Institution: St George's, University of London

Unit of Assessment: A1 Clinical Medicine

Title of case study:

Development of dual targeting antibacterials and the circumvention of resistance

1. Summary of the impact (indicative maximum 100 words)

Genetic, biochemical and structural characterisation of drug targets in the human pathogen *Streptococcus pneumoniae* by Fisher and colleagues at St George's showed that antibacterial quinolones selectively target the enzymes gyrase, topoisomerase IV, or both, and led to the concept that 'dual targeting' drugs minimise the emergence of drug resistance. They demonstrated the potency and the mechanism of action of besifloxacin, a fluoroquinolone developed by Bausch and Lomb which was subsequently approved by the FDA in 2009 for treatment of bacterial conjunctivitis. This has been shown to be a highly efficacious treatment with correspondingly increased usage and sales in the USA.

2. Underpinning research (indicative maximum 500 words)

Quinolones are a class of broad-spectrum antibiotics that act by prevention of bacterial DNA replication by interfering with the action of the bacterial topoisomerase enzyme that acts to unwind DNA prior to replication. *Streptococcus pneumoniae*, the gram-positive bacterium responsible for pneumococcal pneumonia and multiple other infections including some cases of meningitis, is typically sensitive to penicillins, but serious concern has arisen over the emergence of penicillin-resistant strains. Consequently, efforts have been made to develop other antibiotics effective against this pathogen. Throughout the last two decades, the Fisher group based at St George's has made a number of seminal contributions.

The dual targeting hypothesis

Development of quinolones effective against *Streptococcus pneumoniae* was initially hindered by a lack of studies on quinolone action in Gram-positive pathogens. Bacteria usually manufacture two related type II topoisomerases - DNA gyrase, which regulates DNA supercoiling of the circular bacterial chromosome, and topoisomerase IV (topo IV) which unlinks catenated chromosomes allowing their segregation at cell division. Building on expertise developed in *E.coli*, Fisher and colleagues at St George's successfully cloned and sequenced the pneumococcal genes for topoisomerase IV (*parC*/*parE*) and gyrase (*gyrA*/*gyrB*) in 1994, and developed a novel assay to identify mutations causing resistance. In a series of studies, the group established that topoisomerase IV, gyrase, or both, could be quinolone targets depending on the quinolone structure [1,2]. This work led to the suggestion that 'dual-targeting' drugs (acting equally through gyrase and topoisomerase IV) would be clinically advantageous in terms of minimising resistance, as this would require the occurrence of mutations in both targets - a rare event [1] . This concept was validated in 1998 when they demonstrated that clinafloxacin, an experimental fluoroquinolone, satisfied the criteria of a dual-action drug. Clinafloxacin does not select first-step topoisomerase mutants, and mutations in both *parC* and *gyrA* are necessary to register resistance [2]. This work was the result of substantial studentship- and postdoctoral-funding from Parke-Davis over many years, and more latterly from Smith Kline Beecham via an MRC CASE studentship.

Mechanism of quinolone action

In addition to genetic studies, analysis of drug action against their protein targets is an essential and complementary component in understanding how they work. Pan & Fisher successfully expressed recombinant pneumococcal ParC, ParE, GyrA and GyrB proteins in *E. coli*, yielding milligram amounts of proteins that reconstituted highly active enzyme complexes [3]. The group showed that dual acting quinolones exhibited a balanced inhibition of both gyrase and topoisomerase IV and were obstructed by GyrA/ParC mutations [4,5]. Recently, in collaboration

with Sanderson at KCL, Fisher's group supported by successive BBSRC funding awards (PI – Fisher) solved for the first time the structures of quinolone-DNA, and quinazolinedione-DNA complexes with topoisomerase IV, providing the first structural insight into the mode of action of these two classes of antibacterial agent [6,7].

3. References to the research (indicative maximum of six references)

1. Pan XS, Ambler J, Mehtar S, Fisher LM. Involvement of topoisomerase IV and DNA gyrase as ciprofloxacin targets *in Streptococcus pneumoniae*. Antimicrob Agents Chemother. 1996; 40, 2321-2326. No DOI available.

2. Pan XS and Fisher LM. DNA gyrase and topoisomerase IV are dual targets of clinafloxacin action in *Streptococcus pneumoniae*. Antimicrob Agents Chemother. 1998 Nov; 42, 2810-2816. No DOI available.

3. Pan XS and Fisher LM. *Streptococcus pneumoniae* DNA gyrase and topoisomerase IV: overexpression, purification and differential inhibition by quinolones. Antimicrob Agents Chemother. 1999 May;43(5):1129-36. No DOI available

4. Heaton VJ, Ambler JE and Fisher LM. Potent antipneumococcal activity of gemifloxacin is associated with dual targeting of gyrase and topoisomerase IV, an in vivo preference for gyrase and enhanced stabilization of cleavage complexes in vitro. Antimicrob Agents Chemother. 2000, 44, 3112-3117. doi: 10.1128/AAC.44.11.3112-3117.2000, doi: 10.1128/AAC.44.11.3112-3117.2000

5. Yague G, Morris JE, Pan XS, Gould KA, Fisher LM. Cleavable complex formation by wild-type and quinolone-resistant *Streptococcus pneumoniae* type II topoisomerases mediated by gemifloxacin and other fluoroquinolones. Antimicrob Agents Chemother.2002; Sept; 46, 413-419. , doi: 10.1128/AAC.46.2.413-419.2002

6. Laponogov I, Sohi MK, Veselkov DA, Pan XS, Sawhney R, Thompson AW, McAuley KE, Fisher LM, Sanderson MR. Structural insight into the quinolone-DNA cleavage complex of type IIA topoisomerases. Nature Struct Mol Biol. 2009 Jun; 16, 667-669. doi: 10.1038/nsmb.1604

7. Laponogov I, Pan XS, Veselkov DA, McAuley KE, Fisher LM and Sanderson MR. Structural basis of gate-DNA breakage and resealing by type II topoisomerases. PLoS ONE 2010; 5, e11338. DOI: 10.1371/journal.pone.0011338

4. Details of the impact (indicative maximum 750 words)

Gemfloxacin

During the course of studies on *S. pneumoniae* gyrase and topoisomerase IV, Fisher and colleagues were approached by SmithKline-Beecham to define the mode of action of gemifloxacin (Factive), a fluoroquinolone licensed from LG Pharmaceuticals. By using a panel of defined *S. pneumoniae* mutants bearing known alterations in *gyrA* or and *parC* genes, by mutant selection and by studies of pneumococcal gyrase and topoisomerase IV, it was demonstrated that gemifloxacin has a balanced activity through both the gyrase and topoisomerase IV i.e. it fulfils the criteria of a dual targeting antibacterial agent (refs 4 and 5 above). These studies were instrumental in the approval of gemifloxacin (NDA 021158) by the FDA in 2003 [A] for the treatment of community-acquired pneumonia arising from *S. pneumoniae*, from penicillin-resistant *S. pneumoniae* and from multidrug-resistant *S. pneumoniae*.

Gemifloxacin is marketed worldwide by LG and in North America by Vansen Pharma (previously by Oscient Pharmaceuticals and Cornerstone Therapeutics) with companies emphasizing its potency and dual action properties through reference on their websites to Fisher's work [B]. Gemifloxacin

continues to be used in the treatment of refractory pneumococcal infections and to date more than 1.9 million prescriptions have been filled in the United States since it was first launched with sales of $5.1M and $6.3M the US in 2010 and 2011 [C,D].

Besifloxacin – FDA approval 2009

In 2008, Fisher and colleagues established the mode of action of besifloxacin, a novel fluoroquinolone developed by Bausch and Lomb. They determined its potency and target specificity against *S. pneumoniae* and *Staphylococcus aureus*, the two bacterial pathogens most frequently involved in bacterial conjunctivitis [E]. The properties of the drug established by these genetic and biochemical means were consistent with the desired dual targeting of gyrase and topo IV and lower resistance development. The Fisher group used recombinant human topoisomerase II (cloned by them and expressed in yeast) to show that besifloxacin was highly selective in inhibiting bacterial topoisomerases whilst having little effect on human topoisomerase. Their work contributed significantly to the FDA approval for the drug (NDA 22-308) granted in May 2009 [F] - extended in Sept 2012 to other indications including virulent sight-threatening pathogens such as *Pseudomonas aeruginosa* [G]. Besifloxacin (Besivance) has been shown to resolve bacterial conjunctivitis more rapidly and efficiently than other treatments on the market providing significant patient benefit [H]. InSite Vision (which took a single-figure royalty for providing the mucoadhesive formulation) recorded $1.2 million besifloxacin royalty revenues in 2011, and $2.1 million for 2012 [I]. 400,000 prescriptions for besifloxacin were filled in the US from November 2010 through March 2012 [J].

Development of new antibacterials

Fisher and colleagues' work on topoisomerase structures begins to explain how mutations at or near the drug binding pockets induce drug resistance. One insight from the X-ray crystallography work is that rather subtle changes in drug structure are sufficient to avoid cross-resistance with quinolones. This realisation is being exploited by pharmaceutical companies to design a new generation of drugs that target resistant bacteria, in some cases exploiting new binding pockets on the target molecule. Without knowledge of the molecular structure, these approaches would be severely limited, and largely dependent on relatively inefficient trial-and-error screening. These structures are providing key leads for compound development, although industrial collaborators are unwilling to release confidential information on this for the REF. The expectation is that these new drug entities will be valuable in the management of bacterial diseases. Given the close similarities in target enzyme structures between *S. pneumoniae*, *S. aureus* and other species, this approach will benefit the discovery of new drugs in a wide range of bacterial pathogens.

5. Sources to corroborate the impact (indicative maximum of 10 references)

A. http://www.accessdata.fda.gov/drugsatfda_docs/nda/2003/21158_Factive.cfm
Microbiology Reviews-Part 1(PDF) pp11-13, 30-32; Part 2 (PDF) p79, refs 3 and 4; Part 4 p19 and Part 6, ref 95 (subsequently published as Heaton VJ, Goldsmith CE, Ambler JE, Fisher LM. Activity of gemifloxacin against penicillin- and ciprofloxacin-resistant *Streptococcus pneumoniae* displaying topoisomerase- and efflux-mediated resistance mechanisms. Antimicrob Agents Chemother. 1999, Dec;43; 2998-3000). No DOI available.

B. Factive- the fast, active quinolone. www.factive.com (to access information from this url: click in the area of the spinning globe to skip. Then click on the region 'Europe' on the map. Then click on the search for prescribing information in the top right hand corner and type in 'mechanism of actions' and the link will show)

C. http://www.lgmpharma.com/blog/tag/gemifloxacin-mesylate

D. www.faqs.org/sec-filings/120306/CORNERSTONE-THERAPEUTICS-INC_10-K/
(United States Securities and Exchange filing- page 5)

E. Cambau E, Matrat S, Pan XS, Roth Dit Bettoni R, Corbel C, Aubry A, Lascols C, Driot JY, Fisher LM. Target specificity of the new fluoroquinolone besifloxacin in *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Escherichia coli*. J Antimicrob Chemother. 2009 Mar; 63, 443-50. doi: 10.1093/jac/dkn528

F. FDA Drug Approval Package: Besivance (Besifloxacin) www.accessdata.fda.gov/drugsatfda_docs/nda/2009/022308s000TOC.cfm. Fisher and colleagues were responsible for studies PHA-005 and PHA-006 (pp11-13 and 36-39).

G. News from the American Optometric Association. http://newsfromaoa.org/2012/11/10/fda-grants-additional-indications-approval-on-besivance

H. Cornstock TL, Karpecki TM, Morris TW, Zhang JZ. Besifloxacin: a novel anti-infective for the treatment of bacterial conjunctivitis. Clin Opthalmol. 2010; 4, 215-225. No DOI available.

I. http://www.businesswire.com/news/home/20130402005714/en/InSite-Vision-Announces-Sale-Besivance%C2%AE-Royalty-15

J. Moxeza Use Reviews-Food and Drug Administration. www.fda.gov/downloads/Advisory Committees/..../UCM342259.pdf