SUPPLEMENT ARTICLE

Daptomycin: From the Mountain to the Clinic, with Essential Help from Francis Tally, MD

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Daptomycin has been approved and successfully launched for the treatment of complicated skin and skin-structure infections caused by gram-positive pathogens [1] and bacteremia and right-sided endocarditis due to *Staphylococcus aureus,* including strains that are resistant to methicillin or other antibiotics [2]. The development of the drug, however, was not straightforward; it involved a cast of characters, including scientists at Eli Lilly and at Cubist Pharmaceuticals. Of most importance, the development of daptomycin involved the tenacious leadership of Dr. Francis Tally. As a tribute to Dr. Tally, we attempt to reconstruct the path of daptomycin from the mountain to the clinic.

DISCOVERY OF DAPTOMYCIN

Daptomycin (Figure 1) is a natural product of a soil actinomycete, as are most of the important antibiotics developed in the past 50 years [3]. The producing microorganism, *Streptomyces roseosporus,* was isolated by scientists at Eli Lilly from a soil sample from Mount Ararat (Turkey). This sporulating actinomycete produced a family of lipopeptide antibiotics designated A21987C (Figure 1) [4, 5]. Eli Lilly scientists also isolated a strain of *Actinoplanes utahensis* that produced a secreted deacylase that could cleave the natural long-chain lipid side chains from the A21978C factors [6, 7]. This enabled the production of the core cyclic peptide for reacylation with different lipid side chains [8]. Daptomycin, which contains an *n*-decanoyl side chain, was chosen for clinical development because of its in vivo efficacy and low toxicity in animals [9]. Because enzymatic deacylation, coupled with chemical reacylation with decanoate, was not cost-effective to manufacture, scientists at Eli Lilly developed a process that fed decanoic acid during fermentation, to directly produce daptomycin [10].

Scientists at Eli Lilly designed and implemented clinical studies of intravenous (IV) daptomycin during the late 1980s and early 1990s [11]. In the initial phase 1 trials, daptomycin was well tolerated in healthy volunteers at up to 6 mg/kg IV in 2 divided doses per day [9]. For the initial phase 2 clinical trial involving complicated skin and skin-structure infection (SSSI), the scientists at Eli Lilly used a daptomycin treatment regimen of 2 mg/kg given once daily. In later clinical trials, daptomycin was administered twice daily (every 12 h). In another phase 2 trial, a dosage of 6 mg/kg given in 2 divided doses per day to patients with *Staphylococcus aureus* bacteremia provided promising results. Scientists thought that a higher dosage, such as 8 mg/kg per day in 2 divided doses, would be required to effectively treat such infections. However, when that dosage was used for safety testing in a phase 1 study, 2 of 5 volunteers developed the unacceptable adverse effects involving the musculoskeletal system with accompanying increases in creatine phosphokinase (CPK) levels (a marker of potential adverse effects involving the musculoskeletal system) [12]. Subsequently, Eli Lilly voluntarily suspended further trials. Primarily because the therapeutic window between efficacy and safety was thought to be small, a decision was made to discontinue daptomycin development and to put the antibiotic "on the shelf." This decision was helped by the concurring viewpoint of the new Vice President for Infectious Diseases Discovery at Eli Lilly, Barry Eisenstein.

Meanwhile, in early 1992, Richard Baltz and colleagues at Eli Lilly began genetic studies on

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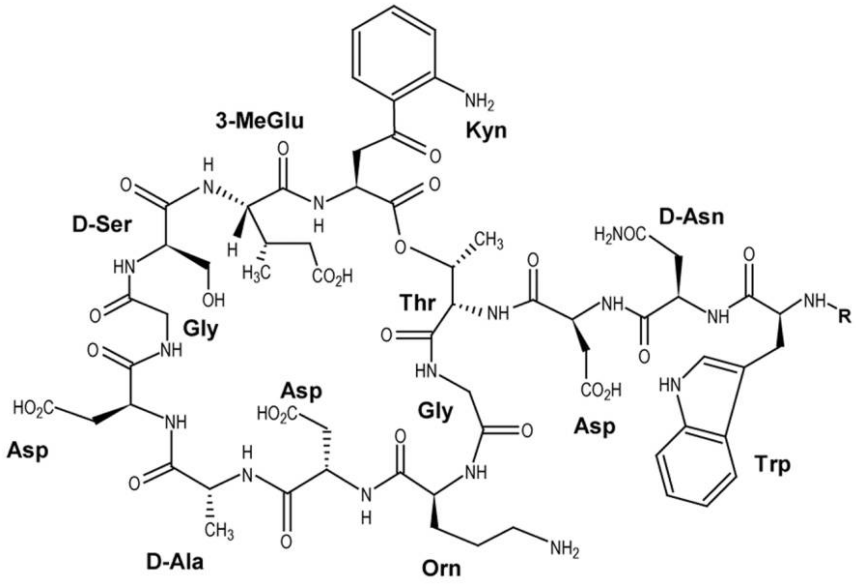

Figure 1. Structure of A21978C factors and daptomycin (Cubicin). Daptomycin, R = n-decanoyl; A21978C_1, R = anteisoundecanoyl; A21978C_2, R = isododecanoyl; A21978C_3, R = anteisotrdecanoyl.

S. roseosporus [13–17] to develop combinatorial biosynthetic approaches of conducting systematic modifications of the nonribosomal peptide synthetase to reprogram the production of novel peptide cores for improvement of the therapeutic index of daptomycin-related lipopeptides. They initiated the cloning of the biosynthetic genes for daptomycin and its related lipopeptide (A54145) to form hybrid molecules [13, 16, 17]. This work was supported primarily as a postdoctoral project and was terminated in 1996. Around that same time, Eli Lilly management decided to discontinue all further work on other natural product antibiotics, including glycopeptides, macrolides, and β-lactams, and to focus on target-based drug discovery using *Streptococcus pneumoniae* genomics [18, 19]. They also initiated a downsizing of the Infectious Diseases Discovery Division, and many of the seasoned researchers were reassigned to other therapeutic areas or, like Eisenstein, departed entirely.

After witnessing the decline of the long-standing and distinguished collaboration between Natural Products Discovery and Infectious Diseases Discovery Divisions at Eli Lilly [20], Baltz attended a scientific meeting in 1996, where he was introduced to Dr. Tally. Tally knew of Baltz's work at Eli Lilly and was aware of the recent downsizing of the Infectious Diseases Discovery group. He inquired about Baltz's interest in a position as Vice President of Molecular Biology at Cubist. Baltz told Tally that he would give it some thought, and a short time later, on a lark, he agreed to interview for the position.

The trip to Cubist (Cambridge, MA) was an interesting experience for Baltz. In the morning, Baltz presented a seminar that briefly summarized 3 projects that he had been working on at Eli Lilly: genetic engineering of lipopeptides related to daptomycin [13–17], genetic engineering of novel glycopeptides [21], and *S. pneumoniae* genomics [18, 19]. He led with a report on his work with daptomycin. In the introduction to the daptomycin molecular genetics portion of the seminar, Baltz mentioned why he thought a derivative of daptomycin might be developed into a useful antibiotic if a sufficient increase in therapeutic index could be achieved to overcome the muscle toxicity that Eli Lilly encountered in the phase 2 clinical trials.

At the end of the day, Tally and Baltz again discussed the vice president position at Cubist. Baltz was within 2 years of retirement from Eli Lilly and its substantial benefits, and he deferred the decision. Tally understood and told him, "Don't be surprised to see me at Lilly, because I am going to license daptomycin for Cubist to develop" (R. H. Baltz, personal recollection). Tally had come to that decision within a few hours after hearing the seminar.

It took Tally and Cubist nearly a year to negotiate a license agreement with Eli Lilly. After the agreement was signed, Tally called Baltz to ask whether he would help Cubist with the daptomycin project as a consultant. Baltz was still 1 year away from early retirement but told Tally that he would inquire with the Eli Lilly upper management. Eli Lilly stood to benefit from a healthy royalty if Cubist was successful; management agreed to let Baltz consult, but on his own time. This started a fruitful scientific relationship and friendship with Tally that eventually brought Baltz to Cubist full-time in 2001, after retirement from Eli Lilly and 3 years of consulting with Cubist. With Tally's support and with the acquisition of TerraGen in 2000, Cubist continued with molecular engineering where Eli Lilly had stopped [13–17] and developed combinatorial biosynthesis as

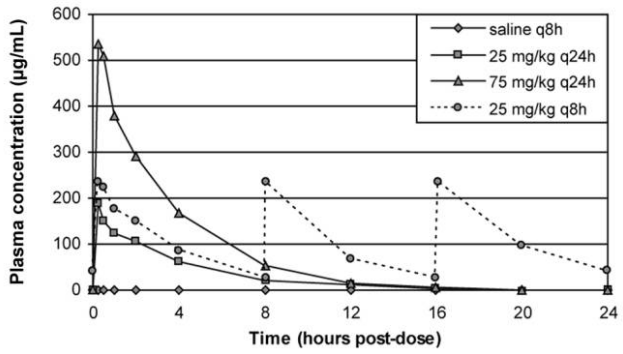

Figure 2. Effect of daptomycin dosing regimen on pharmacokinetics in dogs. q8h, given every 8 hours; q24h, given once daily. Data are from Oleson et al [30]. Reproduced with permission from the American Society of Microbiology.

a robust means to generate derivatives of daptomycin that were not attainable through medicinal chemistry [22–29].

CUBIST IN-LICENSING OF DAPTOMYCIN AND EARLY CLINICAL DEVELOPMENT

The Cubist daptomycin in-licensing agreement was finalized on 7 November 1997, the same day that Frederick Oleson, trained in nonclinical development and toxicology, joined Cubist as a full-time employee to work with Dr. Tally on the development of daptomycin. For several months, Oleson had been consulting with Tally and the Cubist team in the due diligence evaluation process. During the initial 2–3 months after in-licensing, Tally and Oleson—in addition to others at Cubist, including Jan-Ji Lai—focused their efforts on developing daptomycin formulations and treatment approaches for oral and topical clinical indications (ie, elimination of colonization of the gastrointestinal tract with vancomycin-resistant enterococci and treatment of dermal gram-positive bacterial and/or *S. aureus* infections, respectively). One of the main reasons for these treatment approaches was to limit the systemic exposure to daptomycin, which was only minimally absorbed after oral or dermal administration. Thus, these treatment approaches would minimize any risks for the adverse effects involving the musculoskeletal system that resulted in the termination of the IV daptomycin clinical programs at Eli Lilly.

During this initial development phase, Tally and Oleson had numerous discussions about the possibility of reinitiating the IV clinical programs. They, along with leaders in the infectious diseases community, were concerned about the lack of antibiotics in development across the pharmaceutical industry. The Cubist scientists were convinced that daptomycin had good attributes (eg, high potency, bactericidal activity, and high efficacy in animal models against a broad array of gram-positive pathogens, including drug-resistant and drug-susceptible *S. aureus* and vancomycin-resistant enterococci) and that it was worth their further effort to overcome the toxicity problems that had confounded Eli Lilly scientists. A number of factors were considered, including the level of muscle toxicity related to daptomycin (based on the Eli Lilly preclinical and clinical data), the clinical trial efficacy data from the Eli Lilly phase 2 trials involving skin and bloodstream infections, and the increasing rates of vancomycin-resistant or intermediately susceptible gram-positive organisms in the hospitals. The adverse effects involving the musculoskeletal system could be monitored by measuring the level of the serum enzyme CPK. The phase 2 trial results suggested that daptomycin could be an effective therapy for complicated SSSI and for bacteremia (although higher doses would be desirable) [12]. From a medical need viewpoint, the increasing rates of vancomycin-resistant enterococci and methicillin-resistant *S. aureus* made it abundantly clear that a drug such as daptomycin would be an ideal candidate for the treatment of serious and life-threatening gram-positive, hospital-acquired infections. Consequently, in February 1998, only 3 months after in-licensing, Cubist senior

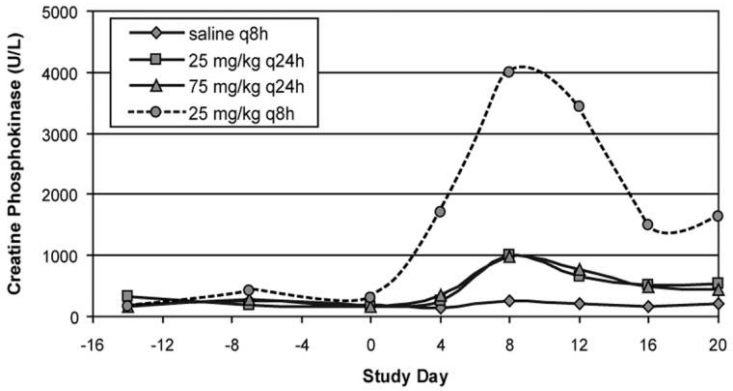

Figure 3. Effect of daptomycin dosing regimen on serum creatine phosphokinase levels in dogs. q8h, given every 8 hours; q24h, given once daily. Data are from Oleson et al [30]. Reproduced with permission from the American Society of Microbiology.

Table 1. Effect of Daptomycin Dosing Regimen on Microscopic Musculoskeletal Findings in Dogs

Dosage	Peak serum creatine phosphokinase level, IU/L	No. of muscle sites affected[a]
0 mg/kg every 8 h	265	1
25 mg/kg every 24 h	~990	3
75 mg/kg every 24 h	~990	8
25 mg/kg every 8 h	~4000	15

[a] Twenty-eight sites were evaluated (7 sites evaluated in each of 4 dogs). Microscopic findings indicated only minimal musculoskeletal degeneration.

management decided to pursue the development of daptomycin as an IV antibiotic for the treatment of serious gram-positive infections, including complicated SSSI and bacteremia with suspected infective endocarditis, and subsequently discontinued the oral and dermal programs.

In March 1998, a preinvestigational new drug document was submitted to the US Food and Drug Administration (FDA) to inform the agency of the Cubist plans to develop daptomycin for the IV treatment of serious gram-positive infections and to solicit its advice on this program. At the same time, Cubist signed a manufacturing agreement with ASC Dobfar in Italy to manufacture daptomycin for its clinical development program.

ONCE-DAILY TREATMENT ADMINISTRATION IS THE KEY THAT IMPROVES THE THERAPEUTIC INDEX

During this time, Tally and Oleson debated the cause of the adverse effects involving the musculoskeletal system that Eli Lilly had experienced in its trials, focusing on whether the adverse effects were caused by peak concentrations of the drug in the blood (C_{max}) or by the total amount of drug exposure over a 24-h period (area under the plasma concentration–time curve [AUC]). Tally thought that the adverse effects were related to C_{max}, whereas Oleson postulated that they were related to AUC or to total daily dose (however, both were incorrect). One extraordinary finding that Oleson noted from the Eli Lilly animal toxicity studies was that the incidence and severity of the daptomycin-associated adverse effects involving the musculoskeletal system increased only marginally as the duration of treatment was extended from 1 month to 6 months with the once-daily regimen for toxicity studies at that time. This result was unusual because increasing the duration of treatment can cause a substantial increase in toxicity; however, this was not the case for the effect of daptomycin on muscle.

In the spring of 1998, Tally and Oleson designed a series of canine studies to determine which pharmacokinetic parameter (C_{max} or AUC) was the key determinant in the daptomycin-associated adverse effects involving the musculoskeletal system [30]. Dogs were selected because the canine model correlated most closely with humans in terms of the adverse effects involving the musculoskeletal system and monitoring through serum CPK levels.

In the key study, a dosage of 75 mg/kg given once daily was selected because the researchers thought that this dose would produce a mild effect on the muscle. The effects at this dosage, using the once-daily treatment regimen, were compared with those of a dosage of 25 mg/kg of daptomycin given every 8 h, for a comparable daily dose of 75 mg/kg. The total daily AUCs for these 2 regimens (75 mg/kg given once daily vs 25 mg/kg given every 8 h) were similar, because the terminal half-life of daptomycin in dogs was 2–2.5 h. Pharmacokinetic profiles for these 2 regimens are shown in Figure 2. The once-daily regimen (which had a higher C_{max} than the other regimen) did not increase the severity of adverse effects involving the musculoskeletal system, as predicted by Tally. In fact, the once-daily regimen, despite its much higher C_{max} (and similar AUC), produced substantially less muscle damage than did the regimen in which treatment was given every 8 h. As shown in Figure 3, serum CPK levels were lower throughout the 20-day treatment period for the once-daily regimen, compared with the regimen in which treatment was given every 8 h, with ~4-fold lower peak levels (~1000 U/L vs ~4000 U/L). In addition, the incidence of microscopic findings (musculoskeletal sites with minimal myofiber degeneration) was increased ~2-fold in the dogs receiving daptomycin 3 times per day, compared with dogs receiving the drug once per day (Table 1).

Therefore, this study demonstrated that once-daily treatment administration minimized the daptomycin-associated adverse effects involving the musculoskeletal system. These results were counterintuitive to industry standards for treatment administration and clearly demonstrated that the daptomycin-associated adverse effects involving the musculoskeletal system were not driven primarily by C_{max} or by AUC but were related to time between administration of doses and time below a critical trough concentration. The research that led Oleson and Tally to determine the nonobvious fact that once-daily administration of daptomycin was the optimal way to increase drug safety solved the problem that stymied Eli Lilly. In 1998, Cubist filed a patent application directed toward methods of administering daptomycin, naming Oleson and Tally as inventors on the basis of the novel treatment regimen to minimize the adverse effects involving the musculoskeletal system. The patent was issued by the United States Patent and Trademark Office in October 2002.

In addition, because the research revealed that the pharmacokinetic mechanism of daptomycin for in vivo efficacy was driven by C_{max} or AUC, not by time over the minimum inhibitory concentration, pharmacodynamic animal model data

indicated that a once-daily regimen should be as effective as a fractionated regimen with the same total daily dose [31, 32].

On the basis of this research, the daptomycin clinical trials were redesigned around a once-daily treatment regimen. In December 1998, the investigational new drug was refiled to support the initiation of phase 3 trials involving complicated SSSI, and these trials were initiated during the first quarter of 1999. The clinical program eventually included 2 different ascending-dose phase 1 trials involving healthy volunteers that demonstrated that once-daily administration of daptomycin caused no elevations in CPK level or symptoms of adverse effects involving the musculoskeletal system in any study participant after 14 days of treatment at doses of 8 and, in a later study, 10 or 12 mg/kg [33, 34]. Thus, the potential for adverse effects involving the musculoskeletal system and CPK level elevations had been assessed using the once-daily treatment regimen in volunteers at total daily doses equal to or exceeding those that had proved to be problematic for Eli Lilly (4 mg/kg twice daily; equivalent to 8 mg/kg per day). These clinical results confirmed the canine study results, demonstrating that the once-daily regimen minimized the potential for daptomycin-associated adverse effects involving the musculoskeletal system.

In phase 3 trials involving complicated SSSI and *S. aureus* bacteremia, including right-sided infective endocarditis, daptomycin was associated with a low incidence of adverse effects involving the musculoskeletal system. In phase 3 trials involving complicated SSSI, CPK level elevations occurred in 15 (2.8%) of 534 patients in the daptomycin-treated group, compared with 10 (1.8%) of 558 in the comparator-treated group [1, 35]. Although the rates of CPK level elevation in the bacteremia trial were higher for daptomycin (9.2%) than for the comparator (1.7%) and daptomycin was discontinued in 3 (2.5%) of 120 patients because of this elevation, the proportion of patients with adverse effects involving the musculoskeletal system and connective tissue was actually higher in the comparator group (36.2%) than in the daptomycin group (29.2%) [2, 35]. On the basis of these studies, daptomycin dosages of 4 and 6 mg/kg per day are indicated by the FDA as safe and effective for treatment of complicated SSSI and *S. aureus* bacteremia (including right-sided endocarditis), respectively [35].

In 2003, daptomycin for injection (Cubicin), a first-in-class acidic lipopeptide IV antibiotic, was approved by the FDA at a dosage of 4 mg/kg given once daily for the treatment of complicated SSSI caused by specific gram-positive bacteria. Since its launch in November 2003, the drug has had the most financial success (in nominal dollars of sales) of any IV antibiotic in US history. In 2006, it was approved in the United States for the treatment of *S. aureus* bacteremia, including right-sided infective endocarditis, at a dosage of 6 mg/kg given once daily [35].

In conclusion, we have attempted to convey the story of the creation of a drug that has become a great commercial success. More importantly, physicians now have an excellent treatment option for life-threatening infections, including increasingly drug-resistant staphylococcal and other infections, which cause thousands of deaths each year. Physicians, along with the scientists and staff at Cubist Pharmaceuticals, will forever be grateful for the visionary leadership of Dr. Francis Tally, an extraordinary microbiologist and infectious diseases physician, who was the right person at the right time and the right place to make a difference of a truly noble kind. Those of us who had the privilege to work with Dr. Tally are thankful for that opportunity, and we will always cherish our memories of having had the high honor to know him.

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