Drug Discovery Within the Context of AMR: Extending Beyond ‘Me Too’ Drugs.

Tom Parr
Outline

• Nothing New Under the Sun
• Stories
  1. Anti-virulence approach, Agr 1993; MvrR 2015
  2. GSK2251052 a Leucyl tRNA Synthetase Inhibitor
  3. Combination therapy to change spectrum -- Potentiator
• The moral of the stories
There is Nothing New Under the Sun

• The scramble for new approaches for the treatment of antibiotic resistant microbes has specific challenges
• There is not a golden bullet guaranteed to overcome these challenges
• If you want to succeed, you need to believe in your approach AND you need to go do it, now.

• Look before you leap
• He who hesitates is lost
Don’t Walk Off a Cliff by Ignoring the Complexity

See for examples:

Challenges of Antibacterial Discovery
Clinical Microbiology Reviews, Jan. 2011, p. 71–109 Vol. 24, No. 1
Lynn L. Silver

Multi-targeting by monotherapeutic antibacterials
Nature Reviews Drug Discovery vol 6, 2001 pp. 41
Lynn L. Silver
Only Ogling the Possibilities
-Won’t Bring About a New Therapy
## Anti-Virulence Strategies

<table>
<thead>
<tr>
<th>Targets</th>
<th>Inhibitors</th>
<th>Mode of Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial adhesion</td>
<td>Pilicides</td>
<td>Decrease pilus formation</td>
</tr>
<tr>
<td></td>
<td>Mono- and oligosaccharides</td>
<td>Block attachment</td>
</tr>
<tr>
<td>Specialized secretory systems</td>
<td>Small molecules</td>
<td>Inhibit assembly or host cell interaction</td>
</tr>
<tr>
<td></td>
<td>Antibodies</td>
<td>Block toxin secretion</td>
</tr>
<tr>
<td>Toxin production</td>
<td>Antibodies</td>
<td>Neutralize toxin</td>
</tr>
<tr>
<td></td>
<td>Toxin analogs</td>
<td>Block toxin activation</td>
</tr>
<tr>
<td>Quorum-sensing</td>
<td>Analogs</td>
<td>Block signal production</td>
</tr>
<tr>
<td></td>
<td>Enzymes</td>
<td>Inactivate signal molecules</td>
</tr>
<tr>
<td></td>
<td>Antagonists</td>
<td>Block transcriptional regulators</td>
</tr>
</tbody>
</table>

So far, no approved anti-virulence strategies available on the market

Adapted from: Microbial pathogens and strategies for combating them: science, technology and education (A. Méndez-Vilas, Ed.) 2013
Example: Anti-Virulence as a Treatment Approach

• **Agr 1993** (Microcide Pharmaceuticals) to **MvfR 2015** (Spero Therapeutics)

  - Expression of virulence factors allow bacteria to
    - Enter host and establish a beneficial niche
    - Avoid the primary defenses of host
    - Counter subsequent immunological responses
    - Multiply and disseminate within host or to new host
  
• Therapeutic intervention can potentially target any of these processes

MvfR Target Background

- MvfR (Multiple Virulence Factor Regulator): transcriptional regulator that controls expression of most *Pseudomonas aeruginosa* virulence genes
- MvfR also regulates the transition of acute into chronic infections
- Rahme laboratory (MGH/Harvard) identified small molecule inhibitors of MvfR in a screening campaign at Harvard
- Compounds were found to inhibit expression of virulence factors and exhibit efficacy in mouse models of *Pseudomonas* infections

Traditional Antibiotics

\[ \text{Acute Infection} \quad \text{Virulence} \quad \text{MvfR inhibitors} \quad \text{No MICs!} \]

\[ \text{Chronic Infection} \quad \text{Persistence} \quad \text{Biofilms} \quad \text{“Persisters”} \]
Screen for MvfR Inhibitors

- Whole cell assay using \textit{mvfR} regulated \textit{pqsA} promoter fusion to \textit{sacB}
- In the presence of sucrose and active MvfR, cell growth is inhibited
- Antibacterial compounds present as inactive
- 300,000 compound screening library provided by ICCB Harvard
  - 6K known bioactives
  - 240K commercial collection
  - 30K natural products
- Initial lead compound, SPR001, basis for Spero analog program
Efficacy and Pharmacodynamic Markers

MoA-specific development paradigm analogous to MIC
- Look to link key MvfR-regulated PD markers to efficacy and PK
- Potential PD markers include signaling molecules such as PQS and HHQ

Ex vivo

Clinical PD

The three principal members of the HAQ family were assessed by LC/MS from murine burn wounds post burn & infection

Que et al, J. Pathogens, 2011

HHQ

eXiao G. et. al. Mol. Micro, 2006

Necrotic muscle + fat

HAQs isolated & detected from human burn wounds. Similar to ratios in burned and infected animals
MvfR Target Rationale

MvfR controls the pathology of acute infections and the persistence of chronic infections caused by *Pseudomonas aeruginosa*

- **Unmet need:** Limited options available or in development for MDR/XDR *Pseudomonas*
- **Novel:** Seminal work is <10 years old
- **Essential for virulence:** Supported by *MvfR KO studies*
- **In vivo proof of principle** in mouse infection models
- **Complementary to bactericidal mechanisms**
  - Reduced resistance pressure and impact on microbiome
- **On-mechanism biomarkers** to guide dosing *in vivo* and in clinic
Supporting Genetic Data for MvfR

- MvfR mutants produce substantially lower levels of virulence factors and are less pathogenic \textit{in vivo}

**Virulence/Secreted Factors**

- Pyocyanin
- Cyanide
- Lectins
- Proteases
- Elastase
- Rhamnolipids
- Peptides
- Chitinases
- Pyochelin
- Pyoverdin
- Type VI secretion
- HAQS, 2-AA

**Impact on Mortality in Lung Infection Model**

Mouse lung infection survival: PA14 vs. PA14\textit{mvfR}

Unpublished results, Laurence Rahme

In vivo Activity of M64 Screen Hit in Mouse Infection Models

MvfR inhibitor used as monotherapy disarms acute infection and improves survival in mouse infections using *P. aeruginosa* as the pathogen.

- **Acute Burn Infection**
- **Acute Lung Infection**

- M64 is formulated in 10% DMSO, 15% ethanol/cremaphore 50:50 in H₂O at 4 mg/kg
- First dose given 6 hours post infection (5x10⁴ CFU for burn model, 5x10⁶ for lung model)
- Then M64 is given q12 h until Day 5.5

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Starkey et al. PLoS Pathog 2014, 10(8): e1004321
Assessing Target Engagement *In Vivo*

- Model designed to assess target engagement
- NOT designed to measure impact on survival or burden based on short duration and high bacterial burden

- Immunocompetent acute thigh infection
- Pa14 – highly virulent, high biomarker producing strain
- Measure HHQ and PQS after two doses of MvfR inhibitor 100 and 200 mg/kg PO (2, 10 hr PI)
- Sacrifice at 12 hr PI
- No difference in burden (expected result)
MvfR Chemistry Advanced

A research career + a company + an alliance + some millions of dollars

Substantial improvements from the chemical lead M64, but solubility limitations continue to dog the efforts to validate the approach and reach the clinic.
Challenges to the Development of a Narrow-Spectrum Anti-virulence Therapeutic

• **Challenge 1: Finding patients** - Need evaluable patients infected with target pathogen
  - *P. aeruginosa* is common VAP pathogen, occurs 18-21% of cases\(^1\)
  - May need to screen over 3000 subjects to find evaluable subjects for a typical VAP trial (300 patients/arm)
  - HAP/VAP studies are hard to recruit in general, typically <0.1 subjects/center/month
    - Recent HAP/VAP study took 5 yrs to enroll ~1200 pts.\(^2\)

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\(^1\)Jones RN, Etiologies of HABP and VABP • CID 2010:51 (Suppl 1)
\(^2\)Wunderink RG, et al. Linezolid in MRSA Nosocomial Pneumonia: A Randomized, Controlled Study. CID 2012 54:621-9
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• **Challenge 2:** Unless you can consider an antivirulence therapy as an alternative to an antibiotic (...) and be certain to only enroll *P. aeruginosa*, you have to consider the anti-virulence therapy as an **adjunct** to antibiotics
  - Must prove superiority
  - SoC + antivirulence > SoC alone

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  - SoC + antivirulence > SoC alone

- **Challenge 3:** Is there enough “headroom” left to actually show superiority
  - Perhaps for some, likely severely ill subjects, i.e. rescue
  - But unlikely all subjects, after all a cure is a cure

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Good Idea, Not so Good Outcome… So Far

• Approach is intriguing
• Methods still need refinement
• Chemistry significantly advanced but still limiting
• Remaining questions about “window” of opportunity
• Clinical development plans still significantly uncharted
Acknowledgements

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  - Damien Maura
GSK2251052 A LEUCYL tRNA SYNTHETASE INHIBITOR

Resistance Emerges in the Clinic

Mechanism of action
- LeuRS is an essential enzyme for protein synthesis
- GSK'052 forms an adduct with the tRNA in the editing site locking LeuRS in an unproductive state
- Mutants are editing deficient
### ‘052 In vitro Activity: Enterobacteriaceae, including Multidrug-resistant Strains

<table>
<thead>
<tr>
<th>Organism (Selected Phenotypes)</th>
<th>No. of Isolates</th>
<th>MIC&lt;sub&gt;90&lt;/sub&gt; (mcg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AN3365</td>
<td>Tigecycline</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>2029</td>
<td>1</td>
</tr>
<tr>
<td>E. coli (WT)</td>
<td>214</td>
<td>1</td>
</tr>
<tr>
<td>E. coli (ESBL)</td>
<td>27</td>
<td>1</td>
</tr>
<tr>
<td>Klebsiella spp. (WT)</td>
<td>159</td>
<td>1</td>
</tr>
<tr>
<td>Klebsiella spp. (ESBL)</td>
<td>71</td>
<td>1</td>
</tr>
<tr>
<td>Klebsiella spp. (KPC)</td>
<td>26</td>
<td>2</td>
</tr>
<tr>
<td>Enterobacter aerogenes (WT)</td>
<td>199</td>
<td>1</td>
</tr>
<tr>
<td>Enterobacter aerogenes (AmpC)</td>
<td>51</td>
<td>1</td>
</tr>
<tr>
<td>Enterobacter cloacae (WT)</td>
<td>202</td>
<td>1</td>
</tr>
<tr>
<td>Enterobacter cloacae (AmpC)</td>
<td>50</td>
<td>0.5</td>
</tr>
<tr>
<td>Citrobacter freundii (AmpC)</td>
<td>60</td>
<td>1</td>
</tr>
<tr>
<td>Morganella morganii (AmpC)</td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td>Proteus mirabilis (WT)</td>
<td>235</td>
<td>1</td>
</tr>
<tr>
<td>Proteus vulgaris (WT)</td>
<td>41</td>
<td>1</td>
</tr>
<tr>
<td>Providencia spp.(all)</td>
<td>68</td>
<td>1</td>
</tr>
<tr>
<td>S. marcescens (AmpC)</td>
<td>26</td>
<td>1</td>
</tr>
</tbody>
</table>

**Key:** Red = resistant, Yellow = intermediate and Green = susceptible based on CLSI interpretive criteria (M100-S21, 2011), except for GSK2251052 (susceptible defined as MICs ≤4 mcg/mL), tigecycline (FDA interpretive criteria used to define susceptibility) and polymixin B (susceptible ≤2mcg/mL , intermediate 4 mcg/mL, resistant ≥8 mcg/mL)
Evolution of ‘052 Efficacy Package Leading to Candidate Selection

- Anacor and GSK data leading to CS decision
  - Efficacy demonstrated vs 1 *P. aeruginosa*, 1 *K. pneumoniae*, 2 *E. coli*, and 1 *E. cloacae* in neutropenic thigh infection murine model, (SQ dosing)
  - 2 *B. fragilis* in non-neutropenic abscess model (PO dosing)
  - Pneumonia (2 *K. pneumoniae*) and skin/soft tissue models (multiple organisms), non-neutropenic, PO and SQ dosing
  - ‘052 was shown to circumvent major GN efflux mechanisms

- As FTIH data became available:
  - Would the human exposure profiles be efficacious?
### ‘052: Pre-Clinical *In vitro* Frequency of Resistance

<table>
<thead>
<tr>
<th>Compound</th>
<th>Frequency of Resistance</th>
<th>E. coli</th>
<th>K. pneumoniae</th>
<th>P. aeruginosa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4xMIC</td>
<td>10xMIC</td>
<td>4xMIC</td>
</tr>
<tr>
<td>AN3365/(‘052)</td>
<td></td>
<td>8x10^{-8}</td>
<td>6x10^{-8}</td>
<td>5x10^{-8}</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2x10^{-7}</td>
<td>7x10^{-8}</td>
<td>8x10^{-7}</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1x10^{-7}</td>
<td>8x10^{-8}</td>
<td>4x10^{-8}</td>
</tr>
<tr>
<td>Ceftazidine</td>
<td>&lt;3x10^{-9}</td>
<td>1x10^{-8}</td>
<td>&lt;4x10^{-9}</td>
<td>2x10^{-7}</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>2x10^{-8}</td>
<td>&lt;4x10^{-9}</td>
<td>5x10^{-7}</td>
<td>&lt;5x10^{-9}</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>2x10^{-8}</td>
<td>&lt;5x10^{-9}</td>
<td>2x10^{-7}</td>
<td>&lt;3x10^{-9}</td>
</tr>
<tr>
<td>Meropenem</td>
<td>&lt;4x10^{-9}</td>
<td>&lt;4x10^{-9}</td>
<td>&lt;3x10^{-9}</td>
<td>&lt;3x10^{-9}</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>1x10^{-5}</td>
<td>4x10^{-6}</td>
<td>1x10^{-6}</td>
<td>5x10^{-8}</td>
</tr>
</tbody>
</table>

Key: Red = High Resistance ≥1x10^{-7}; Yellow = Moderate Resistance; Green = Satisfactory Resistance <1x10^{-8}

- While considered moderate-to-high, spontaneous resistance frequencies for AN3365/(‘052) ranged from 10^{-7}-10^{-8} and were within the range of frequencies for comparator agents.
‘052: Pre-Clinical Mechanism of Resistance

- Laboratory generated mutants resistant to AN3365/‘052 contain mutations in the LeuRS editing domain
  - Single-step mutations in leuS
- AN3365/‘052 MICs of resistant mutants range from 32->256 mcg/mL

<table>
<thead>
<tr>
<th>Organism</th>
<th>AN3365/‘052 MIC (mcg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WT</td>
</tr>
<tr>
<td><strong>E. coli</strong></td>
<td>1</td>
</tr>
<tr>
<td><strong>K. pneumoniae</strong></td>
<td>1</td>
</tr>
<tr>
<td><strong>P. aeruginosa</strong></td>
<td>4</td>
</tr>
</tbody>
</table>

- AN3365/‘052 resistant mutants do not confer cross-resistance to other antibiotics and they appear to be editing deficient as evidenced by their sensitivity to norvaline
Phase II Clinical Studies

- **LRS114688**: Comparative, dose-ranging study of GSK2251052 vs. imipenem-cilastatin in complicated lower urinary tract infection and pyelonephritis
- **LRS114689**: Comparative, dose-ranging study of GSK2251052 vs. meropenem in the treatment of complicated intra-abdominal infection
  - Both studies are dose ranging, 750mg:1500mg twice a day: Active Comparator
  - 210 subjects per study
  - Independent Safety Review Committee
  - Decide on the most appropriate and safe dose for Phase III

- Studies were suspended when the rapid emergence of resistance was detected in four subjects in the cUTI study

  cUTI: (20 patients recruited)  cIAI: (15 patients recruited)
Summary

• A thorough understanding of antibacterial resistance potential is essential
  - Assessing fitness is difficult but an important consideration; better, robust models should be developed
    • Current in vitro systems give mutants not seen in the wild
    • Failure to do so can lead to the emergence of resistance in clinical study
• More needs to be understood about potential of combination therapies to prevent emergence of resistance
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...and many others
Oh My, Polymixins

[Chemical structures of Polymixins]

- PMB
- SPR7061
- PMBN
- SPR826
- NAB815
- SPR741
- NAB739
Potentiator: Another Approach to Treating Gram-Negative Infections

UNTREATED GRAM-NEGATIVE BACTERIA

GRAM-NEGATIVE BACTERIA TREATED WITH SPR741

Potentiators interact with lipopolysaccharide, disrupting the outer cell membrane and allow antibiotics to pass into the cell.
Novel Platform Allows Entry of Antibiotics into Gram-Negative Bacteria

**GRAM-NEGATIVE BACTERIA TREATED WITH AZITHROMYCIN ONLY**

*E. coli* (ATCC25922) bacteria continue to divide during 1 hour period; lack of green in cell signals lack of entry by labeled azithromycin

**GRAM-NEGATIVE BACTERIA TREATED WITH AZITHROMYCIN+SPR741**

Azithromycin enters cell in high concentrations over 1 hour period; cells stop dividing
SPR741 – Very Poor Antibacterial Activity, Poor Cytoplasmic Leakage

ATP Released into Medium (30 min Incubation)

ATP Released (µM)

Concentration (µg/mL)

0 0.05 0.1 0.15 0.2

0 0.5 1 2 4 8 32 64 128 256

PMB

SPR741
SPR741 Headed for Clinical Evaluation

- Much reduced nephrotoxicity
- Much reduced antibacterial activity
- Maintains strong synergy with otherwise “too large” antibiotics
- In combination acts to potentiate the activity of the antimicrobial agents
Potentiator Combinations Demonstrate Killing of MDR/XDR Gram-negative pathogens – Rifampin

A. baumannii (n = 162)

Enterobacter cloacae (n = 31)

MIC of Combination

E. coli (n = 138)

K. pneumoniae (n = 136)
SPR741 Combinations Demonstrate *In Vivo* Efficacy in Lung Infections of MDR/XDR Gram-negative Pathogens

- Neutropenic mouse lung infection, *K. pneumoniae ATCC 43816*, harvested at 25h
- SPR741 SQ 2, 10, 18; rifampin SC at 2, 10, 18h
SPR741 Combinations Demonstrate In Vivo Efficacy in Thigh Infections of MDR/XDR Gram-negative pathogens

- Neutropenic mouse thigh infection, *K. pneumoniae KPC 114*, harvested at 25h
- SPR741 SQ 1, 9, 17; rifampin SC at 1, 9, 17h
GyrB/ParE Inhibitors Potential Partner for SPR741

- Formerly VXc-486/VXc-100
- GyrB/ParE dual targeting
- Modest Gram-negative activity with dramatic potentiation potential
Novel Spero Partner (SPR719), Exceeds Rifampin and Meropenem Spectrum Coverage

**A. baumannii**
- **719**
- **719 +741 @ 8**
- **RIF + 741 @8**
- **MERO**

**K. pneumoniae**
- **719**
- **719 +741 @ 8**
- **RIF + 741 @8**
- **MERO**

**Enterobacter**
- **719**
- **719 +741 @ 8**
- **RIF + 741 @8**
- **MERO**

**E. coli**
- **719**
- **719 +741 @ 8**
- **RIF + 741 @8**
SPR741 On Track for FIH Studies in Q4 2016

- ADME studies completed
- Genotoxicity studies completed
- 14-day repeat dose IV toxicology studies in rat & monkey completed
  - Monkey NOAEL 40 mpk/day → Plasma AUC ~300 μg.hr/mL/day
  - Rat NOAEL 5 mpk/day → Plasma AUC ~12 μg.hr/mL/day
- Safety pharmacology studies completed
- Hemolysis, flocculation & local tolerance studies completed

All data support moving forward based upon the margins compared to anticipated human exposure in the Phase 1 & projected efficacious dose.
Beyond Me Too

- Good Idea!
- Try with your eyes open and be thoughtful
- Do the critical experiment to kill the project early
- Start now and work hard
The Quest for Safe and Effective Novel Classes

"If we pull this off, we'll eat like kings."