



Pitfalls of Target Validation for Novel Antibacterial Strategies

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The Reality:

Novel Targets

GSK discovered >100 targets

Run HTS

Between 1995-2001 GSK ran >70 HTS (massive effort !)

Novel Classes

HTS very unsuccessful few new series discovered

Lead Optimization

Dev't Candidates

Exceptionally more challenging for novel classes than predicted

Clinical Testing

Hard to get resistance claims, unpredictable regulatory environment

Nature Reviews Drug Discovery 6, 29-40 (Jan 2007)

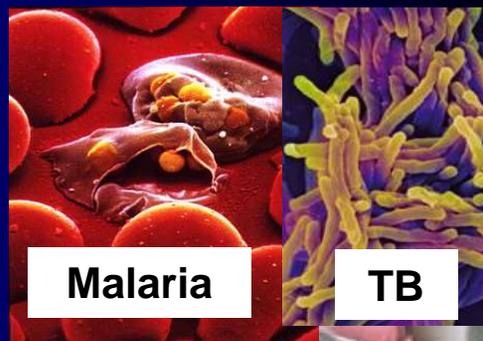
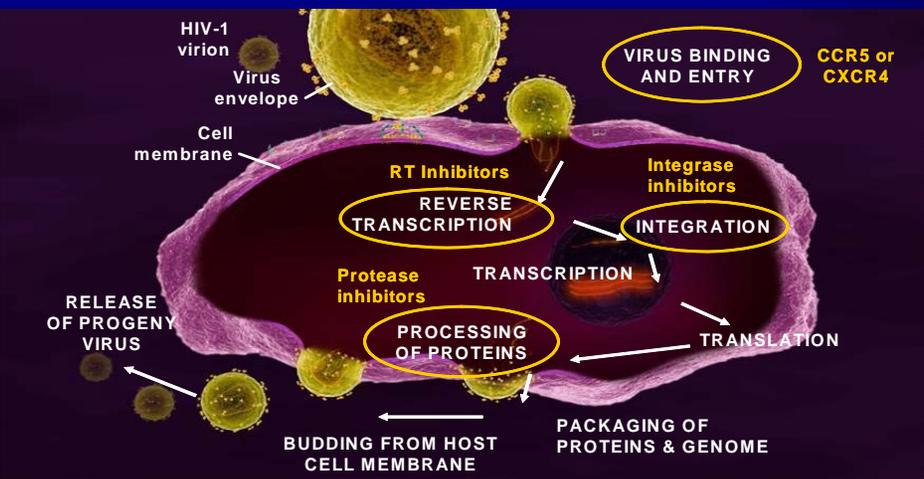
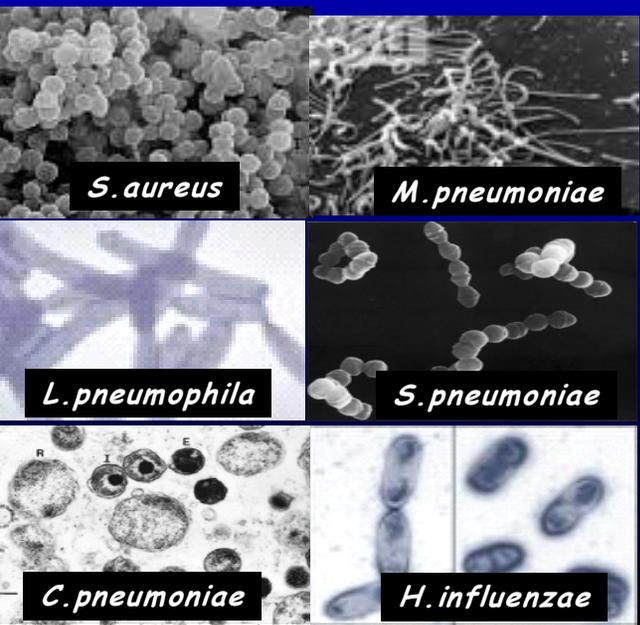
Return on investment low compared to other areas



Overall low pharma investment in antibacterials R&D

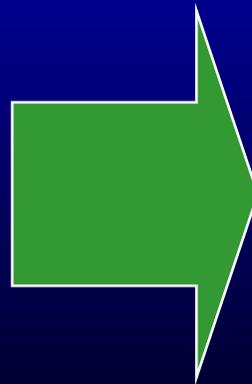
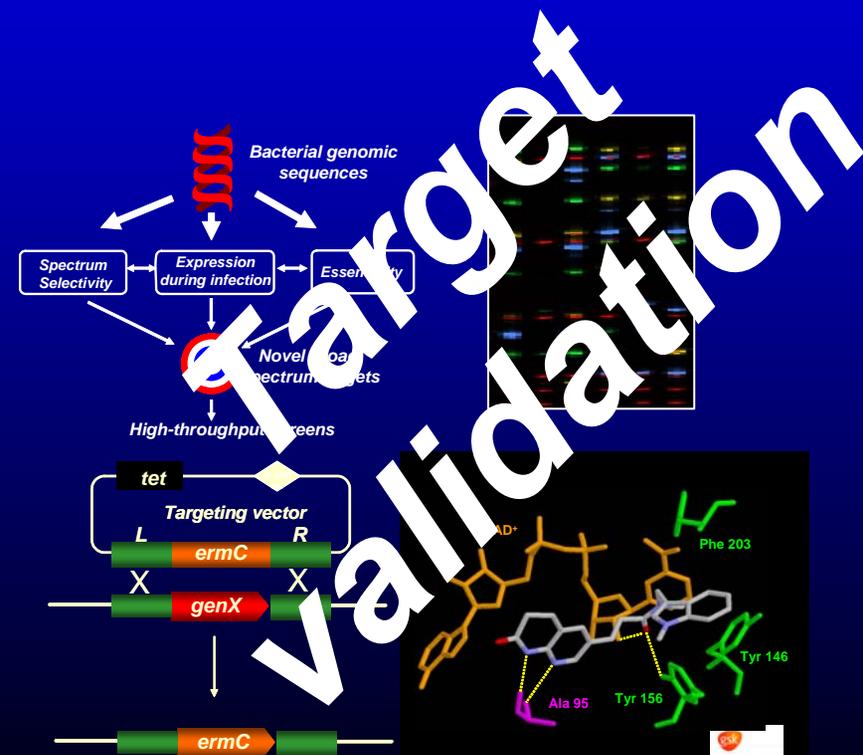
Infectious Diseases Center of Excellence in Drug Discovery (ID CEDD)

Antibacterials Diseases of the Developing World Antivirals



Target Validation

- Demonstrate the target is critically involved in the disease process and modulation will have a therapeutic effect.



Using Bioinformatics to Select targets

Genomic DNA
sequences
from variety
of bacterial
pathogens



Bioinformatics



Is the target
present in a
clinically
relevant
spectrum of
organisms ?

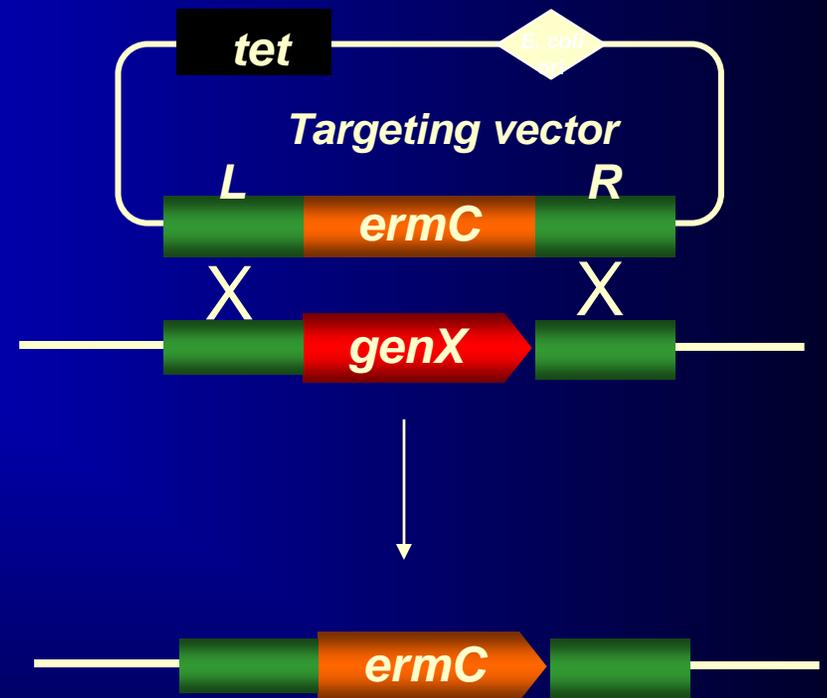
Pathogen coverage required

	Gram +				Gram -							
	Spy	MRSA	Spn	MSSA	Hin	Mca	Kpn	Eco	Pmi	Bfrag	Enteroc	Pae
Community			✓	✓	✓	✓	✓	✓	✓			
Hospital	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Gram Positive	✓	✓	✓	✓								
Gram Negative					✓	✓	✓	✓	✓	✓	✓	✓
Pathogen specific	x	x	x	x	x	x	x	x	x	x	x	✓
Pathogen specific	x	✓	x	x	x	x	x	x	x	x	x	x

Screen genomes for target gene

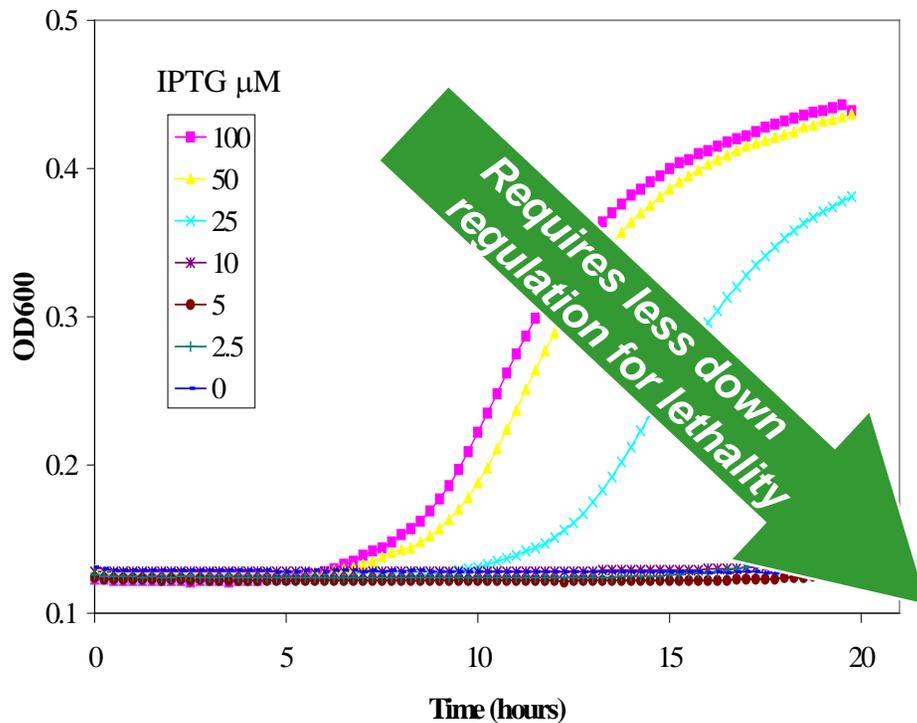
Essentiality Testing

- Genomic based technologies enabled gene knockouts to be performed rapidly.
- Gene knockouts completed in days
- Higher throughput gene knockouts developed for different bacterial species
- Essentiality of 100s genes evaluated

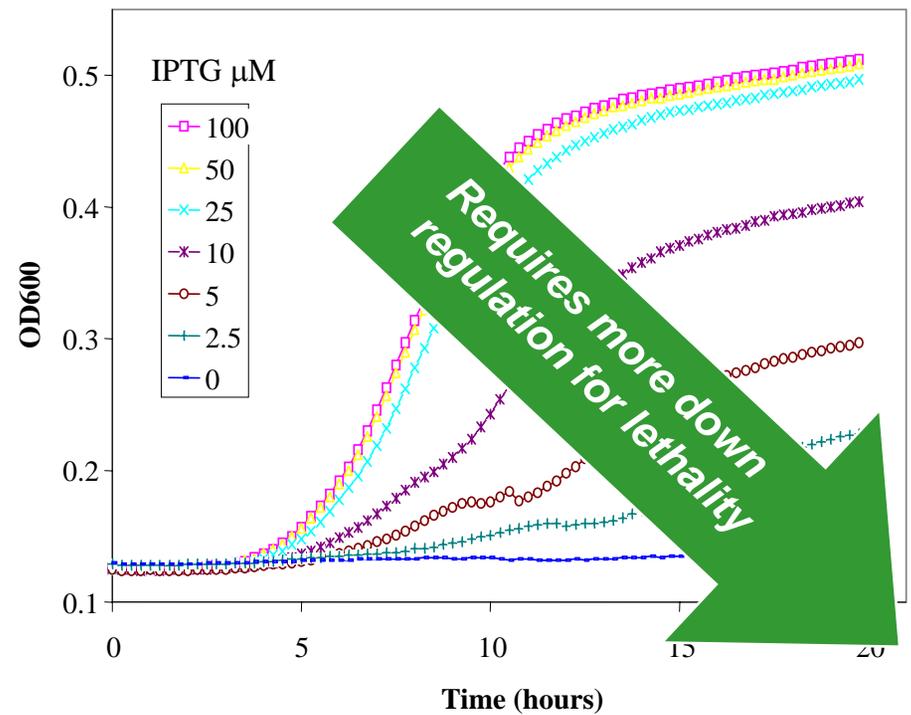


Are some essential targets better than others ?

Expression Titration of MRS



Expression Titration of Def1



One species not representative

Essential ?

Gene:	<i>S. pneumoniae</i>
<i>trmE</i>	✓
<i>ypuL</i>	✓
<i>yaaJ</i>	✓
<i>ykrA</i>	✓
<i>yrrK</i>	✓
<i>ydiB</i>	✓

Novel Target Seduction - PcrA

PcrA is encoded by Gram positive bacterial and is essential for cell growth

helicase in *E. coli* (3). Furthermore, because of its requirement for cell viability, PcrA may also represent an important target for the development of antibacterial agents against Gram-positive organisms.

PcrA is an essential helicase in Gram-positive bacteria, but its precise role in cellular DNA metabolism is currently unknown. The *Staphylococcus aureus*

involved in nucleic acid metabolism. The PcrA DNA helicase is an essential bacterial protein involved in rolling circle plasmid replication and DNA repair.

PcrA is an essential helicase in gram-positive bacteria, and a gene encoding this helicase has been identified in all such organisms whose genomes have been sequenced so far. The precise role of PcrA that makes it

Chorismate Biosynthesis

Phosphoenol pyruvate + erythrose 4-phosphate

↓ 3-deoxy-arabino-heptulosonate 7-P synthase (aroF,G,H)

↓ 3-dehydroquinate synthase (aroB)

↓ 3-dehydroquinate dehydratase (aroD)

↓ Shikimate dehydrogenase (aroE)

↓ Shikimate kinase (aroK/L)

→ ↓ EPSP synthase (aroA)

↓ Chorismate synthase (aroC)

Enterochelin

Ubiquinone ←

Vitamin K

←

Chorismate

↓

Phenylalanine

Tryptophan

Tyrosine

→

→

→

p-aminobenzoic acid (PABA)

↓

↓

↓

↓

Folic acid

Pathway essentiality

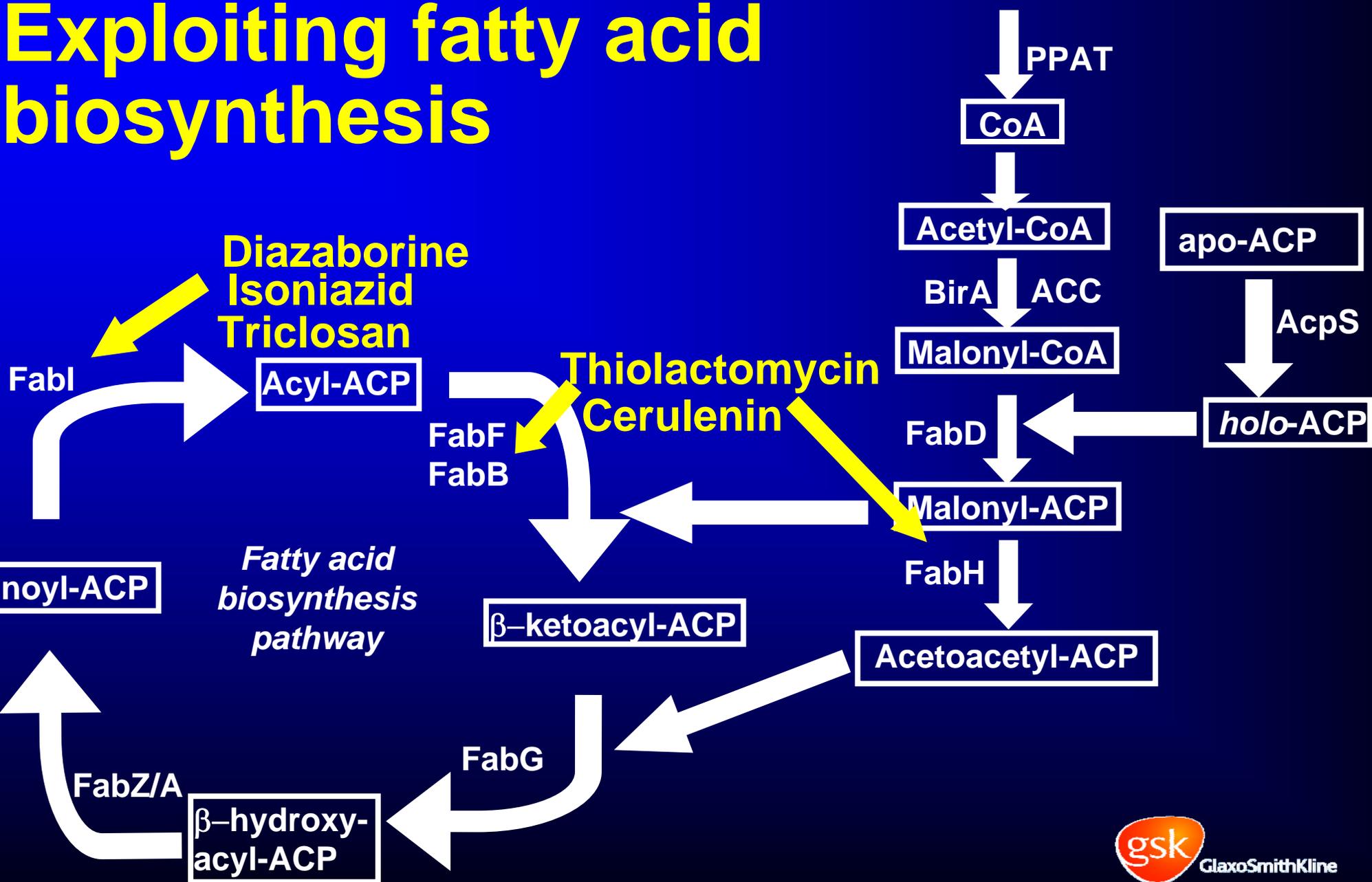
	aroB	aroD	aroE	aroK
Bioinformatics	✓	✓	✓	✓
<i>S.pneumoniae</i> in vivo essential	YES	YES	YES	YES
<i>H.influenzae</i> in vitro essential	YES	YES	YES	YES

Pathway essentiality

	aroB	aroD	aroE	aroK
Bioinformatics	✓	✓	✓	✓
<i>S.pneumoniae</i> <i>in vivo</i> essential	YES	YES	YES	YES
<i>H.influenzae</i> <i>in vitro</i> essential	YES	YES	YES	YES
<i>S.aureus</i> <i>in vivo</i> essential	YES	YES	NO	YES

- One species not representative of all bacteria
- Essentiality of 1 pathway enzyme not representative of all steps in the pathway.

Exploiting fatty acid biosynthesis



FabI target validation 1996

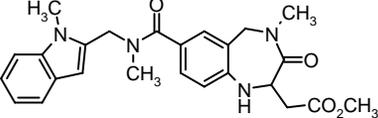
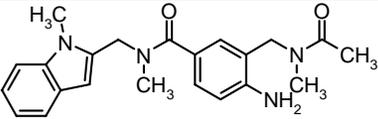
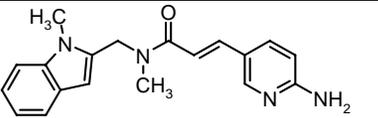
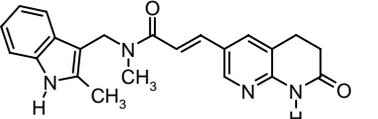
- Present in *E.coli*, *H.influ* & *S.aur* genomes, not yet found in partial *S.pneumo* genome
- Essentiality proven in *S.aureus* (K/O). TS lethal mutants of FabI in *E.coli*
- FabI inhibitors (Diazaborines, Triclosan) were broad spectrum antibacterials.
 - eg: Diazaborines active against *Proteus*, *E.coli*, *Salmonella*, *Klebsiella*, *Enterobacter*, *Neisseria*, *Staph*, *Bacillus*, *Strep*.
- FabI 'unique enoyl ACP reductase' in all bacteria

Enoyl ACP reductase (FabI) - HTS leads



	High throughput screening lead	IC50 (uM)		
		<i>S.aureus</i> FabI	<i>E.coli</i> FabI	Human FAS
Benzodiazepine series		17.1	-	>100
Imidazole series		1.24	13.7	>100

Enoyl ACP reductase (FabI)

Compound	IC ₅₀ (uM)		MIC (ug/mL)
	<i>S.aureus</i> FabI	<i>H.influenzae</i> FabI	<i>S. aureus</i>
1 	17.1	6.9	>64
2 	6.7	4.7	16
3 	2.4	4.2	0.5
4 	0.047	0.13	0.06

MILLER, et al. (2002). J Med Chem 45:3246-3256. PAYNE et al. (2002). AAC 46: 3118-3124.

- Target potency, antibacterial activity & developability characteristics of HTS hit successfully optimized.

Early lead optimization looked promising

	Triclosan	[3]	SB 515905
<i>S.aureus</i>	0.06	0.5	<0.06
<i>E.faecalis</i>	2	>64	64 - >64
<i>S.pneumoniae</i>	8	>64	>64
<i>M.catarrhalis</i>	0.06	4	2
<i>H.influenzae</i>	4	>64	16
(<i>E.coli</i> AcrAB-)	8	8	4)

- Highly potent vs *S.aureus*
- But no success in achieving activity vs *S.pneumo*

Discovery of FabK, unrelated to FabI

	<i>E.faecalis</i>	<i>S.aureus</i>	<i>S.pneumoniae</i>	<i>M.catarrhalis</i>	<i>H.influenzae</i>
Enoyl ACP reductase	FabK & FabI	FabI	FabK	FabI	FabI

(Heath & Rock (2000). Nature 406, 145-146)

	FabI	FabK
Tertiary structure	Rossmann fold	TIM-barrel
Cofactor	-	FMN
NAD(P)H	NADPH or NADH	NADH
Inhibition by Triclosan	YES	NO
MW	28KDa	34KDa
Essentiality	<i>E.coli, H.infl, S.aureus</i>	<i>S.pneumoniae</i>

Marrakchi, Dewolf, Quinn, West, Polizzi, Holmes, Reed, Heath, Payne, Rock, Wallis (2003). *Biochem J* 15;370(Pt 3):1055-62.

- Unlikely single molecule inhibit FabK & FabI, FabI select spectrum target

Aminoacyl tRNA Synthetases

Enzyme recognition and activation:



Enzyme transfer:



Protein Synthesis

Different tRNA synthetase for each amino acid

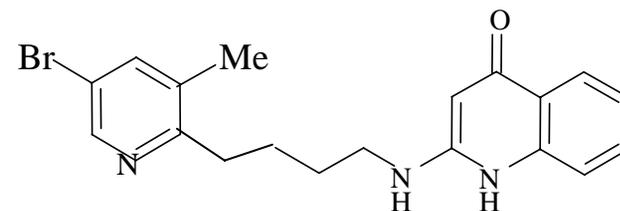
Novel Antibiotic Target Surprises

- Methionyl tRNA synthetase

Gram +				Gram -							
Spy	MRSA	Spn	MSSA	Hin	Mca	Kpn	Eco	Pmi	Bfrag	Entero	Pae
✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

MRS gene identified:

- Hit from comprehensive screening of all 19 *S. aureus* tRNA synthetases
- HTS hit identified, no antibacterial activity



IC50s vs MRS enzyme

S.aureus 350 nM

MICs

S.aureus >64

S.pneumoniae >64

E.faecium >64

Rational Design: Building In Activity Against G+ves & H. influ

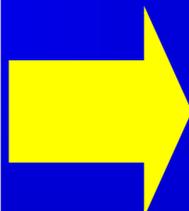
SB-299683

IC50s vs MRS enzyme

S.aureus 5.2 nM
H.influenzae > 500 nM

MICs

S.aureus 4 - 8
S.pneumoniae 0.5, 4, 64
E.faecium 2 - 4
H.influenzae 64, >64



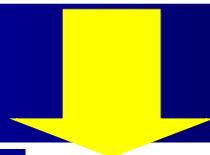
SB-430537

IC50s vs MRS enzyme

S.aureus 9.0 nM
H.influenzae > 1000 nM

MICs

S.aureus <0.06-0.25
S.pneumoniae 2, 2, 64
E.faecium <0.06
H.influenzae >64



SB-655260

MIC90s (ug/ml):

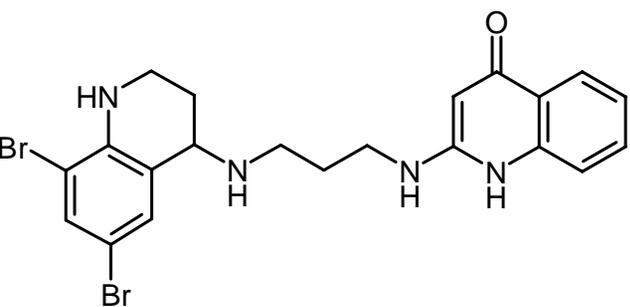
<i>S.aureus</i>	<i>S.epidermidis</i>	<i>E.faecium</i>	<i>E.faecalis</i>
0.5	0.5	0.5	0.5

MICs

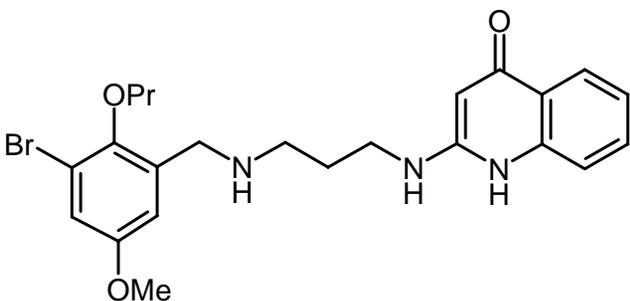
<i>S.aureus</i>	<i>S.pneumoniae</i>	<i>E.faecium</i>	<i>H.influenzae</i>
0.5	2-4, >64	2, 0.5	2, 4

PK parameters optimized to achieve in vivo efficacy

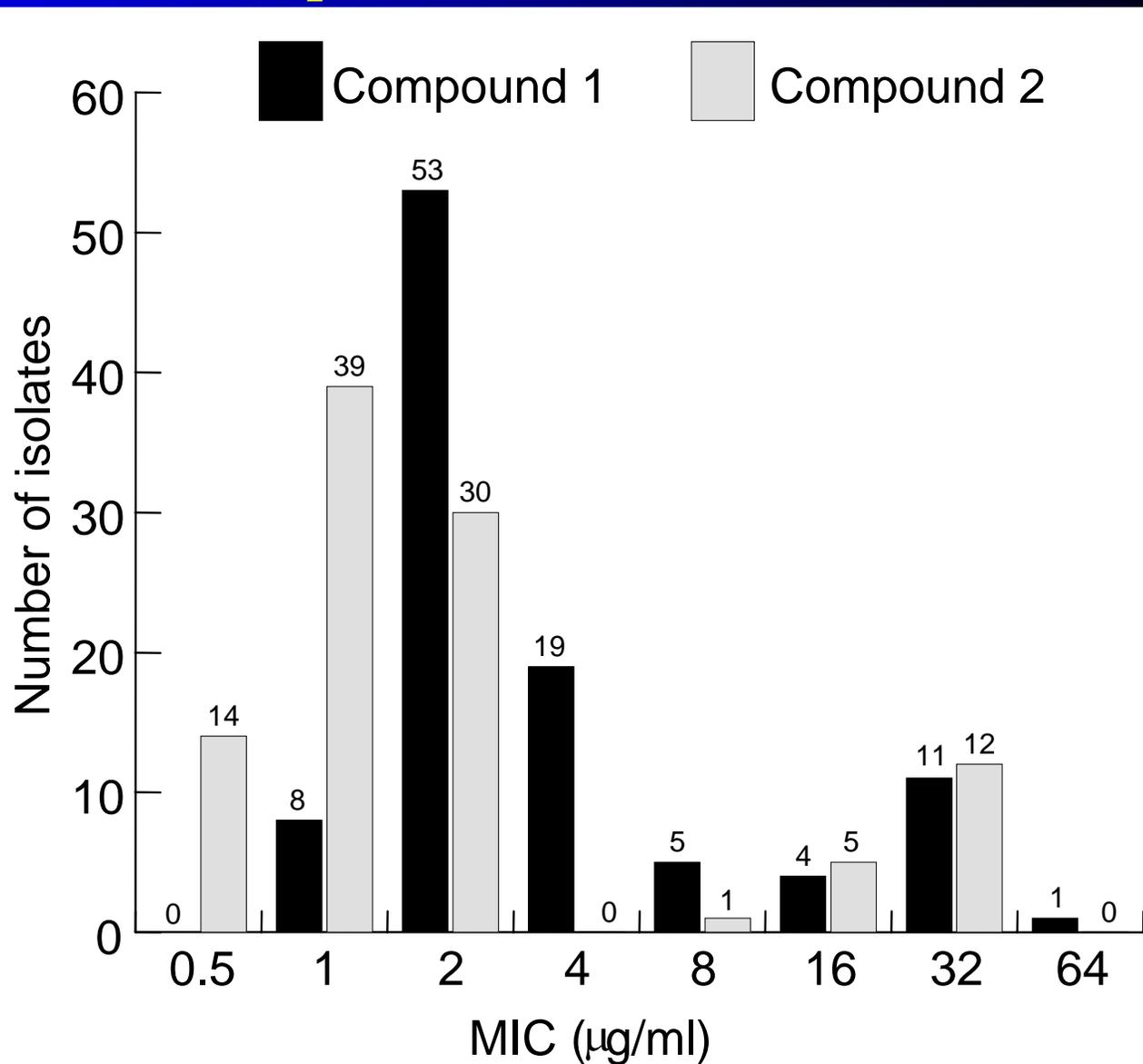
MRS leads vs *S.pneumoniae* (n=101)



Compound 1



Compound 2



Discovery of MRS1 & MRS2

<i>S.pneumo</i> strain	MIC (ug/ml)	
	Compound 1	Tetracycline
R6	4	0.5
QA1442	>64	0.5
QA1442 $\Delta mrs1$	>64	0.5
QA1442 $\Delta mrs2$	4	0.5

- Resistant strains have 2 MRS (MRS1 & MRS2)
 - MRS2 resistant to inhibition by leads !
- Survey of 315 isolates (1992-98, 7 countries), showed prevalence of MRS2 46%

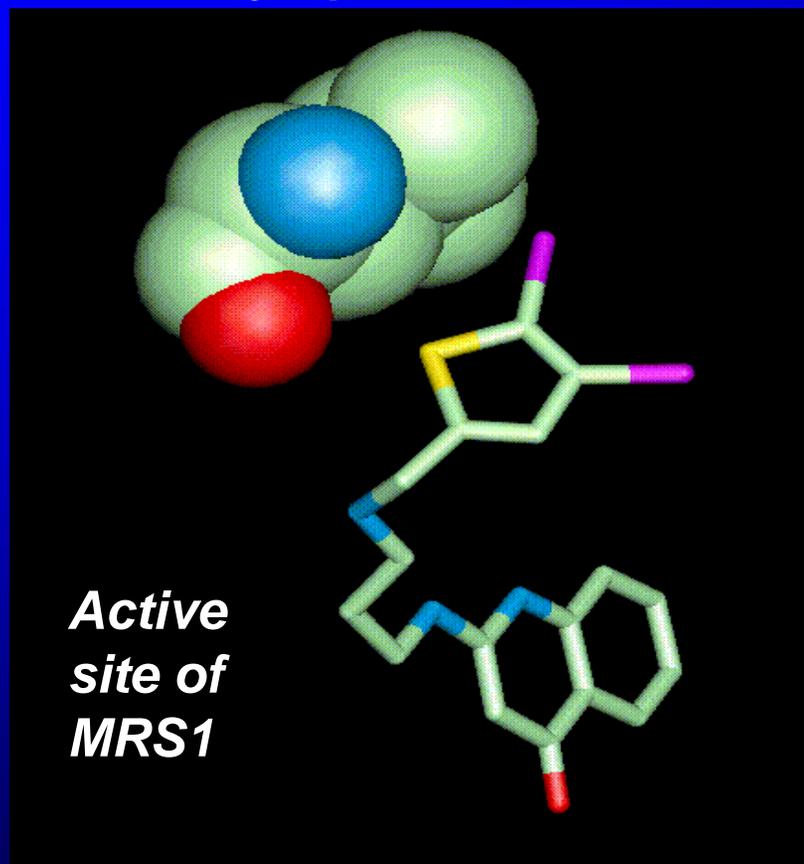
Geographic distribution of MRS2 by (determined by PCR)

- Survey of 315 isolates (1992-98, 7 countries), showed prevalence of MRS2 46% *S.pneumoniae* isolates

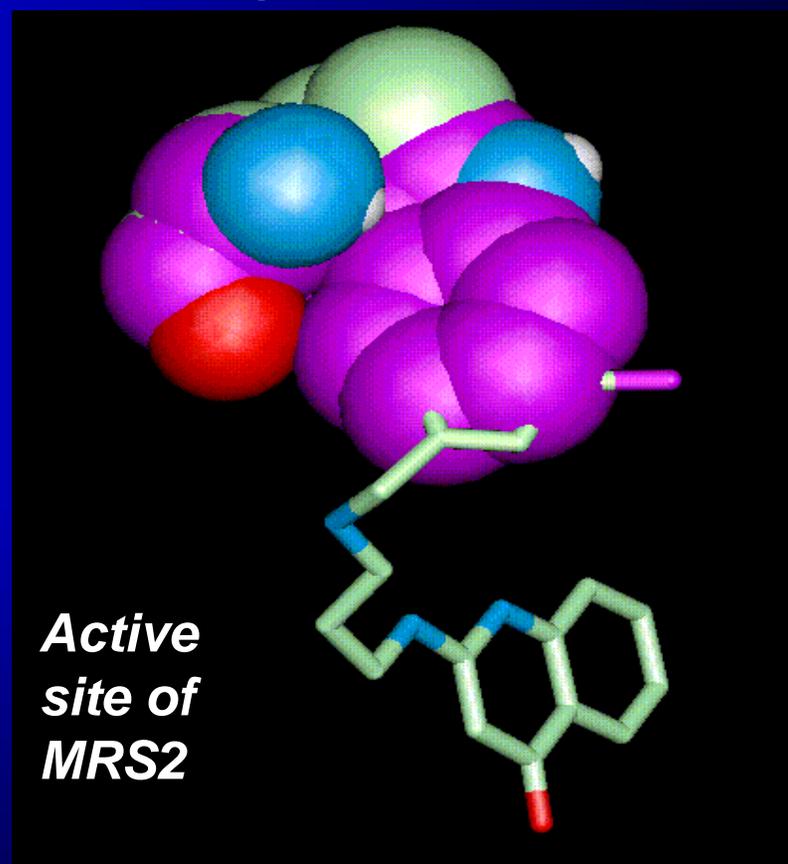
Country	Year	Present	Absent	# Isolates	Frequency
France	1992	3	8	11	0.27
	1993	9	11	20	0.45
	1994	7	7	14	0.50
	1995	9	4	13	0.69
	1996	7	6	13	0.54
	1997	6	9	15	0.40
	1998	4	11	15	0.27
Germany	1992	1	3	4	0.25
	1994	0	5	5	0.00
	1997	2	1	3	0.67
	1998	1	5	6	0.17
Italy	1994	1	1	2	0.50
	1995	3	2	5	0.60
	1996	3	7	10	0.30
	1997	3	4	7	0.43
	1998	0	3	3	0.00
Spain	1992	2	3	5	0.40
	1993	3	4	7	0.43
	1994	6	1	7	0.86
	1995	9	1	10	0.90
	1996	4	1	5	0.80
	1997	6	2	8	0.75
	1998	21	38	59	0.36
UK	1992	2	2	4	0.50
	1993	1	7	8	0.13
	1994	1	2	3	0.33
	1995	10	2	12	0.83
	1996	6	2	8	0.75
	1997	7	3	10	0.70
	1998	21	38	59	0.36
USA	1992	0	1	1	0.00
	1997	7	10	17	0.41
Hong Kong	1997	0	5	5	0.00
Totals		144	171	315	0.46

Two types of MRS exist in *S.pneumo*

- LHS aryl-pocket occluded by Leu->Trp in MRS2



Novel MRS antibiotics inhibit MRS 1



Novel MRS antibiotics do not inhibit MRS2

- Unable to optimize leads to encompass MRS1 and MRS2

Implications.....

- Antibiotic from current series would only be active vs 50% of *S.pneumoniae* (project terminated).
- **Implications for target validation**
 - MRS target demonstrated to be broad spectrum by genomic analysis.
 - Bacterial genomes provide the genetic make up for only ONE strain from the species
 - Target validation ‘beyond the genome’ required to minimize risk of ‘surprises’ !

What we learned ?

- Novel targets have surprises
 - 1 species not representative of all species
 - Pathways – not all enzymes essential
 - Validation beyond the genome
- LO tough enough, robust target validation is imperative.
- Assumptions made in the place of incomplete information

There are known knowns. These are things we know that we know. There are known unknowns. That is to say, there are things that we know we don't know. But there are also unknown unknowns. There are things we don't know we don't know.

Donald Rumsfeld

