

**MUTANT SELECTION WINDOW:  
LIST OF PUBLICATIONS AND MAJOR STATEMENTS**

Drlica Updated: 6/11/08

**General Disclaimer:** This is a working document that should be checked for accuracy before use; it should not be considered comprehensive.

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### **Highlights since last update:**

animal studies now support the hypothesis beyond fluoroquinolones

window extended to vancomycin and daptomycin *in vitro*

yeast-miconazole data finally published

AUC/MPC as the upper boundary of the window for concentration-dependent killers

liquid assay for MPC when inoculum effects are extreme

inverted U-shape curve (mutant freq. vs. dose) demonstrates window *in vitro* and *in vivo*

use of MPC for finding new drugs

combination of MPC and dose-escalation modeling

## 1. Pre-MPC work

Baquero, F. and M. C. Negri. 1997. Strategies to minimize the development of antibiotic resistance. *J. Chemother.* 9: Suppl 29-37.

- ◆Proposed the existence of a “dangerous window” in which mutants are selected
- ◆Proposed that time in the window would be important

Zhao, X., Xu, C., Domagala, J., and Drlica, K. 1997. DNA topoisomerase targets of the fluoroquinolones: a strategy for avoiding bacterial resistance. *Proc. Natl. Acad. Sci. U.S.A.* 94: 13991-13996.

- ◆Drug (fluoroquinolone) concentrations that require a cell to obtain 2 concurrent resistance mutations severely restricted the recovery of resistant mutants.
- ◆Fluoroquinolones differ in their ability to kill resistant mutants

Dong, Y., Xu, C., Zhao, X., Domagala, J., and Drlica, K. 1998. Fluoroquinolone action against mycobacteria: effects of C8 substituents on bacterial growth, survival, and resistance. *Antimicrob. Agents Chemother.* 42: 2978-2984.

- ◆Compounds (fluoroquinolones) with greater activity against first-step resistance mutants allow the recovery of fewer resistant mutants at the same drug concentration.

Sieradzki, K., R. Roberts, S. Haber, and A. Tomasz. 1999. The development of vancomycin resistance in a patient with methicillin-resistant *Staphylococcus aureus* infection. *N. Engl. J. Med.* 340: 517-523.

- ◆Effect of vancomycin concentration on mutant selection measured with *S. aureus*. Description of population analysis.

## 2. Initial definition of MPC

Dong, Y., Zhao, X., Domagala, J., and Drlica, K. 1999. Effect of fluoroquinolone concentration on selection of resistant mutants of *Mycobacterium bovis* BCG and *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* 43: 1756-1758.

- ◆The term MPC was coined and defined.
- ◆Effect of fluoroquinolone structure on MPC (related to activity against mutants)
- ◆Mutant selection curves for fluoroquinolones shown for single-target (*M. bovis* BCG) and double-target (*S. aureus*) bacteria
- ◆Known resistance mutants alter selection curve as expected

## 3. Mutant selection window hypothesis: general theory

Zhao, X., and Drlica, K. 2001. Restricting the selection of antibiotic-resistant mutants: a general strategy derived from fluoroquinolone studies. *Clin. Inf. Dis.* 33(Suppl. 3): S146-S157.

- ◆General theory of selection window extended to all single-step mutants
- ◆General ideas about pharmacokinetic mismatch

Baquero, F. 2001. Low-level antibacterial resistance: a gateway to clinical resistance. *Drug Resistance Updates* 4; 93-105.

- ◆Each mutant type has a “selection window,” and high-level resistance arises gradually through “hill climbing.”

Drlica, K. 2003 The mutant selection window and antimicrobial resistance. *J. Antimicrob. Chemother.* 52: 11-17.

- ◆ Updated with supporting evidence
- ◆ Consideration of whether monotherapy or combination therapy should be used
- ◆ MPC compared to MIC and C<sub>max</sub> for various compound-bacterium combinations

Drlica, K., and Zhao, X. 2004. Is “dosing-to-cure” appropriate in the face of increasing antimicrobial resistance? *Rev. Med. Microbiol.* 15: 73-80.

- ◆ Development of resistance and factors affecting mutant enrichment; mutant selection window as a context for mutant enrichment and ways to slow it

Drlica, K. and Zhao, X. 2007. Mutant selection window hypothesis updated. *Clin. Inf. Dis.* 44: 681-688.

- ◆ logic for AUC/MPC as upper boundary of window; AUC/MIC as lower boundary

Drlica, K. and Zhao, X. 2008. Unifying anti-mutant antimicrobial dosing strategies. *J. Antimicrob. Chemother.* (in press).

- ◆ three general approaches for restricting emergence of resistance described
- ◆ MPC combined with dose-escalation modeling

#### 4. Characterization of the selection window *in vitro*: static studies with bacteria

Zhou, J., Dong, Y., Zhao, X., Lee, S., Amin, A., Ramaswamy, S., J. Domagala, J. Musser, and Drlica, K. 2000. Selection of antibiotic-resistant bacterial mutants: allelic diversity among fluoroquinolone-resistant mutants. *J. Infect. Dis.* 182: 517-525.

- ◆ Different mutants are selected at different drug concentrations, i.e. at different regions of selection window (fluoroquinolones with mycobacteria)
- ◆ Non-target mutants are selectively enriched by low fluoroquinolone concentrations

Sindelar, G., Zhao, X., Liew, A., Dong, Y., Lu, T., Zhou, J., Domagala, J., and Drlica, K. 2000. Mutant prevention concentration as a measure of fluoroquinolone potency against mycobacteria. *Antimicrob. Agents Chemother.* 44: 3337-3343.

- ◆ Fluoroquinolone structure affects the mutant selection curve
- ◆ MIC of least susceptible mutant correlates with MPC ( $r^2 = 0.92$ ) better than does wild-type MIC ( $r^2 = 0.39$ ) for several closely related C-8-H and C-8-Ome compounds
- ◆ Correlation with C-8-F derivatives is good with both wild type and mutant

Dong, Y., Zhao, X., Kreiswirth, B., and Drlica, K. 2000. Mutant prevention concentration as a measure of antibiotic potency: studies with clinical isolates of *Mycobacterium tuberculosis*. *Antimicrob. Agents Chemother.* 44: 2581-2584.

- ◆ Ratio of MPC to C<sub>max</sub> for anti-tuberculosis agents
- ◆ Mutant selection curves for non-quinolones with *M. tuberculosis*

Negri, M.-C., Lipsitch, M., Blazquez, J., Levin, B., and Baquero, F. 2000. Concentration-dependent selection of small phenotypic differences in TEM  $\beta$ -lactamase-mediated antibiotic resistance. *Antimicrob. Agents Chemother.* 44: 2485-2491

- ◆ Optimal concentration seen for selective enrichment of low-level mutant
- ◆ With extended incubation an even less susceptible low-level mutant is enriched

Blondeau, J., Zhao, X., Hansen, G., and Drlica, K. 2001. Mutant prevention concentrations of fluoroquinolones for clinical isolates of *Streptococcus pneumoniae*. *Antimicrob. Agents Chemother.* 45: 433-438.

- ◆ Method for measuring MPC with large numbers of clinical isolates
- ◆ Ranking fluoroquinolones with respect to MPC using pharmacokinetics and data from clinical isolates

Drlica, K. 2001. A Strategy for Fighting Antibiotic Resistance. *ASM News* 67: 27-33.

- ◆ Discussion of Vernon's HIV<sup>+</sup> tuberculosis experiment that provides support for the potential importance of pharmacokinetic mismatch

Drlica, K. 2001. Antibiotic resistance: can we beat the bugs? *Drug Discovery Today* 6: 714-716.

- ◆ Relationship between traditional pharmacodynamics and mutant selection window; restricting resistance requires dosing higher than is needed to cure.

Tillotson, G., Zhao, X., and Drlica, K. 2001. Fluoroquinolones as pneumococcal therapy: closing the barn door before the horse escapes. *Lancet Inf. Dis.* 1: 145-146.

- ◆ Use of the selection window to predict the development of levofloxacin resistance among isolates of *S. pneumoniae*

Zhao, X. and Drlica, K. 2002. Restricting the selection of antimicrobial-resistant mutants: measurement and potential use of the mutant selection window. *J. Inf. Dis.* 185: 561-565.

- ◆ Experimental demonstration of selection window
- ◆ Consideration of MPC for combinations of compounds (combination MPC)
- ◆ MPC determined with compounds other than fluoroquinolones with *S. aureus*.
- ◆ Selection window can be narrowed by altering fluoroquinolone structure

Li, X., Zhao, X., and Drlica, K. 2002. Selection of *Streptococcus pneumoniae* mutants having reduced susceptibility to moxifloxacin and levofloxacin. *Antimicrob. Agents Chemother* 46: 522-524.

- ◆ With *S. pneumoniae* showed how a single-step mutation in a target gene can have a dramatic effect on the shape of the mutant selection curve that is likely to increase the selective enrichment of mutants.
- ◆ Measurement of MPC for moxifloxacin and levofloxacin with laboratory strain using single-colony detection
- ◆ Target mutants recovered with levofloxacin at much higher frequency than with moxi
- ◆ Non-topoisomerase mutants recovered at low drug concentrations

Hansen, G., Metzler, K., Drlica, K., and Blondeau, J.M. 2003. Mutant prevention concentration for gemifloxacin with clinical isolates of *Streptococcus pneumoniae*. *Antimicrob. Agents Chemother* 47: 440-441.

- ◆ Ranking of fluoroquinolones with clinical isolates of *S. pneumoniae* using time above MPC

Zhao, X., Eisner, W., Perl-Rosenthal, N., Kreiswirth, B., and Drlica, K. 2003. Mutant prevention concentration for garenoxacin (BMS-28475) with ciprofloxacin-susceptible and ciprofloxacin-resistant *Staphylococcus aureus* *Antimicrobial Agents Chemother.* 47: 1023-1027

- ◆ Use of selection window to guide use of a new fluoroquinolone (garenoxacin)
- ◆ MPC is more sensitive than MIC to creeping resistance
- ◆ example of using MPC to evaluate a new indication

Lu, T., Zhao, X., Li, X., J., Hansen, G., Blondeau, J., and K. Drlica. 2003. Effect of chloramphenicol, erythromycin, moxifloxacin, penicillin, and tetracycline concentration on the recovery of resistant mutants of *Mycobacterium smegmatis* and *Staphylococcus aureus* J. Antimicrob. Chemother. 52: 61-64.

- ◆MPC extended to compounds other than fluoroquinolones with *S. aureus* and *M. smegmatis* (erythromycin, tetracycline, penicillin, chloramphenicol)

Hovde, L., Rotschafer, S., Ibrahim, K., Gunderson, B., Hermsen, E., and Rotschafer, J. 2003. Mutation prevention concentration of ceftriaxone, meropenem, imipenem, and ertapenem against three strains of *Streptococcus pneumoniae*. Diagn. Microbiol. Inf. Dis. 45: 265-267.

- ◆showed that MPC, MIC, MBC were within one dilution
- ◆argues against applicability of MPC to  $\beta$ -lactams, but had not seen Lu 2003

Li, X., Mariano, N., Rahal, J.J., Urban, C.M. and Drlica, K. 2004. Quinolone-resistant *Haemophilus influenzae*: determination of mutant selection window for ciprofloxacin, garenoxacin, levofloxacin, and moxifloxacin. Antimicrob. Agents Chemother. 48: 4460-4462.

- ◆Mutant selection window defined for garenoxacin, ciprofloxacin, levofloxacin, moxifloxacin; explains low prevalence of quinolone resistance
- ◆Mutant selection window defined for step-wise selection of mutants (*gyrA*, *parC*, *gyrA*, *parC*) using ciprofloxacin, compared to pharmacokinetic curves to show process for highly susceptible pathogen; defines one path to resistance

Randall, L.P., Cooles, S.W., Piddock, L.J.V., and Woodward, M.J. 2004. Mutant prevention concentrations of ciprofloxacin and enrofloxacin for *Salmonella enterica*. J. Antimicrob. Chemother. 54: 688-691.

- ◆cipro and enro generally had same MIC, but enro had higher MPC
- ◆MAR mutant has exceptionally high MPC re: MIC

Linde, H.-J. and Lehn, N. 2004. Mutant prevention concentration of nalidixic acid, ciprofloxacin, clinafloxacin, levofloxacin, norfloxacin, ofloxacin, sparfloxacin or trovafloxacin for *Escherichia coli* under different growth conditions. J. Antimicrob. Chemother. 53: 252-257.

- ◆With *E. coli* under aerobic conditions MPC/MIC differed substantially for the different quinolones emphasizing that structure affects the window opening.
- ◆MPC was affected differently by anaerobic conditions for the different compounds: lower MPC for nor, spar, clin, levo; higher MPC for cipro; about the same for nal, oflox. Cipro effects could reflect overall increase in susceptibility under anaerobic conditions

Metzler, K., Hansen, G., Hedlin, P., Harding, E., Drlica, K., and Blondeau, J. 2004. Comparison of minimal inhibitory and mutant prevention concentrations of 4 fluoroquinolones: methicillin-susceptible and -resistant *Staphylococcus aureus*. Int. J. Antimicrob. Agents 24: 161-167.

- ◆122 MSSA and 22 MRSA were examined for MIC and MPC with 4 quinolones
- ◆MRSA generally showed higher values

Smith, H., Walters, M., Hisanaga, T., Zhanel, G., and Hoban, D. 2004. Mutant prevention concentrations for single-step fluoroquinolone-resistant mutants of wild-type, efflux-positive, or ParC or GyrA mutation-containing *Streptococcus pneumoniae* isolates. Antimicrobial Agents Chemother. 48: 3954-3958.

- ◆First-step mutants recovered from clinical isolates; extends Li 2002, which was done with lab strain
- ◆Some clinical isolates were *gyrA*<sup>R</sup>, *parC*<sup>R</sup>, or *efflux*<sup>R</sup> as starter strains

- ◆ Window expansion for parC<sup>R</sup> as starter, not much for gyrA<sup>R</sup> as starter (different from Li 2002)
- ◆ For pooled treatments, “wt” and efflux<sup>R</sup> gave similar results; parC<sup>R</sup> gave mainly gyrA<sup>R</sup>; gyrA<sup>R</sup> gave mainly parC<sup>R</sup>
- ◆ No PK in compound ranking

Cui, J., Liu, Y., Wang, R., Liang, B., Pei, F., and Zheng, Z.J. 2004. [Mutant prevention concentrations of fluoroquinolones for *Staphylococcus aureus*] *Zhonghua Yi Xue Za Zhi* 84: 1863-1866.

- ◆ MPC for moxi, gati, pasufloxacin, cipro with lab isolate were 0.18, 0.3, 0.75, 1.8
- ◆ MPC<sub>90</sub> for moxi, gati, pasufloxacin, cipro with 20 clinical isolates were 1, 1, 4, 8
- ◆ MPC<sub>90</sub>/MIC<sub>99</sub> for moxi, gati, pasufloxacin, cipro with lab isolate were 9, 7.5, 8, 10.6

Rodriguez, J., Cebrian, L., Lopez, M., Ruiz, M., Jimenez, I., and Royo, G. 2004. Mutant prevention concentration: comparison of fluoroquinolones and linezolid with *Mycobacterium tuberculosis*. *J. Antimicrob. Chemother.* 53: 441-444.

- ◆ MPC measured for cipro > levo > moxi > gati with 244 clinical isolates
- ◆ AUC/MPC<sub>90</sub> is about 30 for moxi and gati;
- ◆ MPC for linezolid shows high ratio C<sub>max</sub> to MPC; AUC/MPC is about 116 hr
- ◆ example of using MPC to evaluate a new indication

Rodriguez, J., Cebrian, L., Lopez, M., Ruiz, M., and Royo, G. 2004. Mutant prevention concentration: a new tool for choosing treatment in nontuberculous mycobacterial infections. *Int. J. Antimicrob. Agents.* 24: 352-356.

- ◆ MPC<sub>50</sub>/MPC<sub>90</sub> and AUC/MPC<sub>90</sub> for 9 agents with 16 nontuberculous mycobacteria

Rodriguez, J., Cebrian, L., Lopez, M., Ruiz, M., and Royo, G. 2005. Usefulness of various antibiotics against *Mycobacterium avium-intracellulare*, measured by their mutant prevention concentration. *Int. J. Antimicrob. Agents.* 25: 221-225.

- ◆ MPC<sub>50</sub> for linezolid, rifabutin, levo, gati, moxi, clarithromycin, cipro, rif, azithromycin with 20 *M. avium* and 12 *M. intracellulare* isolates
- ◆ MPC was generally quite high, indicating high propensity of resistance development

Hermesen, E., Hovde, L., Konstantinides, G., and Rotschafer, J. 2005. Mutant prevention concentration of ABT-492, levofloxacin, moxifloxacin, and gatifloxacin against three common respiratory pathogens. *Antimicrob. Agents Chemother.* 49: 1633-1635.

- ◆ ABT-492 has low MPC and MIC
- ◆ With *S. pneumoniae*, MPC/MIC ratios were 8 for ABT-492, moxi; 4 for levo, gati
- ◆ With *H. influenzae* MPC/MIC ratios were 16 for ABT; 4,8 for levo, moxi; 8,16 for gati
- ◆ With *M. catarrhalis* MPC/MIC ratios were 4 for ABT; 16 for levo, moxi; 8 for gati

Roveta, S., Schito, A., Marchese, A., and Schito, G. 2005. Microbiological rationale for the utilization of prulifloxacin, a new fluoroquinolone, in the eradication of serious infections caused by *Pseudomonas aeruginosa*. *Int. J. Antimicrob. Agents.* 26: 366-372.

- ◆ *P. aeruginosa* MPC for cipro, levo, prulifloxacin, 15 isolates

Marcusson, L., Olofsson, S., Lindgren, P., Cars, O., and Hughes, D. 2005. Mutant prevention concentrations of ciprofloxacin for urinary tract infection isolates of *Escherichia coli*. *J. Antimicrob. Chemother.* 55: 938-943

- ◆ Examined agar after no colonies at MPC and found only wild-type susceptibility
- ◆ MIC of some mutants exceeded MPC, suggesting multiple mutations
- ◆ MPC gives good kill (10<sup>-8</sup>)
- ◆ suggests stable genetic change that increases survival at MPC

- ◆ Ratio of MIC to MPC can vary considerably for clinical *E. coli*;  $R^2 = 0.58$
- ◆ urinary infection could have large numbers of cells, on the order of  $10^9$

Drlica, K., Zhao, X., Wang, J.-Y., Malik, M., Lu, T., Logan, C., Park, S., Li, X., and Perlin, D. An anti-mutant approach for antimicrobial use (in preparation), in *Antimicrobial Resistance: Implications for the Twenty-first Century*, I. Fong and K. Drlica, eds.

- ◆ Non-topoisomerase mutants of *S. pneumoniae* change the shape of the mutant selection curve slightly and increase the frequency at which target mutants are obtained by orders of magnitude.

Hansen, G., Zhao, X., Drlica, K., and J. Blondeau. Enrichment of fluoroquinolone-resistant mutant subpopulations of *Pseudomonas aeruginosa*. 2006. *Int. J. Antimicrob. Agents* 27: 120-124.

- ◆ Population analysis for ciprofloxacin and levofloxacin with clinical isolates of *P. aeruginosa*; MPC lower for ciprofloxacin and fewer moderately susceptible mutants enriched
- ◆ Survey of 151 clinical strains shows MPC for ciprofloxacin is lower than levofloxacin
- ◆ In vitro data correlates with hospital studies in which shift from ciprofloxacin to ofloxacin/levofloxacin increases resistance.
- ◆ *gyrA* mutants are selected as single-step mutants

Wetzstein, H.-G. 2005. Comparative mutant prevention concentrations of pradofloxacin and other veterinary fluoroquinolones indicate differing potentials in preventing selection of resistance. *Antimicrob. Agents Chemother.* 49: 4166-4173.

- ◆ Consideration of factors involved in measurement of MPC
- ◆ Inoculum effect seen with *S. aureus* not *E. coli*
- ◆ Relative MIC vs MPC varies among organisms and among quinolones

Hansen, G., and Blondeau, J. 2005. Comparison of the minimum inhibitory, mutant prevention and minimum bactericidal concentrations of ciprofloxacin, levofloxacin, and garenoxacin against enteric gram-negative urinary tract infection pathogens. *J. Chemother.* 17: 484-492.

- ◆ MPC determined by survey method for cipro, levo, and garenoxacin using 20 isolates each of *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *C. freundii*, *E. cloacae*

Drlica, K., Zhao, X., Blondeau, J., Hesje, C. 2006. Low correlation between minimal inhibitory concentration (MIC) and mutant prevention concentration (MPC). *Antimicrob. Agents Chemother.* 50: 403-404.

- ◆ regression analysis shows poor correlation between MIC and MPC with clinical isolates for fluoroquinolones and macrolides

Blondeau, J., Blondeau, L., Hesje, C., and Borsos, S. 2006. Application of two methods to determine killing of *Streptococcus pneumoniae* by various fluoroquinolones. *J. Chemother.* 18: 366-372.

- ◆ killing of *S. pneumoniae* is faster and more extensive at MPC than MIC for 4 fluoroquinolones

Quinn B, Hussain S, Malik M, Drlica K, Zhao X. 2007. Daptomycin inoculum effects and mutant prevention concentration with *Staphylococcus aureus*. *J. Antimicrob. Chemother.* 60: 1380-1383.

- ◆ daptomycin MPC determined by liquid method; extreme inoculum effect
- ◆ single-step daptomycin-resistant mutants

Pasquali, F., and Manfreda, G. 2007. Mutant prevention concentration of ciprofloxacin and enrofloxacin against *Escherichia coli*, *Salmonella* Typhimurium, and *Pseudomonas aeruginosa*. *Veterinary Microbiol.* 119: 304-310

- ◆MPC determined in vitro for cipro and enro for *E. coli*, *Salmonella* Typhimurium, and *P. aeruginosa*.

Hedlin, P. and Blondeau, J. 2007. Comparative minimal inhibitory concentration and mutant prevention concentration of four fluoroquinolones against ocular isolates of *Haemophilus influenzae*. *Eye Contact Lens* 33: 161-164.

- ◆26 clinical isolates of *H. influenzae* MPC for gati, moxi, oflox, and cipro. Gati was lowest

## 5. Dual targeting: a special case

Pan, X.-S, Ambler, J., Mehtar, S., and Fisher, L.M. 1996. Involvement of topoisomerase IV and DNA gyrase as ciprofloxacin targets in *Streptococcus pneumoniae*. *Antimicrobial Agents Chemother.* 40: 2321-2326.

- ◆Fluoroquinolones are dual targeting, with *parC* being the primary target of cipro *in vitro*
- ◆Sequential clinical isolates from one patient showed *parC* mutation first, then *gyrA*
- ◆Pointed out that equal activity could restrict emergence of resistance

Ng, E., Trucksis, M., and Hooper, D. 1996. Quinolone resistance mutations in topoisomerase IV: relationship to the *flqA* locus and genetic evidence that topoisomerase IV is the primary target and DNA gyrase is the secondary target of fluoroquinolones in *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* 40: 1881-1888.

- ◆Fluoroquinolones are dual targeting, with *parC* being the primary target of some *in vitro*
- ◆Pointed out that equal activity could restrict emergence of resistance

Pan, X.-S. and Fisher, L.M. 1998. DNA gyrase and topoisomerase IV are dual targets of clinafloxacin action in *Streptococcus pneumoniae*. *Antimicrob. Agents Chemother.* 42: 2810-2816.

- ◆ Evidence for clinafloxacin targeting both *gyrA* and *parC* almost equally

Takei, M., Fukuda, H., Kishii, R., and Hosaka, M. 2001. Target preference of 15 quinolones against *Staphylococcus aureus*, based on antibacterial activities and target inhibition. *Antimicrob. Agents Chemother.* 45: 3544-3547.

- ◆Related biochemical activity with MIC for the two targets for 15 compounds
- ◆Compounds fall in categories

Li, X., Zhao, X., and Drlica, K. 2002. Selection of *Streptococcus pneumoniae* mutants having reduced susceptibility to moxifloxacin and levofloxacin. *Antimicrob. Agents Chemother.* 46: 522-524.

- ◆Mutate on of the two targets and the window increases strikingly

Okumura, R., Hirata, T., Onodera, Y., Hoshino, K., Otani, T., and Yamamoto, T. 2008. Dual-targeting properties of the 3-aminopyrrolidyl quinolones, DC159a and sitafloxacin, against DNA gyrase and topoisomerase IV: contribution to reducing in vitro emergence of quinolone-resistant *Streptococcus pneumoniae*. *J. Antimicrob. Chemother.* 62: 98-104.



## 6. Pharmacodynamic considerations *in vitro*

Allen, G., Kaatz, G., and Rybak, M. 2003. Activities of mutant prevention concentration-targeted moxifloxacin and levofloxacin against *Streptococcus pneumoniae* in an *in vitro* pharmacodynamic model. *Antimicrob. Agents Chemother.* 47: 2606-2616.

- ◆ Fluoroquinolones can differ in ability to select resistant mutants even when concentrations are normalized to AUC/MPC

Firsov, A., Vostrov, S., Lubenko, I., Drlica, K., Portnoy, Y. and Zinner, S. 2003. *In vitro* pharmacodynamic evaluation of the mutant selection window hypothesis for four fluoroquinolones against *Staphylococcus aureus*. *Antimicrobial Agents Chemother.* 47: 1604-1613.

- ◆ Dynamic *in vitro* model shows for fluoroquinolones with *S. aureus* that mutants are selected by drug concentrations in the window, not above or below
- ◆ Ranking of fluoroquinolones using dynamic model and MPC
- ◆ Demonstration that concentrations below MIC do not enrich mutants, as expected from selection window theory but counter to popular opinion

Zinner, S., Lubenko, I., Gilbert, D., Simmons, K., Zhao, X., Drlica, K., and Firsov, A. 2003. Emergence of resistant *Streptococcus pneumoniae* in an *in vitro* dynamic model that simulates moxifloxacin concentrations in and out of the mutant selection window: related changes in susceptibility, resistance frequency, and bacterial killing. *J. Antimicrob. Chemother.* 52: 616-622.

- ◆ Dynamic *in vitro* model shows for moxifloxacin with *S. pneumoniae* that mutants are selected by drug concentrations in the window, not above or below

Firsov, A., Vostrov, S., Lubenko, I., Zinner, S., and Portnoy, Y. 2004. Concentration-dependent changes in the susceptibility and killing of *Staphylococcus aureus* in an *in vitro* dynamic model that simulates normal and impaired gatifloxacin elimination. *Internat. J. Antimicrobial Agents* 23: 60-66.

- ◆ Dynamic *in vitro* model shown for gatifloxacin with *S. aureus* with two half-lives. Mutants are collected when concentrations fall inside the selection window.
- ◆ Less drug needed for impaired than normal elimination.

Firsov, A., Vostrov, S., Lubenko, I., Portnoy, Y., and Zinner, S. 2004. Prevention of the selection of resistant *Staphylococcus aureus* by moxifloxacin plus doxycycline in an *in vitro* dynamic model: an additive effect of the combination. *Internat. J. Antimicrobial Chemother.* 23: 451-456.

- ◆ Dynamic *in vitro* model shown for moxifloxacin and doxycycline, resistant mutants when in the window, but no single drug controls performed outside the window.
- ◆ Dual drug with both drugs in the window most of the time still gave resistance. MIC may not be restrictive for the model due to 100x inoculum differences.
- ◆ Dual drug with one drug above the window most of the time restricted resistance.

Firsov, A., Vostrov, S., Lubenko, I., Arzamastsev, Portnoy, Y., and Zinner, S. 2004. ABT492 and levofloxacin: comparison of their pharmacodynamics and their abilities to prevent the selection of resistant *Staphylococcus aureus* in an *in vitro* dynamic model. *J. Antimicrob. Chemother.* 54: 178-186.

- ◆ Dynamic *in vitro* model shows bell-shaped curve for resistance vs. AUC/MIC for levofloxacin and a new C-8-Cl quinolone ABT492.
- ◆ Resistance was related to time above MPC
- ◆ Less time above MPC was required for ABT492 to restrict resistance.

- ◆ ABT492 shows more pronounced killing, as we would expect for a C-8-Cl compound. This probably means better killing of mutants (not cited), which would explain item 3 above.
- ◆ Example of using MPC to evaluate a new indication

Allen, G., Kaatz, G., and Rybak, M. 2004. In vitro activities of mutant prevention concentration-targeted concentrations of fluoroquinolones against *Staphylococcus aureus* in a pharmacodynamic model. *Int. J. Antimicrob. Agents* 24: 150-160.

- ◆ A blood clot model was used to measure the effect of dose on recovery of resistant mutants.
- ◆ A resistant mutant produced less susceptible cells as expected; a susceptible strain did not.

Campion, J., McNamara, P., and Evans, M. 2004. Evolution of ciprofloxacin-resistant *Staphylococcus aureus* in *in vitro* pharmacokinetic environments. *Antimicrob. Agents Chemother.* 48: 4733-4744.

- ◆ No resistant subpopulations expanded when cipro was kept above the window (AUC/MIC = 584)
- ◆ Later regrowth and slower as dose exceeds MPC for only part of time.
- ◆ Mutants selected when concentrations were inside the window (at least at AUC/MIC 159)
- ◆ Near bottom of the window the mutants have the lowest increase in MIC
- ◆ Time in the window is not by itself related to level of resistance (position in window is probably important)
- ◆ Mutants selected when concentrations were inside the window
- ◆ double mutants come up later and only when conc are in middle of window; heterogeneous pathways to get the double mutations

Campion, J., McNamara, and Evans, M. 2005. Pharmacodynamic modeling of ciprofloxacin resistance in *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* 49: 209-219.

- ◆ no mutant recovered with low population size
- ◆ time in window did not correlate with enrichment of resistant mutants

Campion, J., Chung, P., McNamara, P., Titlow, W., and Evans, M. 2005. Pharmacodynamic modeling of the evolution of levofloxacin resistance in *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* 49: 2189-2199.

- ◆ levo in window and below (AUC/MIC 33; AUC/MPC 9) lots of mutant growth
- ◆ levo in window and both above and below (AUC/MIC 64; AUC/MPC 18) less outgrowth
- ◆ levo in window and above (AUC/MIC 126; AUC/MPC 36)
- ◆ levo in window less and above (AUC/MIC 188; AUC/MPC 54)
- ◆ total population tested 1.5 to 5 x 10<sup>8</sup>
- ◆ heterogeneous pathways to resistance
- ◆ time in the window did not correlate, but not done as by Firsov and pop was smaller here

Tam, V., Louie, A., Deziel, M., Liu, W., Leary, R., and Drusano, G. 2005. Bacterial-population responses to drug-selective pressure: examination of garenoxacin's effect on *Pseudomonas aeruginosa*. *J. Inf. Dis.* 192: 420-428.

- ◆ In vitro model with *P. aeruginosa*, assayed at 3 X MIC, which gave pump mutants only
- ◆ Mutants restricted with AUC/MIC = 200
- ◆ Mutants enriched concurrently with killing of susceptible cells

Gumbo, T., Louie, A., Deziel, M., Parsons, L., Salfinger, M., and Drusano, G. 2004. Selection of a moxifloxacin dose that suppresses drug resistance in *Mycobacterium tuberculosis*, by use of an in vitro pharmacodynamic infection model and mathematical modeling. *J. Inf. Dis.* 190: 1642-1651.

- ◆ dosage escalation, not window to determine threshold
- ◆ used MIC<sub>99</sub>, not the usual MIC; resistance was scored on plates at 6 x MIC; pop size 1.7 x 10<sup>7</sup>

- ◆ showed that eradication and acquisition of resistance can occur concurrently
- ◆ AUC/MIC assoc with suppression of resistance was 102 for free moxi or 204 for total;
- ◆ equations to calculate resistance breakpoint are 53 for free.
- ◆ Monte Carlo to deal with patient heterogeneity gives 800 mg moxi daily to suppress resistance; 400 mg daily will suppress in 60% of people

Olofsson, S., Marcusson, L., Komp-Lindgren, P., Hughes, D., and Cars, O. 2006. Selection of ciprofloxacin resistance in *Escherichia coli* in an in vitro kinetic model: relation between drug exposure and mutant prevention concentration. *J. Antimicrob. Chemother.* 57: 1116-1121.

- ◆ dynamic model, time > MPC needed to be only 18 hr, not 24
- ◆ raise C<sub>max</sub> and shorted time > MPC needed to block mutant growth
- ◆ restrictive AUC/MPC is 22, best correlate
- ◆ persists seen in liquid cultures

Firsov, AA, Smirnova MV, Lubenko IY, Vostrov SN, Portnoy YA, Zinner SH. 2006. Testing the mutant selection window hypothesis with *Staphylococcus aureus* exposed to daptomycin and vancomycin in an in vitro dynamic model. *J. Antimicrob. Chemother.* 58: 1185-1192.

- ◆ selection window demonstrated for vancomycin with *S. aureus*
- ◆ selection window demonstrated for daptomycin with *S. aureus*
- ◆ relevance of protein binding calculations is questioned
- ◆ time in window correlation seen and other work discussed
- ◆ bell-shaped curve for AUC/MPC

Tam, V. Louie, A., Deziel, M. Liu, W., and Drusano, G. 2007. The relationship between quinolone exposures and resistance amplification is characterized by an inverted U: a new paradigm for optimizing pharmacodynamics to counterselect resistance. *Antimicrob. Agents Chemother.* 51: 744-747.

- ◆ bell-shaped curve for AUC/MIC for garenoxacin with 2 strains of *S. aureus* and one of *Klebsiella*, thereby confirming with an in vitro dynamic model the existence of the selection window as shown by many other systems

Homma, T. Hori, T., Sugimori, G. and Yamano, Y. 2007. Pharmacodynamic assessment based on mutant prevention concentrations of fluoroquinolones to prevent the emergence of resistant mutants of *Streptococcus pneumoniae*. *Antimicrob. Agents Chemother.* 51: 3810-3815.

- ◆ moxi gave no mutant enrichment with *S. pneumoniae* when AUC/MPC > 13.4 and C<sub>max</sub>/MPC > 1.2
- ◆ mutants enriched when moxi AUC/MPC < 0.84 and C<sub>max</sub>/MPC < 0.08
- ◆ no correlation with t . MPC or t in MSW.

Henrichfreise, B., Wiegand, I., Luhmer-Becker, I., Wiedemann, B. 2007. Development of resistance in wild-type and hypermutable *Pseudomonas aeruginosa* strains exposed to clinical pharmacokinetic profiles of meropenem and ceftazidime simulated in vitro. *Antimicrob. Agents Chemother.* 51: 3642-3649.

- ◆ made conclusion that it would be better for antibiotic concentration to remain above MPC longer than seen with current dosing

Olofsson, S., Marcusson, L., Stromback, A., Hughes, D., and Cars, O. 2007. Dose-related selection of fluoroquinolone-resistant *Escherichia coli*. *J. Antimicrob. Chem.* 60: 795-801.

- ◆ in vitro PD model with mixture of wt, single mutant (*gyrA* A83L) and double mutant (*gyrA* S83L and *marR* deletion) as mixed infection (1% mutant with wt).
- ◆ norfloxacin at approved human dose (200 mg) failed to suppress mutant outgrowth
- ◆ moxifloxacin at approved human dose (400 mg) allowed double mutant to survive

- ◆ cipro at 750 mg 2 x daily eliminated single mutant (AUC/MPC between 14 and 35); eliminated double mutant (AUC/MPC<sub>single</sub> between 8 and 14)

## 7. Characterization of the mutant selection window *in vitro*: studies with eukaryotic organisms

Drlica, K., Wang, J.-Y., Malik, M., Lu, T., Park, S., Li, X., Perlin, D., and Zhao, X. 2008. An anti-mutant approach for antimicrobial use. In *Antimicrobial Resistance and Implications for the 21<sup>st</sup> Century*, I.W. Fong and K. Drlica, eds. Springer, New York., p.371-400.

- ◆ The effect of miconazole concentration on mutant selection with wild-type *C. glabrata* (haploid) and *C. albicans* (diploid) was similar to mutant selection window data seen for fluoroquinolone-mycobacterial combinations
- ◆ Resistant mutants behave as expected from selection window theory.

## 8. Characterization of the mutant selection window *in vitro*: studies with viruses

To be done

## 9. Characterization of mutant selection window *in vivo*

Negri, M.-C., Lipsitch, M., Blazquez, J., Levin, B., and Baquero, F. 2000. Concentration-dependent selection of small phenotypic differences in TEM  $\beta$ -lactamase-mediated antibiotic resistance. *Antimicrob. Agents Chemother.* 44: 2485-2491

- ◆ Plasmid-borne low-level resistance shows drug concentration optimum in mouse thigh model of infection with *E. coli*

Jumbe, N., A. Louie, R. Leary, W. Liu, M. Deziel, V. Tam, R. Bachhawat, C. Freeman, J. Kahn, K. Bush, M. Dudley, M. Miller, and G. Drusano. 2003. Application of a mathematical model to prevent *in vivo* amplification of antibiotic-resistant bacterial populations during therapy. *J. Clin. Invest.* 112: 275-285.

- ◆ Mouse thigh model infected with *P. aeruginosa* and treated with levo
- ◆ Escalating doses used until point is reached at which no mutants are recovered; related to susceptible cell MIC.
- ◆ Drug concentrations that kill wild type cells allow survival of resistant mutants
- ◆ Mutants recovered at 3 X MIC, no gyrase mutants; thus probably underestimate of threshold (max infection size was  $10^8$ )
- ◆ Efflux mutant restricting threshold estimated at total drug AUC/MIC = 157 hr
- ◆ not intended to measure window

Croiser, D., Etienne, M., Bergoin, E., Charles, P.-E., Lequeu, C., Piroth, L., Portier, H., and Chavanet, P. 2004. Mutant selection window in levofloxacin and moxifloxacin treatments of experimental pneumococcal pneumonia in a rabbit model of human therapy. *Antimicrob. Agents Chemother.* 48: 1699-1707.

◆

Croiser, D., Etienne, M., Piroth, L., Bergoin, E., Lequeu, C., Portier, H., and Chavanet, P. 2004. *In vivo* pharmacodynamic efficacy of gatifloxacin against *Streptococcus pneumoniae* in an experimental model of pneumonia: impact of the low levels of fluoroquinolone resistance on the enrichment of resistant mutants. *J. Antimicrob. Chemother.* 54: 640-647.

- ◆ Resistant mutants recovered in most animals when starter is parC<sup>R</sup>, not wt or gyrA<sup>R</sup>.
- ◆ Resistant *gyrA parC* mutants recovered in some animals when starter is efflux and dose is low.
- ◆ analysis argues that mutants are selected when doses are inside the window

Etienne, M., Croisier, D., Charles, P.-E., Lequeu, C., Piroth, L., Portier, H., Drlica, K., and Chavanet, P. 2004. Effect of low-level resistance on subsequent enrichment of fluoroquinolone-resistant *Streptococcus pneumoniae* in rabbits. *J. Inf. Dis.* 190: 1472-1475

- ◆ Human pharmacokinetics of ciprofloxacin and moxifloxacin simulated in rabbits; ciprofloxacin readily selects mutants from wild-type cells, moxifloxacin does not.
- ◆ With a *parC* resistance mutant moxifloxacin readily enriches resistant (*gyrA*) mutants
- ◆ Data consistent with agar plate behavior of *parC* mutants (see Li, 2002).
- ◆ Upper boundary of mutant selection window defined at 220% of standard human dose.

Cui, J., Liu, Y., Wang, R., Tong, W., Drlica, K., and Zhao, X. 2006. The mutant selection window in rabbits infected with *Staphylococcus aureus*. *J. Inf. Dis.* 194: 1601-1608.

- ◆ whiffle ball model with *S. aureus* in rabbit with levofloxacin
- ◆ PK measured and MIC of cells recovered from model after treatment
- ◆ amplification of mutants only when levofloxacin concentration inside the window
- ◆ correction for protein binding
- ◆ first to measure window *in vivo*

Almeida, D., Nuermberger, E., Tyagi, S., Bishai, W., and Grosset, J. 2007. In vivo validation of the mutant selection window hypothesis with moxifloxacin in a murine model of tuberculosis. *Antimicrob. Agents Chemother.* 51: 4261-4266.

- ◆ mice infected with  $\log_{-10} = 7.9$  cfu and treated with moxi in food at 0.25% (produces human concentrations) or 1.5%; resistant mutants recovered from lower dose, none from upper
- ◆ mutants were GyrB E512D and GyrA D94N
- ◆ higher dose much more bactericidal
- ◆ MPC for moxi is 4-8 ug/ml; upper dose keeps serum above 8 ug/ml

Stearne, L., Goessens, W., Mouton, J., and Gyssens, I. 2007. Effect of dosing and dosing frequency on the efficacy of ceftizoxime and the emergence of ceftizoxime resistance during the early development of murine abscesses caused by *Bacteroides fragilis* and *Enterobacter cloacae* mixed infection. *Antimicrob. Agents Chemother.* 51: 3605-3611.

- ◆ Ceftizoxime treatment of murine subcutaneous mixed infection with *B. fragilis* and *E. cloacae* – mutant frequency shows inverted U-shape with mutant frequency vs dose (window)
- ◆ MPC for *E. cloacae* = 384

## **10. Correlation between anti-mutant activity *in vitro* and clinical selection of antimicrobial resistance**

Liu, Y., Cui, J., Wang, R., Wang, X., Drlica, K., and Zhao, X. 2005. Selection of rifampicin-resistant *Staphylococcus aureus* during tuberculosis therapy: concurrent bacterial eradication and acquisition of resistance. *J. Antimicrob. Chemother.* 56: 1172-1175

- ◆ nasal colonization; rifampicin resistance acquired by *S. aureus* is 8%, concurrent with eradication (92%) (n = 58)
- ◆ PFGE and spa typing show resistance acquired, not primary
- ◆ No resistance when *S. aureus* was susceptible to drugs in addition to rifampicin
- ◆ These data are counter-example to the “dead bugs don’t mutate” idea

## **11. Mutant selection window as it applies to resistance to anticancer therapy**

To be done

## **12. Use of anti-mutant strategy to obtain new antimicrobials**

German, N., Malik, M., Drlica, K., and Kerns, R. Use of gyrase resistance mutants to guide synthesis of quinolone-like 8-methoxy-quinazoline-2,4-diones (submitted for publication)

### **13. Mutant selection window as it applies to insecticide resistance**

McKenzie, J.A. Applying the theory: the better management of resistance and pests. In *Ecological and Evolutionary Aspects of Insecticide Resistance*, p. 149-173

◆ Ideas developed that are parallel to selection window hypothesis

### **14. Alternate points of view and commentary**

Drusano, G. 2003. Prevention of resistance; a goal for dose selection for antimicrobial agents. *Clin. Inf. Dis.* 36(Suppl. 1): S42-S50

◆ Pharmacodynamic approach is described using activity against susceptible cells rather than resistant mutants as a reference point.

### **15. Other Refinements**

Smith, H., Nichol, K., Hoban, D., and Zhanel, G. 2003. Stretching the mutant prevention concentration (MPC) beyond its limits. *J. Antimicrob. Chemother.* 51: 1323-1325.

◆ Previous work has not pointed out that for clinical application the *in vitro* studies should be measuring MPC for the type of resistance actually found clinically.

Livermore, D. 2003. Overstretching the mutant prevention concentration. *J. Antimicrob. Chemother.* 52; 732.

◆ Treatment to exceed MPC may result in collateral damage through resistance developing in organisms that are not the initial target of the treatment.

### **16. Other Reviews and Commentary (also see section 3)**

Allen, G. P. 2003. The mutant prevention concentration (MPC): a review. *J. Inf. Dis. Pharmacother.* 6: 27-47.

Drlica, K. and Zhao, X. 2003. Controlling antibiotic resistance: strategies based on the mutant selection window. In *Reemergence of Established Pathogens in the 21<sup>st</sup> Century*, Ed. I.W. Fong and K. Drlica. 295-331.

Zhao, X. 2003. Clarification of the MPC and the mutant selection window concept. *J. Antimicrob. Chemother.* 52: 731. (response to Smith *et al.* 2003)

Smith, H., Hoban, D., and Zhanel, G. 2003. Overstretching the mutant prevention concentration. *J. Antimicrob. Chemother.* 52: 732-733. (response to Zhao and Livermore).

Epstein, B., Gums, J., and Drlica, K. 2004. The Changing Face of Antibiotic Prescribing: the Mutant Selection Window. *Annals of Pharmacotherapy* 38: 1675-1682.

Blondeau, J., Hansen, G., Metzler, K., and Hedlin, P. 2004. The role of PK/PD parameters to avoid selection and increase of resistance: mutant prevention concentration. *J. Chemother. Suppl.* 3: 1-19.

Blondeau, J., 2005. Differential impact of macrolide compounds in the selection of macrolide nonsusceptible *Streptococcus pneumoniae*. *Therapy* 2: 813-818. (azithromycin as inferior drug re: resistance; MPC data for 3 macrolides and 170 strains)

Hesje, C., Tillotson, G., and Blondeau, J. 2007. MICs, MPCs, and PK/PDs: a match (sometimes) made in hosts. *Exp. Rev. Resp. Med.* 1: (in press). (survey of MIC, MPC)

Drlica, K., Wang, J.-Y., Malik, M., Lu, T., Park, S., Li, X., Perlin, D., and Zhao, X. 2008. An anti-mutant approach for antimicrobial use. In *Antimicrobial Resistance and Implications for the 21<sup>st</sup> Century*, I.W. Fong and K. Drlica, eds. Springer, New York., p.371-400.

Drlica, K., and Zhao., X. 2008. Mutant selection window hypothesis: a framework for anti-mutant dosing of antimicrobial agents. In *National Institute of Allergy and Infectious Diseases, NIH Volume 1, Frontiers in Research*, V. Georgiev, Editor. Humana Press Inc., Totawa, NJ. pp 101-106. (history of MSW hypothesis)