Antimicrobial peptides stage a comeback

Better understanding of the mechanisms of action, modification and synthesis of antimicrobial peptides is reigniting commercial development. Jeffrey L. Fox reports.

Against a background of US federal government incentives providing additional years of market exclusivity for antimicrobials (Box 1) and a more flexible stance at the US Food and Drug Administration (FDA) with respect to clinical trial design, efforts to bring antimicrobial peptides into clinical use are accelerating. Unlike the exuberance accompanying forays into antimicrobial peptide development a decade ago, current optimism is tempered by fuller recognition of the challenges in translating R&D leads into a new class of products.

Negatives and positives

Antimicrobial peptides are based on natural molecules—found in anything from lowly microorganisms to the human innate immune response—that contain from 15 to nearly 50 amino acids and are generally positively charged. Typically, they are ribosomally synthesized, post-translationally modified molecules, unlike classical natural product-based peptide antibiotics, such as vancomycin, that are made in part by peptide synthetases. Some of these peptides kill microbial pathogens directly, whereas others act indirectly by modulating host-defense systems, according to Robert Hancock of the University of British Columbia (UBC) in Vancouver, Canada. More than 2,000 such peptides are known and are cataloged in The Antimicrobial Peptide Database (http://aps.unmc.edu/AP/main.php) established and maintained by researchers at the University of Nebraska Medical Center in Omaha, who have been collecting sequences since 2003.

Toxicity can be a problem when such peptides are administered systematically, says Steven Projan, a senior vice president at MedImmune in Gaithersburg, Maryland, where he heads research on antimicrobial products and vaccines. “They’re fine when ingested because they’re broken down, and several are used as food preservatives, where they can do a good job when used, for instance, with high salt in soft cheeses,” he adds, pointing to nisin, a 34-amino-acid lantibiotic that has been widely used in foods for decades.

But proponents insist that breakdown by hydrolysis can be controlled and that toxicities are being overcome. One major problem is context, says Michael Yeaman of the University of California, Los Angeles (UCLA). Many of these peptides are derived from all manner of organisms or tissues, simply tested for activity in bacterial cultures and then moved too rapidly into the “complicated context” of preclinical and clinical testing without being fully optimized, he suggests.

Another drawback of many antimicrobial peptides is that they lack potency against Gram-negative bacterial pathogens, says David Shlaes, principal of Anti-infective Consulting in Stonington, Connecticut. “Our greatest medical need is for antibiotics targeting resistant Gram-negative pathogens and for oral alternatives to treat resistant infections.” Many hospital-acquired infections are attributable to Gram-negative bacterial pathogens for which there is a dwindling supply of antibiotics to which they are susceptible. Such infections take a heavy toll in terms of extending illnesses of patients being treated for other diseases or injuries, and in lives lost. In the aggregate, treating such infections costs an estimated $45 billion per year, according to a report from the US Centers for Disease Control and Prevention (CDC) in Atlanta.

On the shelf

The field suffered a major setback in 1999 when the developers of the broad-spectrum antimicrobial peptide pexiganan (a 22-amino-acid membrane disruptor analog of the Xenopus peptide magainin) for the topical treatment of diabetic foot ulcers received a nonapproval letter from the FDA. In classic fashion, the principal developer, Migenix Pharmaceuticals, a biotech company that is now defunct, had partnered with SmithKline Beecham (which later became GlaxoSmithKline; GSK) to take the product through clinical trials. Early manufacturing difficulties, a belated demand to change the design of a clinical trial (which some critics considered unethical in terms of participating patients), shifting commercial priorities of the pharma partner and waning resources led to pexiganan being shelved.

Pexiganan is, however, now getting another lease on life as Locilix, under the sponsorship of Dipexium Pharmaceuticals of White Plains, New York. This company is negotiating with FDA to bring pexiganan through clinical trials, as a way of treating bacterial infections associated with diabetic foot ulcers—particularly when the bacteria are resistant to standard antibiotics (Table 1).

UBC’s Hancock shares “the sad story” of another such peptide that was being developed to disinfect catheters but ran into regulatory barriers. That peptide, omiganan, was sponsored by Micrologix Biotech (later Migenix) of British Columbia. “It missed its primary clinical trial endpoint ‘physician determined infections,’ although it showed statistically significant efficacy in preventing catheter colonization and preventing microbiologically confirmed infections,” he adds. “I do not precisely know why its developers were told ‘it would never get approval.’ That’s been the fate of nearly every antibiotic before FDA during the past few years. FDA is not just a nightmare; it’s refractory. That may be why there is less than stellar progress.”

Other antimicrobial peptides, such as Plecasin (a defensin isolated from the fungus Pseudoplectania nigrella) that Novozymes of Bagsvaerd, Denmark, licensed in 2008 to Sanofi-Aventis of Paris, was also shelved by the pharma partner, according to Soren Kjærulf, a senior director at Novozymes. “We did a lot of work on this,” he says, adding that in this case commercial (rather than scientific) issues were the main reason that the pharmaceutical company chose not to take the program forward.

“I know Sanofi-Aventis put Plecasin back on their shelf in terms of clinical development,” says Arnold Bayer, a colleague with Yeaman at UCLA, whose group conducted some of the
preclinical studies evaluating it. “However, it was one of the single best agents we have ever evaluated in our rabbit model of staphylococcal endocarditis.”

Despite such setbacks, the alarming rise in antibiotic resistance and the sporadic emergence of new pathogens are among the forces driving the quest for new antimicrobial products. Much of this activity focuses on Gram-negative bacteria and those that form biofilms. But there is also interest in tailoring antimicrobial peptides to combat fungal pathogens or parasites, such as those that cause malaria. Among recent developments that indicate promise are “the demonstration in realistic models that the peptides show excellent efficacy against biofilm infections, an ability to favorably modulate immunity by increasing protective immunity while suppressing potentially harmful inflammation and an ability to enhance wound healing,” Hancock says. “These value-added areas provide both new directions for development as well as the potential to produce dual- or multiple-purpose peptides.”

Under the radar

Pfizer acquired the Milan-based biotech company where Daniela Jabes was working in 2005 but showed no interest in the novel microbial metabolites she was then investigating. Some were lantibiotics—peptide antibiotics, both linear and looped, so called for the lanthionine (polycyclic thioether) amino acids they contain—that derive from lactococcal bacteria and can be lethal for various Gram-positive bacterial pathogens. She soon formed her own company and uncovered additional antimicrobial peptides. Although this work continues at Milan-based NeED Pharmaceuticals, the commercial rights to one of the first “druggable” compounds were transferred to Sentinella Pharmaceuticals in Princeton, New Jersey.

“Sentinella is a virtual company,” says its principal officer Lorenzo Pellegrini, a partner at Care Capital, a venture capital group, in Princeton. Along with that lead lantibiotic peptide, Nai-107, Sentinella has rights to as many as 20 additional compounds, he says. “We’ve done some IND [investigational new drug] work already but parts are missing. The profile of Nai-107 is interesting, and [it] is very potent not just against MRSA [methicillin-resistant Staphylococcus aureus] but also vancomycin-resistant and other glycopeptide antibiotic-resistant pathogens. When this product hits the market, it will be a useful tool in hospitals. No approved antibiotic is like it.” For now, however, much of the crucial developmental work is being outsourced, including to Jabes and her collaborators in Italy, whereas Sentinella remains mostly “under the radar,” he adds.

Oragenics, headquartered in Tampa, Florida, is another low-profile company developing antimicrobial peptides. In this case, its lead compound MU1140, another lantibiotic, derives from Streptococcus mutans, a bacterial species that grows in the mouth, according to vice president for research Martin Handfield, who is based in Alachua, Florida. Until recently, the main hurdle blocking its development was its shortage, with no way to make it in substantial amounts, he says. However, with federal Small Business Innovative Research (SBIR) grants, the company developed and patented a “bold and proprietary” technique for making this or other peptides in amounts adequate for pursuing preclinical research, he says.

In mid-2012, Oragenics teamed up with Intrexon of Germantown, Maryland, to produce and develop this and other lantibiotics for human and veterinary medical uses. “[Intrexon’s] know-how is mind-blowing,” Handfield says. “I can say this collaboration will set out one of the largest pipelines and will allow us to get into clinical trials.” Meanwhile, he adds, MU1140 shows promising activity against both actively growing and dormant Mycobacterium tuberculosis, which causes tuberculosis (TB). It also is active against MRSA as well as Bacillus anthracis, which causes anthrax and can be used as a bioterrorist agent. Although Oragenics is not ready to set a timeline for seeking an IND from FDA, that possibility now looms because a “commercially viable means to produce MU1140” will likely soon be in hand.

Despite past frustrations with trying to usher an antimicrobial peptide into commercial use through Micrologix Biotech, UBC’s Hancock and his collaborators are pursuing a nine-amino-acid synthetic peptide, designated 1037, based on bovine bactericin, which shows only weak activity against free-living cells of both Gram-negative and Gram-positive bacteria, but at low concentrations “significantly inhibits” an array of such pathogens within biofilms. “We don’t completely understand why, but it’s pretty exciting,” he says. Part of this activity stems not from deranging membranes but from disrupting the signaling known as quorum sensing that takes place among densely growing bacteria. “It also kills bacteria in pre-grown biofilms, which is unprecedented,” he adds. However, he does not see this agent acting as a “stand-alone” drug but rather in synergy with conventional antibiotics.
In terms of commercial development, Hancock says, “Stay tuned. I’m rushing forward but with no company yet, and I don’t want to go the VC [venture capital] route. I’m working on a model that means keeping it virtual as long as there’s no massive overhead. We just might get this [peptide] through phase 2 before we form an actual company.”

Yeaman of UCLA is developing antimicrobial peptides, again under the auspices of a virtual company, but with a different explanation for what might prove crucial for the successful clinical development of these molecules. “I don’t make light of what others are doing, but if you need to treat infections in the blood, you need a different approach,” he says. Part of that approach is to start with peptides—specifically those produced in and released from platelets, he believes—that are naturally active against pathogens in the bloodstream.

This approach also entails looking closely at the three modular domains that these antimicrobial peptides contain, especially their cysteine-stabilized γ-core segments that share structural similarities across all kingdoms of life in which they are found (Fig. 1). Yeaman says, “At the site of infection, these [platelet-derived] peptides act as a microbicidal symphony—engaging the bacterial pathogens directly, decorating it and making it more digestible by white cells.” One platelet-based but synthetic peptide, designated RP-1, is effective when used to treat S. aureus biofilms in mice, he says. Not only is it as active as vancomycin, but the peptide navigates whatever barriers are present and drives the infection below the detectable level within three days.

“We’re getting there but we’re not there yet, and it’s not exactly as simple as people thought it would be,” says Yeaman. “These molecules are more sophisticated than people gave them credit for. They’re not just membrane detergents. A number of different peptide groups don’t stop at membranes but reach into cells to inhibit critical cell functions.”

Above the radar
Farthest along of systemically administered drugs in this class is the Novartis thiopetide, LFF551, which is in phase 2 clinical trials for infections caused by Clostridium difficile, a bacterial pathogen of the gastrointestinal (GI) tract that can cause persistent and recurrent, sometimes life-threatening diarrhea, particularly among elderly patients following antibiotic therapy for other conditions. The drug developers have “changed its structure so profoundly that it looks very much like a typical small-molecule, natural product peptide, not a peptide-peptide,” according to Michael Fischbach, professor of microbiology at the University of California, San Francisco. In so doing, they have eliminated the problem of immunogenicity and stability, which developers of all manner of peptide drugs must face.

Cubist Pharmaceuticals of Lexington, Massachusetts, is taking the lipopeptide surfomycin (CB-315) through phase 3 clinical trials as a treatment for C. difficile infections. So far, the lipopeptide compares favorably with vancomycin, a conventional antibiotic, and may provide more durable protection against recurrences. And, what should be another plus for this drug candidate, FDA granted qualified infectious disease product (QIDP) status (Box 1) to CB-315 last December, making it eligible for priority review and fast-track status, as well as for an added five years of exclusivity if it is licensed.

“We are delighted that our phase 3 antibiotic candidate CB-315 received QIDP designation under the GAIN Act,” says Cubist’s CSO Steve Gilman. “With antibiotic resistance rates on the rise and many companies having already left antibiotic R&D altogether, we believe the provisions of the bipartisan GAIN Act are a critical first step in our country’s efforts to spur meaningful investment into this space.”

Several other compounds among the 1,700 or so within the mimetics series are active against fungal pathogens or the parasites that cause malaria, according to Scott. The compounds “have built-in specificity against different types of membranes, which is exciting,” he says. These mimetic compounds appear to act directly on membranes of target cells, but there is “also some immunomodulatory activity as well.” Another plus is that it is difficult to select for pathogens with resistance against the mimetics, he adds.

Novexatin, the lead product of NovaBiotics of Aberdeen, UK, is a cyclic and highly cationic (arginine-rich) peptide based on human α and β defensins (among others), to target stubborn fungal infections in toenails. This toenail condition affects an estimated 12% of the global population and represents a substantial unmet medical challenge; other products, such as topical antifungals, systemic antifungals or laser treatments, are either ineffective, toxic or too expensive. “They are not appropriate—full stop,” says Deborah O’Neil,
NovoBiotics’ CEO. Cyclizing the peptide not only improves its stability but also increases its antifungal potency (it acts by hydrolyzing fungal cell membranes). The methods for cyclizing “are not proprietary but the application of cyclic antimicrobial peptides is covered by our IP [intellectual property],” she adds. The product is to begin phase 2b clinical trials later this year.

Other peptides that NovoBiotics is developing are active against Gram-negative bacterial pathogens, O’Neil adds. Arenicin-3 (isolated from the marine lugworm Arenicola marina) is active against Gram-negative bacteria and is now under study at Adenium Biotech in Copenhagen, a spin-off, according to Kjaerulff from Novozymes. “It’s very active, and we out-licensed it about one year ago.”

“You would think we’re very competitive, but we’re a friendly bunch and there’s not that much of a clash,” says O’Neil, speaking more generally about the companies working on antimicrobial peptides. “We’re still relatively small, and there are not that many working on these antimicrobial peptides.”

**Peptoids and bacteriocins**

A separate set of antimicrobial peptide candidates are the peptoids, comprising a natural amino acid backbone with synthetic side chain residues that confer protease resistance and increased hydrophobicity (enhancing membrane permeability). Annelise Barron of Stanford University in California is studying peptides with poly-N-substituted glycines in the side chains, including LL-37, a compound that has promising activity against M tuberculosis. Whereas antimicrobial peptides are widely believed to act by disrupting membranes, LL-37 and other peptoids are thought to act by inducing bacterial ribosomes to aggregate, according to Barron. “There is not that much leakage from the microbial cell after treatment with peptoids,” she says. Rather, what correlated with their antimicrobial activity was ribosomal aggregation. “And we are quite confident that LL-37 works this way.”

A final class of antimicrobials currently receiving attention are the bacteriocins. Strictly speaking, these are full-blown proteins and thus too big to be considered peptides. Moreover, they act on pathogens in different ways than do some among other antimicrobial peptides, says James Knighton, president of AvidBiotics of San Francisco, California. They “bind, punch a hole and kill bacteria by depolarizing cell membranes,” he says. As with other antimicrobial peptides, commercial interest in bacteriocins has waxed and waned over several decades. “We’ve learned a lot lately about dosing, and can make this old technology very viable,” he says. “It’s a very targeted therapy, and is in a whole new realm.”

Indeed, the high specificity of bacteriocins was off-putting until recently because they need to be used along with molecular diagnostics capable of precisely identifying which specific pathogens need to be treated, says Knighton’s colleague David Martin. This challenge is met, in part, by producing cocktails containing several types of bacteriocins whose specificities can be tailored to include variety among the pathogens being treated. Another part of their specific pathogen—targeting strategy calls for having these proteins bind to virulence factors, he says. “If pathogens become resistant by losing those receptors, it compromises virulence.” Either way, the treatment counters the infectious agent.

The company is partnering with Wilmington, Delaware–based DuPont to work on drug-tissue delivery issues and also to develop some products for food and agricultural applications, for which regulatory hurdles are lower. The initial goal is to develop ways to treat beef before slaughter or meat products to rid them of Escherichia coli 0157:H7 or comparable contaminants, Knighton says. “It’s a real problem, and traditional antibiotics are unacceptable; it needs a new solution. This would be a nice way to validate our technology for eventual use in humans.”

In terms of human use, one of the early targets is C difficile. Bacteriocins can be administered orally, survive passage through the upper GI tract simply by neutralizing stomach acids, and make their way to the lower tract to counteract C difficile. Such treatments work well in rabbits, mice and pigs, Martin says.

Beijing-based Pheroxomincin Biotech has a technology for combining bacteriocins with targeting peptides, which obviates the need for diagnostics. This approach was pioneered by the company’s CSO Xiao-Qing Qiu, when he was at Sichuan University in Chengdu. In early work, Qiu employed the targeting capability of bacterial pheromones, in later work, antibody mimetics. The most advanced programs are veterinary products; also under investigation are pheroxomicins targeting MRSA.

**Going forward**

Even the companies with promising candidate products in clinical trials admit they are scrambling for financial support, depending on federal programs for research support but also seeking corporate partners to see them through the late and most expensive stages of clinical evaluations. The companies are also depending on FDA to reconfigure its standards in some cases when dealing with products that do not readily fit into categories that were set decades ago for conventional antibacterial or antifungal drugs (Box 1). According to NovaBiotics’ O’Neil, “peptides of the size we’re developing tend to be considered as being a ‘mix’ of both traditional small molecules and biologics.” They are synthetic molecules but based on or having the same mode of action as endogenous molecules. Thus, she anticipates regulators will apply a “mix and match’ of components of International Conference on Harmonisation guidelines for both molecular classes.”

The problem of square pegs and round holes applies not only to the FDA but also to pharmaceutical partners. According to O’Neil, her company is in discussions with big pharma. But as these peptides “can’t be plugged into traditional” pathways for development and review, “people take more convincing” to support them, she says. “Licensing is still a few years away. When it happens, it won’t be just one or two products but a whole generation.”

Jeffrey L Fox, Washington, DC