Not so FAS

By Tim Fulmer, Senior Writer

The importance of fatty acid biosynthesis to bacterial survival has made the pathway fertile ground for antibiotic targets, and at least three companies are pursuing compounds that block the process. French researchers, however, have challenged the approach with a paper in *Nature* that describes how Gram-positive bacteria can assimilate host fatty acids to ensure bacterial survival.¹

Companies working in the space said the surprising results are still preliminary and may not be applicable to all Gram-positive species.

Synthesis of fatty acids in the fatty acid synthase II (FASII) pathway allows bacteria to modify membrane lipid composition and survive changes in temperature, pH and other environmental factors (see **Figure 1, “Antibacterial targets in fatty acid biosynthesis”**). The organization of the FASII pathway is conserved across many bacterial species but is distinct from the analogous FASI pathway in mammals, thus making FASII enzymes good antibiotic targets.^{2,3}

But the French researchers found that the FASII pathway may not be required for survival in some contexts because bacteria can take up fatty acids directly from host serum rather than synthesizing them *de novo*.

The group's *in vitro* experiments revealed that multiple Gram-positive bacteria could survive inhibition by two different FASII-targeting compounds—cerulenin and triclosan—so long as the strains were cultured in the presence of a fatty acid-based emulsifying agent or human serum. This suggested that the bacteria survived by taking up fatty acids from outside sources.

To confirm those results, a series of FASII pathway knockout mutants were created in *Streptococcus agalactiae*. In the presence of human serum or exogenously added fatty acids, all strains had growth comparable to wild-type *S. agalactiae*. In neonatal rats, wild-type and *S. agalactiae* knockout strains caused the same levels of mortality. Similarly, in adult mice, serum and tissue bacterial counts were comparable between wild-type and knockout strains.

The authors concluded that their findings “strongly predict that FASII-directed drugs would be ineffective under natural infection conditions.”

Casting a wider net

Company researchers polled by SciBX were not so sanguine, suggesting that the paper's findings were too limited to warrant the general conclusion that FASII inhibitors are unsuitable antibiotics for all Gram-positive bacteria.

“The results reported by Brinster *et al.* are interesting, yet they represent an incomplete story due to the limited number of bacterial

organisms and compounds that were used in their *in vivo* work,” said Sheo Singh, a senior investigator at **Merck & Co. Inc.** “One should not over-generalize the conclusion that all Gram-positive pathogens are poor candidates for FASII inhibition until broader studies are performed.”

Those broader studies could include looking at other, more specific, FASII inhibitors, said Barry Hafkin, CMO of **Affinium Pharmaceuticals Inc.** “The two inhibitors used in the paper—triclosan and cerulenin—are research tools that are inadequate to support the paper's general conclusions regarding FASII-targeting antibiotic strategies,” he said.

“The compounds are nonspecific, killing all bacteria, Gram-positive and Gram-negative, and likely by mechanisms in addition to inhibiting FASII,” said Nachum Kaplan, VP of microbiology at Affinium. “Secondly, both compounds are highly hydrophobic, and in the presence of serum they are sequestered by plasma proteins. As a result, only small

Figure 1. Antibacterial targets in fatty acid biosynthesis. Brinster *et al.* suggest in *Nature* that, at least in some instances, the fatty acid synthase II (FASII) pathway may not be essential for bacterial survival, thus throwing some doubt on FASII-targeting strategies. All bacteria rely on the FASII pathway to synthesize fatty acids for incorporation into the bacterial cell membrane. The paper's findings suggest that bacteria may be able to survive by using fatty acids derived from the infected host.

The synthesis of fatty acids is a cyclic process that involves the successive addition of carbon atoms by four enzymes: FabF (or FabB) 3-oxoacyl-acyl carrier protein synthase II, FabG 3-oxoacyl-acyl carrier protein reductase, FabZ (or FabA) β -hydroxyacyl-acyl carrier protein dehydratase, and FabI (or FabK) enoyl-acyl carrier protein reductase. Blocking any of the four steps can shut down fatty acid biosynthesis and potentially kill bacteria.

amounts of the compounds will be available to inhibit FASII. This gives the false impression that bacteria survive because FASII is nonessential whereas in fact they may survive because the inhibitors aren't reaching their targets in the first place."

Affinium's AFN-1252, a small molecule that targets the enoyl-acyl carrier protein reductase (FabI) enzyme in the *Staphylococcus aureus* FASII pathway, has shown efficacy in rodent models of *S. aureus* infection and is in a Phase I safety trial. The company plans to start additional trials in methicillin-resistant *S. aureus* (MRSA) in 1H09, said Hafkin.

Merck has isolated a number of natural products, including platensimycin and platencin, that inhibit components of the FASII pathway.⁴ The company did not disclose the status of its pathway inhibitor program.

Other key next steps include looking at Gram-positive strains beyond *S. agalactiae*—in particular *S. aureus*, said Sonia Escaich, CSO of **Mutabilis S.A.** "The *Nature* paper at best shows that the FASII pathway may be non-essential to *S. agalactiae*. However, we and others have shown that FASII inhibitors significantly reduce the growth and survival of other highly infectious Gram-positives such as *Staphylococcus aureus*," she said.

The company's MUT56-399, a small molecule that targets the FabI enzyme in the *S. aureus* FASII pathway, has shown efficacy in both septicemia and thigh abscess models of infection in mice, Escaich said. Mutabilis plans to start a Phase I safety trial in France this year.

Alexandra Gruss, a corresponding author on the *Nature* paper, acknowledged that the findings may primarily apply to blood-borne—as opposed to local—bacterial infections.

"Serum is particularly rich in fatty acids. If infection occurs via the oral route, for example, the pathogen may not be initially exposed to enough fatty acids to circumvent inhibition by FASII-targeting antibiotics," Gruss told *SciBX*. In that case, she said, FAS-targeted antibiot-

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ics have shown efficacy, including the generic isoniazid, a first-line antibiotic used to treat *Mycobacterium tuberculosis* infection.

Although FASII inhibitors have shown efficacy in systemic infection models, Gruss thinks this may be because the inhibitor was given too soon—before the bacteria have fully adapted to serum fatty acids. The main point of the work published in *Nature*, she said, is that treatment of a real systemic infection would probably come much later, when bacteria have

fully adapted to the presence of serum fatty acids and might thus be able to circumvent FASII inhibition.

Gruss and colleagues are now examining what membrane-protein functions are specifically altered when exogenous fatty acids are integrated into the membrane of *S. agalactiae*. They plan to do a similar characterization of *S. aureus*.

Gruss is a researcher at the **National Institute for Agricultural Research**. She said the *Nature* findings have not been patented.

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Contact: Claire Poyart, University Paris Descartes, Paris, France
e-mail: claire.poyart@cch.aphp.fr
Contact: Alexandra Gruss, National Institute for Agricultural Research, Paris, France
e-mail: alexandra.gruss@jouy.inra.fr
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COMPANIES AND INSTITUTIONS MENTIONED

Affinium Pharmaceuticals Inc., Toronto, Ontario, Canada
Merck & Co. Inc. (NYSE:MRK), Whitehouse Station, N.J.
Mutabilis S.A., Paris, France
National Institute for Agricultural Research, Paris, France