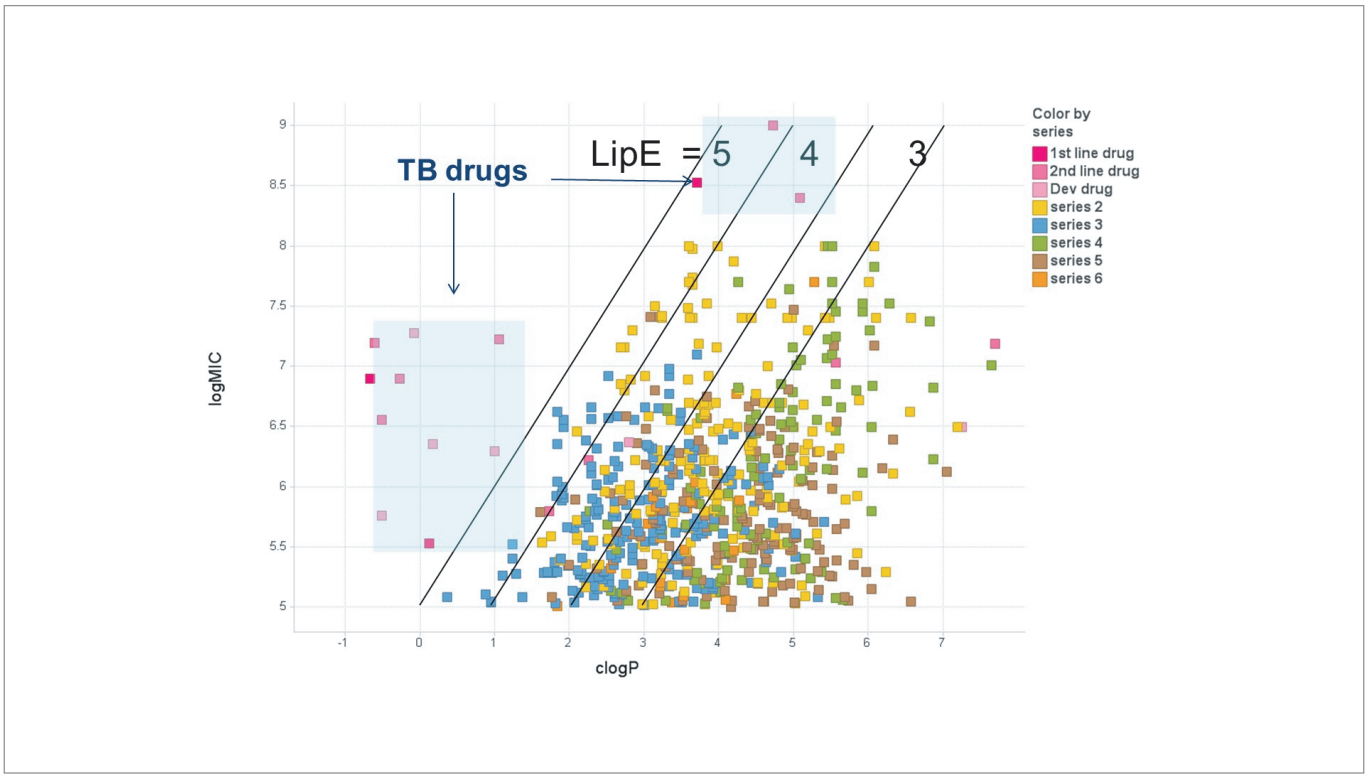

Figure 2. Log MIC vs. clogP for series 2-6 analogues and established TB drugs.

In a collaborative effort between Medivir and Tibotec, the published NS3 protease noncovalent macrocyclic inhibitor BILN-2061 was truncated and functional groups optimized to provide a lead acyl sulfonamide that was a potent NS3 inhibitor but had very poor oral bioavailability in rats. Systematic optimization of the central cyclopentane scaffold, the size and structure of the macrocyclic ring, the P2-mimic heterocycle and isosteres of the P1 acidic acyl sulfonamide led to the discovery of TMC-435 (simeprevir) (Figure 3), a potent and efficacious noncovalent NS3 protease inhibitor that was safe and well tolerated in healthy volunteers and was shown to be suitable for once-daily dosing. As monotherapy, 5-day treatment of TMC-435 200 mg once daily produced a 4 $\log_{10}$ reduction in viral load and showed equivalent efficacy against both genotypes 1a and 1b.

Dr. Raboisson also described a discovery program to identify macrocyclic inhibitors of the HCV NS5B polymerase based on a substituted indole starting point. Several macrocyclic linkage designs were developed using structure-assisted hypothesis generation to identify a candidate compound, TMC-647055, that binds to the thumb-1 domain of the HCV NS5B protein. Combination studies of various compounds that targeted different viral proteins were carried out in replicon-resistant cell lines, and showed that TMC-435 had an additive effect when combined with ribavirin or a non-nucleoside NS5B inhibitor such as TMC-647055, but was synergistic when combined with interferon or a nucleoside NS5B inhibitor. TMC-647055 has been advanced into a phase Ib study in HCV-infected patients, and showed an approximately 5-hour terminal half-life. Doses of up to 1000 mg administered twice daily over 6 days to genotype 1b patients produced up to 3.4 $\log_{10}$ reductions in HCV RNA and similar levels of antiviral efficacy in genotype 1a patients. TMC-647055 was well tolerated and efficacy was found to be dose-dependent and related to plasma levels of active component. It is currently being evaluated in phase II clinical studies in combination with simeprevir and the cytochrome P450 CYP3A4 inhibitor ritonavir dosed once daily, with and without ribavirin, to patients chronically infected with HCV genotype 1.

Discovery of a novel allosteric mechanism for the regulation of HCV NS3/4A protein using fragment screening and structure-based design

Susanne Saalau-Bethell from Astex Pharmaceuticals, U.K., presented a project that used fragment-based screening methods applied to the full-length HCV NS3 protease to identify inhibitors that bound to a novel and functionally relevant allosteric binding site. Fragment screening offers a highly efficient means of sampling chemical space against a target protein, and the Astex approach is built on their X-ray crystallography platform. Using crystallography alongside alternative biophysical techniques, Astex screened the full-length HCV NS3 protease (a protein that comprises two domains, a helicase and a protease domain) with a small collection of 176 fragments. Hits were identified as binding at one of three sites, including the RNA binding groove and the ATP binding pocket, but most intriguing was a site located at the junction of the two protein domains (9). Some 16 fragments were found that bound to this site, with binding affinities of

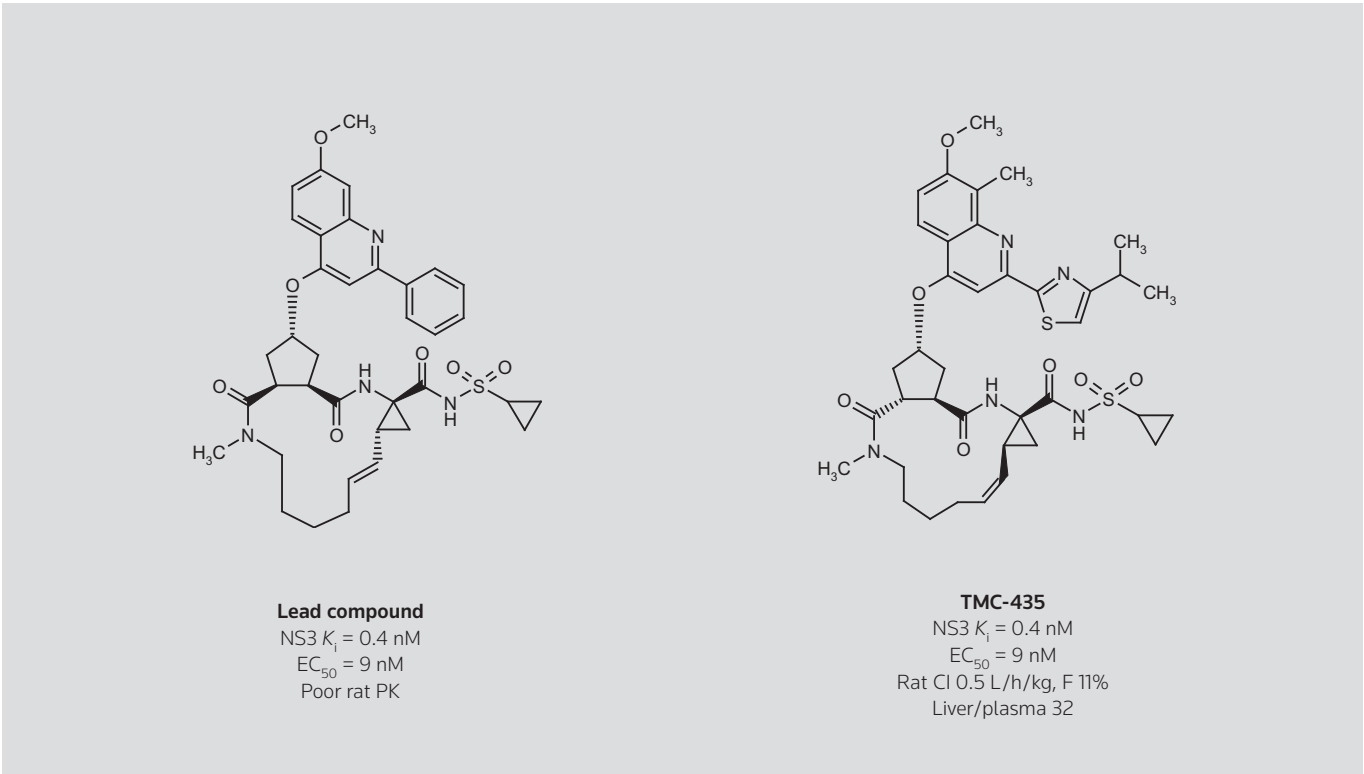

Figure 3. Development of NS3 lead inhibitor to TMC-435 (simeprevir).

between 5 mM and 100 µM and low to moderate ligand efficiencies. They were confirmed active only to the full-length protein and not to the protease domain alone. The working hypothesis that Astex developed was that the HCV NS3 protein could populate either an open active state or a closed inactive state via a hinge-like movement of the two linked domains. It was the latter state that was believed to be stabilized by the fragment hits and was also sensitive to salt concentration in the buffer conditions used for protein preparation (10). This interfacial binding site was found to be highly conserved across HCV genotypes. Fragment optimization was carried out to provide several leads with low micromolar or even submicromolar activity, which were used to raise mutations in replicon cells that mapped to the interfacial binding site, e.g., V630L. Two preclinical candidates were eventually identified with low nM replicon cell-based potency, excellent physicochemical properties and a predicted human dose of between 300 and 600 mg. The structures of the preclinical candidate molecules have not yet been disclosed.

LEDGINs, a novel class of antivirals targeting HIV integration

Professor Zeger Debyser, KU Leuven (Belgium), started with a summary of the current HIV treatment strategies and the clinical inhibitors with their targets, and then focused on integrase, its role in the HIV life cycle and the current integrase inhibitors. The integrase strand transfer inhibitors (INSTIs) raltegravir and elvitegravir are already licensed for use, and a third agent, dolutegravir, is in clinical trials; all of these agents inhibit strand transfer, resulting in a strong reduction in viral load. However, resistance and cross-resistance between the members of this class of agents has already been noted, and next-generation INSTIs are under development. To avoid the issue of increasing resistance, new targets are required that do not show cross-resistance with existing drugs.

Professor Debyser presented a rationale for a novel class of anti-HIV agents targeted at cellular cofactors. These host-derived proteins are hijacked by HIV proteins, such as integrase, and are essential to assist the viral proteins in performing their function; agents designed to inhibit the interaction between proteins and their cofactor could reduce the development of resistance, as these binding sites are likely to be conserved. However, such targets are associated with specific challenges: protein–protein interactions are notoriously difficult to target efficiently and inhibiting the interactions of a host protein could lead to cellular toxicity. The group focused on the steps catalyzed by integrase, particularly the transport of DNA into the nucleus and integration, using a workflow decision tree approach, which identified several possible targets, such as LEDGF/p75 and transportin SR2. Validation and characterization of the role of the targets, including co-immunoprecipitation, knock down experiments, structural and cell biology, led to LEDGF/p75 being identified as a suitable target (11, 12). Lens epithelium-derived growth factor (LEDGF) is a transcriptional coactivator involved in the normal mammalian cellular stress response; it is hijacked by the HIV preintegration complex as a molecular tether for the viral cDNA, as it is transported into the nucleus and inserted into the host DNA. Through structure-based drug design, medicinal chem-

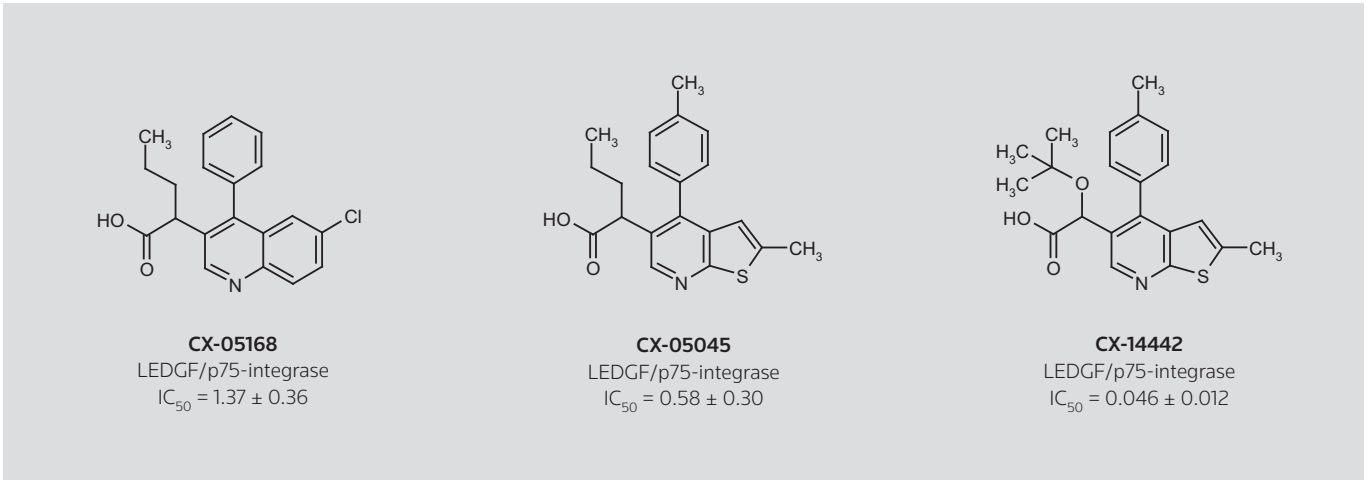

Figure 4. Development of the LEDGIN class of inhibitors; from the hit CX-05168 to the lead CX-14442.

istry, structural biology and virology, a family of inhibitors was developed –the LEDGINs (Figure 4) (13).

Co-crystallization of a lead LEDGIN with LEDGF/p75 and HIV-1 integrase confirmed that the inhibitor directly binds to the LEDGF/p75 binding pocket of HIV-1 integrase (hence the name), allosteric to the active site, preventing the LEDGF/p75–integrase interaction; inhibition of integrase activity was also noted, despite their allosteric binding site (13). Development of further LEDGINs with structural diversity, nanomolar potency and good selectivity for the integrase–LEDGF/p75 interaction (12, 14) allowed investigation into the mechanism of integrase inhibition, showing that the LEDGINs stabilize the dimer form of integrase in a concentration-dependent manner, which interferes with catalytic activities (14, 15). Furthermore, LEDGINs decrease the infectivity of HIV viral progeny, results that may indicate a role for LEDGF/p75 in virion assembly (14).

The LEDGINs exhibit activity across a broad range of HIV-1 subtypes and display no cross-resistance with other integrase inhibitors; in fact, additive to synergistic activity was shown for lead compound CX-14442 with the integrase inhibitor raltegravir, with no antagonism (14). Thus, the LEDGINs represent a novel class of antiretroviral (ARV) agents, with a novel and multimodal mechanism of action, synergistic with existing ARVs, and with the potential to make an important contribution to the future treatment of HIV.

ADVANCES IN VACCINES TARGETING INFECTIOUS DISEASE

Advances in meningococcal vaccine research

Dr. Peter Dull, Head of Development Meningitis and Sepsis Vaccines, Novartis Vaccines and Diagnostics (Novartis, U.S.), gave an overview of the development of 4CMenB (Bexsero®), a serogroup B meningococcal disease vaccine. Meningococcal disease (meningitis) is a sudden, unpredictable and potentially fatal disease which typically occurs in otherwise healthy people without identified risk factors. Early symptoms are flu-like before rapid progression to death within 24 to 48 hours, with hospitalization only usually when late-stage symptoms appear at, on average, 19 hours. Meningococcal disease can lead to significant morbidity and mortality among adolescents (15-24 years) and infants (0-4 years), where 5 to 15% of cases are fatal and 11 to 19% of survivors have significant sequelae, such as amputations, deafness and neurological defects. A successful vaccination campaign against serogroup C meningococcal disease in the U.K. in 1999 led to its rapid decline and near eradication. Similarly, vaccines have dramatically reduced *Haemophilus influenzae* type b and pneumococcal disease rates where the vaccines were widely implemented from the mid-1980s to the early 2000s. However, serogroup B meningococcal disease (MenB) remains a significant medical need and alternative vaccine strategies have been required to address it. Glycoconjugate vaccines against meningitis-causing pathogens have been successful by utilizing the polysaccharide capsule; however, in the case of MenB, the capsule was found to be nonimmunogenic. Therefore, Novartis adopted an innovative, genomic-based approach to identifying broadly conserved protein antigens which induce bactericidal antibodies through a process called "reverse vaccinology". The complete sequencing of the *Neisseria meningitidis* genome and bioinformatics analysis allowed the identification of 570 potential antigens, of which 350 were expressed in *E. coli* and tested for immunogenicity in mice. Confirmation of surface exposure and bactericidal activity led to the selection of 3 proteins: *Neisseria* adhesin A, factor H binding protein and *Neisseria* heparin-binding antigen, which are combined with the outer membrane vesicle component NZ PorinA 1.4 in the final vaccine formulation (16). Novartis has completed clinical trials of 4CmenB in 1,712 adults and adolescents, 250 children and 5,850 infants with a variety of vaccination schedules and has compiled an extensive clinical database. 4CMenB demonstrated a protective immune response across all age groups, with, for example, 95-100% of infants having sufficient bactericidal titers after a 2-, 4-, 6- and 12-month dosing schedule alongside routine vaccines. Novartis has also validated and standardized the Meningococcal Antigen Typing System (MATS), an ELISA test which gives predictive coverage estimates for 4CMenB, by examining the presence of antigens used in 4CMenB in circulating meningitis strains. MATS results from 8 European countries, Australia, the U.S., Brazil and Canada on over 2,000 MenB strains estimate that 66 to 91% would be covered by 4CMenB (17). In November 2012, the Committee for Medicinal Products for Human Use (CHMP) adopted a

positive opinion, recommending the granting of a marketing authorization for 4CMenB (Bexsero®) and a license was granted in January 2013 after the E.U. commission provided endorsement. Therefore, the potential for broad protection from the last of the major causes of childhood bacterial meningitis is now available.

RTS,S: the first licensed malaria vaccine?

Professor Eleanor Riley, London School of Hygiene and Tropical Medicine, U.K., described the burden of disease for malaria; there are annually 216 million clinical episodes and 655,000 deaths in 2010 predominantly in children under 5 years in sub-Saharan Africa (WHO figures). However, a recent paper in The Lancet (18) cited 1.2 million malaria deaths in 2010 due to a higher incidence in Asia and in adults than have been estimated by the WHO. There is also rising awareness of increasing malaria deaths in Southeast Asia due to *Plasmodium knowlesi*, an emerging zoonotic species of malaria with high fatality rates. Patterns of malarial disease show severe malaria mainly in children under 10 years of age, but then a very large adult population with mild or asymptomatic disease. The most frequent causes of hospitalization are severe anemia, cerebral malaria and severe respiratory distress. Professor Riley described the lifecycle of *Plasmodium falciparum*, a protozoan parasite that is transmitted by the female mosquito. Sporozoites released from the salivary glands of the mosquito enter the human bloodstream during feeding, quickly invading liver cells, where they undergo asexual multiplication. This results in merozoites, which are released from the hepatocytes and invade more red blood cells (RBCs). There is then an obligate sexual reproduction in the mosquito which leads to very high genetic diversity of the parasite. Sequestration and rosetting (clumping of cells) also occur, whereby the infected RBCs adhere tightly to endothelial cells in the vasculature, leading to obstruction of the microcirculation, and result in dysfunction of multiple organs, such as brain, lungs and also the placenta during pregnancy. There are several points during the lifecycle at which malarial vaccination intervention can be directed; the pre-erythrocytic stage, the blood stage and during transmission or sexual reproduction, and all of these are being investigated for vaccine development. However, malaria vaccine development has proved to be very challenging and as yet there is no licensed, clinically effective malaria vaccine. The complex life cycle of malaria parasites and the stage-specific nature of immune responses pose great challenges for vaccine development and there are also no good animal models for the disease. There are no malaria "strains", since every infection is genetically unique due to extensive polymorphism; clinical disease is typically associated with infection by a new parasite genotype. Also, conserved antigens/regions tend to be poorly immunogenic and many antigens are highly polymorphic, especially those expressed at the surface of the parasite. In the last 40 years, all vaccine types have been tried, and synthetic peptides and DNA vaccines proved unsuccessful. Live, attenuated vaccines, virally vectored vaccines and recombinant proteins are all in clinical trials or have been tested in humans. For example, the most effective vaccine to date is attenuated (irradiated) whole sporozoites; this approach is under development by Sanaria (www.sanaria.com). The vaccine is administered via bites of infected mosquitoes giving 100% protection over 12 weeks against homologous and heterologous strains of parasites, with protection up to 42 weeks in some individuals. Drug-attenuated sporozoite infection and ultra-low-dose blood-stage infection are also being studied. RTS,S has the potential to be the first licensed malaria vaccine and is in development by GlaxoSmithKline and the Walter Reed Army Institute of Research with funding also from the Bill & Melinda Gates Foundation via the PATH Malaria Vaccine Initiative (http://www.malariavaccine.org). RTS,S is a recombinant chimeric protein representing the repetitive B cell (R) and T cell (T) epitopes of the CS protein of the 3D7 genotype of *P. falciparum* linked to hepatitis B virus surface (S) antigen. Extensive phase I and II trials have been carried out and the vaccine is currently in phase III. In adult males in the Gambia, the vaccine efficiency was found to be 34% over 16 weeks of surveillance, while in a long-term follow-up study in children in Mozambique the vaccine efficiency against mild malaria episodes was 29% and the efficiency against severe malaria was 44%. The vaccine was still effective in the second year post-vaccination via an unknown mechanism. The phase III trial is a multicenter study at 11 sites with 16,000 children vaccinated at 6-12 weeks or 5-17 months on a 0-, 1- and 2-month vaccination schedule. The initial results were promising of partial efficacy, with 50.4% efficacy against clinical malaria episodes in the 14 months after the first dose of vaccine (19). However, next-generation vaccines need to include blood-stage antigens to mop up breakthrough infections and a transmission blocking component, and complementation of RTS,S may be the most efficient way to achieve this. However, most blood-stage vaccines have failed; at least 10 candidate vaccines were discontinued at or before phase II trials and only 3 of the 20 remaining candidates have so far reached phase IIb. There is hope, however, with the recent discovery of basigin as an essential receptor of which the parasite ligand (PfRH5) is relatively invariant (20), which may prove successful in combating this deadly disease in combination with RTS,S.

CONCLUSIONS

Resistance of microorganisms to the current arsenal of clinical anti-infective agents continues to rise, leading to the threat of untreatable infections. "Big Pharma" research into new antibacterials has, unfortunately, concomitantly declined due to the general challenges of antibiotic research, the low return on investment and a difficult regulatory environment. These themes have, to some extent, been mirrored in the antiviral and antiinfective vaccinology fields. However, more recently the "green shoots" of a new era may be sprouting. Firstly, multidisciplinary research groups straddling academia and industry have appeared offering creative solutions to target identification, validation and drug discovery. Secondly, international collaborations have led to sustained progress in drug and vaccine development. Thirdly, pragmatic solutions to the current mismatch and complexity of regulatory authority requirements for new antiinfective agents are on the near-term horizon through European-led initiatives such as IMI. The challenges, solutions and progress shared in this SMR meeting provided encouraging examples of prevention and treatment developments and a welcome optimistic counter to the near-future, worldwide threat of pan-resistant microorganisms.

DISCLOSURES

W.K. Alderton is an employee of Abcodia, S.P. Collingwood is an employee of Novartis and D. Pryde is an employee of Pfizer. R.J. Anderson states no conflicts of interest.

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