Ceftaroline fosamil: a new broad-spectrum cephalosporin

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Ceftaroline fosamil, the prodrug of the active metabolite, ceftaroline, is a new, broad-spectrum cephalosporin recently approved in the USA for the treatment of acute bacterial skin and skin structure infections (ABSSSIs) and community-acquired bacterial pneumonia (CABP). Ceftaroline has potent in vitro activity against Gram-positive organisms, including methicillin-resistant Staphylococcus aureus and Streptococcus pneumoniae, as well as common Gram-negative organisms. The high affinity of ceftaroline for penicillin-binding proteins is responsible for the potent activity observed against clinically relevant pathogens. With respect to the treatment of CABP, the activity of ceftaroline against pathogens such as S. pneumoniae, S. aureus, Haemophilus influenzae and Moraxella catarrhalis demonstrates coverage across a broad range of pathogens typically encountered in clinical practice. Ceftaroline is also very active against common pathogens seen in ABSSSIs such as S. aureus (methicillin-susceptible S. aureus and methicillin-resistant S. aureus) and Streptococcus pyogenes. Ceftaroline exhibits a dose-proportional pharmacokinetic profile, similar to other recently excreted cephalosporins, and has a well-tolerated safety profile consistent with the cephalosporin class. Ceftaroline fosamil is compatible via Y-site administration with many other commonly administered parenteral drugs.

**Keywords:** CAP, community-acquired pneumonia, CABP, MRSA, Streptococcus pneumoniae

**Introduction**

The cephalosporin class of antimicrobial agents is known for its broad spectrum of activity, proven efficacy and favourable safety profile, making it the most commonly prescribed class of antimicrobials (data on file, Forest Laboratories, Inc. and references herein1–5). There are four recognized class generations of cephalosporins based on spectra of activity.6 First-generation cephalosporins are active against Gram-positive cocci; second-generation agents retain this Gram-positive activity and have increased activity against Gram-negative organisms. Third-generation cephalosporins have decreased activity against Gram-positive organisms relative to first- and second-generation agents, but improved Gram-negative coverage. Spectrum of activity is further expanded in the fourth-generation agents, which have increased activity against Gram-negative organisms compared with first- and second-generation agents and greater coverage of Gram-positive organisms than third-generation agents, as well as activity against Pseudomonas spp. and some Enterobacteriaceae, including those that produce β-lactamases. Ceftaroline fosamil (TAK-599 or PPI-0903), the prodrug of the active metabolite, ceftaroline, was synthesized by Takeda Pharmaceutical Co., Ltd and developed by Cerexa, Inc. and Forest Laboratories, Inc.7 The CLSI designates ceftaroline as a member of a new subclass of antimicrobials, cephalosporins with anti-methicillin-resistant Staphylococcus aureus (MRSA) activity.8 Ceftaroline has also been described in the literature as a ‘fifth-generation’ cephalosporin.9 However, such classification suggests a broader Gram-negative profile whereas ceftaroline’s spectrum of activity is truly unique for its expanded Gram-positive activity beyond all other presently available cephalosporins (i.e. MRSA).

Four pivotal Phase III clinical trials were conducted as part of the New Drug Application submitted for approval to the US FDA (two trials for complicated skin and skin structure infections (CANVAS 1 and CANVAS 210,11) and two trials for community-acquired pneumonia (FOCUS 1 and FOCUS 212,13)). Based on these trials, ceftaroline fosamil gained approval from the FDA on 29 October 2010 for treatment of acute bacterial skin and skin structure infection (ABSSSI) and community-acquired bacterial pneumonia (CABP).

The purpose of this article is to provide an overview of the mechanism of action, spectrum of activity, basic pharmacokinetic and pharmacodynamic profile, stability, administration and safety of ceftaroline with respect to the treatment of CABP.

**Mechanism of action**

Ceftaroline was developed by modifying the structure of the fourth-generation cephalosporin cefozopran.14 The prodrug, ceftaroline fosamil, which contains a phosphono group to increase water solubility, is rapidly converted in plasma into the bioactive agent, ceftaroline (Figure 1).15 The 1,3-thiazole ring attached to the 3-position of the cephalosporin nucleus and the oxime group in the C7 acyl moiety are responsible for the enhanced anti-MRSA activity observed with ceftaroline.
Structure

Like other β-lactams, ceftaroline exerts its rapid bactericidal effect by binding to key penicillin-binding proteins (PBPs). However, unlike most other cephalosporins and β-lactams, ceftaroline exhibits enhanced binding affinity via the 3′ side chain to PBPs in key pathogens such as MRSA and penicillin-resistant pneumococci. Methicillin resistance is associated with PBP 2A, for which most β-lactams have low affinity; however, competition assay studies have demonstrated that ceftaroline has a high affinity for staphylococcal PBPs 1, 2, 2A and 3, indicating good binding affinity. The low IC₅₀s correspond to low MICs. CPT, ceftaroline; IC₅₀, drug concentration required to block 50% of Bocillin FL binding to each PBP.

Spectrum of activity

In vitro studies have demonstrated the broad-spectrum activity of ceftaroline against Gram-positive and Gram-negative organisms (Table 1). With respect to clinically important, contemporary CABP pathogens, ceftaroline has activity against the Gram-positive organisms S. pneumoniae, S. aureus and Streptococcus pyogenes, and Gram-negative species (Haemophilus influenzae and...
Ceftaroline fosamil

Table 1. In vitro activity of ceftaroline against Gram-positive and Gram-negative organisms²⁶,²⁸

<table>
<thead>
<tr>
<th>Gram-positive organisms</th>
<th>Gram-negative organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>Enterobacter cloacae</td>
</tr>
<tr>
<td>MRSA</td>
<td>Citrobacter freundii</td>
</tr>
<tr>
<td>MSSA</td>
<td>ESBL-negative Escherichia coli</td>
</tr>
<tr>
<td>VISA</td>
<td>ESBL-negative Klebsiella pneumoniae</td>
</tr>
<tr>
<td>VRSA</td>
<td>Proteus mirabilis</td>
</tr>
<tr>
<td>Coagulase-negative staphylococci</td>
<td>Providencia rettgeri</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>Providencia stuartii</td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>Seratia marcescens</td>
</tr>
<tr>
<td>Streptococcus agalactiae</td>
<td>Moraxella catarrhalis</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>Haemophilus influenzae</td>
</tr>
<tr>
<td>levofloxacin resistant</td>
<td>β-lactamase positive</td>
</tr>
<tr>
<td>penicillin resistant</td>
<td>β-lactamase negative</td>
</tr>
<tr>
<td>penicillin intermediate</td>
<td>β-lactamase resistant</td>
</tr>
<tr>
<td>penicillin susceptible</td>
<td>Pasteurella multocida</td>
</tr>
<tr>
<td>Streptococcus pyogenes</td>
<td></td>
</tr>
<tr>
<td>macrolide resistant</td>
<td></td>
</tr>
<tr>
<td>macrolide susceptible</td>
<td></td>
</tr>
<tr>
<td>Viridans group streptococci</td>
<td></td>
</tr>
<tr>
<td>β-Haemolytic group A</td>
<td></td>
</tr>
<tr>
<td>streptococci</td>
<td></td>
</tr>
<tr>
<td>β-Haemolytic group B</td>
<td></td>
</tr>
<tr>
<td>streptococci</td>
<td></td>
</tr>
</tbody>
</table>

Moraxella catarrhalis), including resistant phenotypes.¹⁹,²⁸–³⁰ Similarly, it is relevant to consider ceftaroline in the context of skin and skin structure infections because S. aureus and S. pyogenes, along with other Gram-positive and Gram-negative species, are common aetiological agents in this type of infection.

Ceftaroline has potent in vitro activity against multidrug-resistant strains of S. pneumoniae.¹⁹,³¹–³⁸ The MIC₉₀ for most isolates tested has been ≤0.5 mg/L (MIC range, ≤0.003–2 mg/L). The activity of ceftaroline has been compared with that of ceftobiprole, the MIC₉₀ against penicillin-, cephalosporin- and levofloxacin-resistant S. pneumoniae and the MIC₉₀ values of ceftaroline are at least 2 double dilutions less than that of ceftaxone.³⁷,³⁸ Ceftaroline also has in vitro activity against MSSA.¹⁹,²⁸–³⁰ The majority of isolates in microbiological studies have been inhibited by MIC₉₀ values of ≤1 mg/L (MIC range, ≤0.12–2 mg/L). Similarly, ceftaroline MIC₉₀ for heteroresistant vancomycin-intermediate S. aureus (HVISA), vancomycin-intermediate S. aureus (VISA) and vancomycin-resistant S. aureus (VRSA) have been reported as ≤2 mg/L (MIC range, ≤0.25–4 mg/L).³²,³³ Furthermore, in a study comparing in vitro activities of ceftaroline and ceftepibopirone, the MIC₉₀ for 19A serotypes and ceftriaxone- or amoxicillin-resistant isolates of S. pneumoniae were 0.25 mg/L and 1 mg/L, respectively.³⁷

An in vivo comparison of ceftaroline fosamil with daptomycin and tigecycline against methicillin-susceptible S. aureus (MSSA), MRSA and glycopeptide-intermediate S. aureus (GISA) was conducted in the rabbit endocarditis model.³⁰ Ceftaroline and daptomycin showed the greatest reduction in bacterial titres in vegetations after 4 days of treatment. Only ceftaroline demonstrated 100% sterilization of vegetations in all strains.

Ceftaroline has been shown to have synergistic activity against Gram-negative species in combination with an aminoglycoside.⁴¹ In an in vitro study, ceftaroline plus amikacin was synergistic against 90% of isolates tested, including Pseudomonas aeruginosa, extended-spectrum β-lactamase (ESBL)-producing Escherichia coli, ESBL-producing Klebsiella pneumoniae and AmpC-derepressed Enterobacter cloacae. Synergy was also demonstrated for ceftaroline in combination with meropenem against all E. coli isolates tested. A similar study showed synergy with this combination against ESBL-producing K. pneumoniae and community-associated MRSA. No evidence of antagonism was noted in the various combinations tested.⁴²

Resistance to ceftaroline is expected to be limited, as demonstrated in multistep resistance selection studies.⁴³–⁴⁶ Serial passages were performed to determine the probability of development of resistance to ceftaroline in H. influenzae, M. catarrhalis, MRSA, MSSA, Enterobacter faecalis, S. pneumoniae and S. pyogenes. The experiments maintained low ceftaroline MICs and did not yield clones with increased MICs, with the exception of vancomycin-resistant E. faecalis, which slowly developed resistance during serial passage, and vancomycin-susceptible E. faecalis, which demonstrated spontaneous resistance development.

Basic pharmacokinetic and pharmacodynamic profile

Pharmacokinetics

Ceftaroline exhibits dose-proportional pharmacokinetics following intravenous (iv) administration similar to other renally excreted cephalosporins (Table 2).⁴² After administration of a single 500 mg dose of ceftaroline fosamil, maximum ceftaroline plasma concentration (C₉₀) was 16.6 mg/L and AUC from time zero to infinity (AUC₀–∞) was 44.8 h·mg/L.⁴⁷ In a multiple-dose study, C₉₀ of ceftaroline at day 14 was 21.3 mg/L and AUC at steady-state (AUC₀–∞) was 56.2 h·mg/L after administration of 600 mg of ceftaroline fosamil every 12 h.⁴⁷ The volumes of distribution in the central and peripheral compartments were 17.3 L and 4.89 L, respectively.⁴⁸ Protein binding was ~20%.⁴⁹ The half-life of ceftaroline after a single 500 mg dose of ceftaroline fosamil was 2.53 h; on day 14 after administration of 600 mg every 12 h, it was 2.66 h. Renal clearance of ceftaroline was 93.5 mL/min after a single 500 mg dose and 118.9 mL/min at the end of the multiple-dose study.⁴⁷

Because ceftaroline is excreted renally, the pharmacokinetic parameters were examined in subjects with mild and moderate renal impairment.⁵⁰–⁵⁴ The AUC and half-life were 25% higher and 14% longer, respectively, in subjects with mild renal impairment [creatinine clearance (CLCR) >50–80 mL/min]; no dosage adjustment of ceftaroline fosamil is recommended in these patients. The AUC was 50% higher in subjects with moderate renal impairment (CLCR >30–50 mL/min), therefore, dosage adjustment is recommended in patients with moderate to severe renal impairment (CLCR 15–30 mL/min).
Table 2. Pharmacokinetic parameters (means ± SD) of ceftaroline after ascending single doses or multiple doses of ceftaroline fosamil administered iv over 60 min in healthy subjects (reproduced from Steed and Rybak,67 with permission)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>50 mg</th>
<th>100 mg</th>
<th>250 mg</th>
<th>500 mg</th>
<th>750 mg</th>
<th>1000 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{max}$ (mg/L)</td>
<td>1.5 ± 0.25</td>
<td>3.1 ± 0.96</td>
<td>10 ± 1.68</td>
<td>16.6 ± 2.11</td>
<td>23.4 ± 4.92</td>
<td>30.5 ± 4.32</td>
</tr>
<tr>
<td>$t_{1/2}$ (h)</td>
<td>2.03 ± 0.15</td>
<td>2.23 ± 0.42</td>
<td>2.33 ± 0.26</td>
<td>2.53 ± 0.28</td>
<td>2.62 ± 0.29</td>
<td>2.9 ± 0.14</td>
</tr>
<tr>
<td>AUC$_{0→T}$ (h·mg/L)</td>
<td>4.0 ± 0.7</td>
<td>6.7 ± 1.7</td>
<td>23.4 ± 5.4</td>
<td>44.8 ± 2.9</td>
<td>57.6 ± 9.8</td>
<td>80.9 ± 8.6</td>
</tr>
<tr>
<td>CL/Fm (mL/min)</td>
<td>192.1 ± 38.9</td>
<td>228.9 ± 49.1</td>
<td>164.6 ± 39.2</td>
<td>164.8 ± 10.6</td>
<td>196.5 ± 34.5</td>
<td>183.7 ± 19.6</td>
</tr>
<tr>
<td>Urinary recovery (%)</td>
<td>48.7 ± 11.5</td>
<td>42.5 ± 10.9</td>
<td>48.0 ± 15.9</td>
<td>56.6 ± 5.7</td>
<td>55.2 ± 12.4</td>
<td>71.0 ± 6.9</td>
</tr>
<tr>
<td>Metabolic ratio</td>
<td>28.2 ± 8.0</td>
<td>27.6 ± 11.0</td>
<td>29.4 ± 10.0</td>
<td>40.2 ± 14.8</td>
<td>19.8 ± 3</td>
<td>25.2 ± 5.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>300 mg every 12 h</th>
<th>600 mg every 12 h</th>
<th>800 mg every 24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{max}$ (mg/L)</td>
<td>10 ± 0.76</td>
<td>8.5 ± 1.85</td>
<td>19.0 ± 0.71</td>
</tr>
<tr>
<td>$t_{1/2}$ (h)</td>
<td>2.56 ± 0.47</td>
<td>2.62 ± 0.41</td>
<td>1.6 ± 0.38</td>
</tr>
<tr>
<td>AUC$_{0→T}$ (h·mg/L)</td>
<td>25.8 ± 3.8</td>
<td>—</td>
<td>56.8 ± 9.3</td>
</tr>
<tr>
<td>AUC$_{ss}$ (h·mg/L)</td>
<td>—</td>
<td>24.3 ± 3.7</td>
<td>—</td>
</tr>
<tr>
<td>CL/Fm (mL/min)</td>
<td>174.6 ± 27.0</td>
<td>184.9 ± 26.9</td>
<td>159.7 ± 30.9</td>
</tr>
<tr>
<td>CLR (mL/min)</td>
<td>92.8 ± 69.3</td>
<td>75.3 ± 19.9</td>
<td>68.8 ± 19.8</td>
</tr>
<tr>
<td>Urinary recovery (%)</td>
<td>51.4 ± 36.8</td>
<td>40.6 ± 8.8</td>
<td>42.1 ± 9.8</td>
</tr>
<tr>
<td>Metabolic ratio</td>
<td>23.9 ± 7.7</td>
<td>23.0 ± 8.1</td>
<td>20.3 ± 9.0</td>
</tr>
</tbody>
</table>

$C_{max}$, maximum concentration; $t_{1/2}$, terminal half-life; AUC$_{0→T}$, AUC from time zero extrapolated to infinity; AUC$_{ss}$, AUC at steady-state; CL/Fm, metabolic clearance, where Fm is the fraction of the dose metabolized; CLR, renal clearance.

**Pharmacodynamics**

In vivo data from murine thigh infection models and lung penetration in rabbit models demonstrate a favourable pharmacodynamic profile for ceftaroline.55–57 Murine thigh and lung infection models using S. pneumoniae, S. aureus and E. coli demonstrated that the pharmacokinetic–pharmacodynamic index that best correlated with efficacy was the percentage of time the serum concentration was greater than the MIC (%T > MIC).55 The mean %T > MIC values for stasis, 1 log kill and 2 log kill for S. pneumoniae were 39, 43 and 50, respectively; these values were 26, 33 and 45 for S. aureus. A rabbit model showed 4% lung penetration after a short iv infusion of ceftaroline fosamil.58 Monte Carlo estimates of probability of target attainment correlate well with data generated in human subjects.58,59

In murine thigh and lung infection models, a modest post-antibiotic effect (PAE) was observed for S. pneumoniae and E. coli; a somewhat longer PAE was observed for S. aureus.55 Escalating doses produced PAEs of −1.9–1.5 h for S. pneumoniae, −0.33–5.7 h for E. coli and 0.8–7.2 h for S. aureus. An in vitro study demonstrated that the PAE of ceftaroline is extended by sub-MIC levels of the drug against Gram-positive cocci.59 PAEs for ceftaroline against pneumococci, staphylococci and enterococci were 0.7–1.8 h, 0.7–2.2 h and 0.2–1.1 h, respectively. Growth continued to be suppressed after the drug levels fell below 0.4× MIC, for a total duration of suppression of 2.5–8.4 h in pneumococci, 2.9–10.8 h in staphylococci and 7.9–10.6 h in enterococci.

With respect to normal gut flora, ceftaroline fosamil was administered to healthy subjects, and it was determined that there was no significant ecological impact on the human intestinal microflora.59 There was a slight decrease in the number of bifidobacteria and lactobacilli was observed during the first 7 days of administration, and the numbers of clostridia increased. No effect on Bacteroides bacteria was noted, and no new colonizing aerobic or anaerobic bacteria resistant to ceftaroline were observed. In Phase III clinical trials of ceftaroline fosamil for the treatment of complicated skin and skin structure infections (cSSSIs), Clostridium difficile toxin was found in two ceftaroline fosamil-treated patients (out of 693 patients who received ceftaroline fosamil); these infections were resolved successfully.61 C. difficile toxin was not identified in any patients in the clinical trials of ceftaroline fosamil for the treatment of community-acquired pneumonia (CAP).62

**Dosing and administration**

Ceftaroline fosamil is dosed at 600 mg iv every 12 h over 1 h in adults ≥18 years of age. Dosage adjustment is necessary in...
patients with moderate to severe renal impairment. In patients with moderate renal impairment (CL\textsubscript{CR} >30–50 mL/min), the dosage should be reduced to 400 mg iv (over 1 h) every 12 h. Patients suffering from severe renal impairment (CL\textsubscript{CR} 15–30 mL/min) should have the dose reduced to 300 mg iv (over 1 h) every 12 h. For patients with end-stage renal disease, including those on haemodialysis, the dose should be further reduced to 200 mg iv (over 1 h) every 12 h.

Ceftaroline fosamil is available in 600 mg and 400 mg single-use vials of sterile powder. The vial contents should be reconstituted with 20 mL of sterile water and further diluted in 250 mL of normal saline, 5% dextrose solution, 2.5% dextrose and 0.45% sodium chloride solution, or Lactated Ringer’s Injection. The resulting solution should be used within 6 h if stored at room temperature or within 24 h if refrigerated.

Systemic exposure following intramuscular administration of 600 mg of ceftaroline fosamil to healthy adults was equivalent to systemic exposure following iv administration.\textsuperscript{63}

**Safety**

The most common adverse events reported in clinical trials of ceftaroline fosamil for the treatment of cSSSI and CAP were diarrhoea, nausea and headache.\textsuperscript{61,62} Treatment was discontinued because of an adverse event in 4% of patients receiving ceftaroline fosamil and 5% of patients receiving comparator therapy; the most common adverse event leading to discontinuation of study drug was hypersensitivity (0.3% in patients treated with ceftaroline fosamil and 0.5% in patients treated with comparator). Serious adverse events occurred in 8% of patients in each treatment group.

Ceftaroline fosamil has been classified as pregnancy category B, and has not been studied in paediatric populations.

**Drug interactions**

No clinical drug–drug interaction studies have been conducted with ceftaroline fosamil. There were no clinically relevant differences in ceftaroline exposure (C\textsubscript{max} or AUC) noted in the Phase II and III patients included in the skin and pneumonia trials who were taking concomitant medications known to be inducers, inhibitors or substrates for cytochrome P450 isozymes or drugs that undergo active renal secretion or drugs that modify renal blood flow. When ceftaroline was incubated in vitro with pooled human liver microsomes, it did not induce or inhibit P450 isozymes. Available data suggest that ceftaroline fosamil has a low propensity for drug interaction.\textsuperscript{64}

**Physical compatibility**

Simulated Y-site co-administration of ceftaroline fosamil with 73 common iv drugs in 219 different admixtures demonstrates good compatibility.\textsuperscript{65} Sixty-four drugs in 192 admixture combinations were found to be compatible with ceftaroline fosamil after a 4 h observation period. The drugs that were incompatible with ceftaroline fosamil included amphotericin B, caspofungin acetate, diazepam, filgrastim, labetalol, potassium phosphates and sodium phosphates (all drugs were tested in admixtures of 5% dextrose, 0.9% sodium chloride and Lactated Ringer’s solution). Dobutamine and magnesium sulphate were incompatible when mixed in 5% dextrose and Lactated Ringer’s solution, respectively.

**Role in therapy and antimicrobial stewardship**

In response to increasing concern about antimicrobial resistance, patterns in prescribing of initial antimicrobial therapy for cSSSI and CAP have evolved during the past decade.

Antimicrobial stewardship programmes can be implemented to reduce inappropriate use of antimicrobials, thereby controlling the development of resistance.\textsuperscript{66,67} These programmes are also useful in limiting toxicity and overgrowth of pathogenic organisms such as *C. difficile*. Typical stewardship programmes target antimicrobials that pose a risk of development of resistance, are associated with significant toxicity, require therapeutic drug monitoring, have the potential to select for pathogenic organisms or have a high cost.

The low potential for development of resistance, favourable safety and tolerability profile, and low incidence of *C. difficile*-associated diarrhoea in clinical trials are positive attributes of ceftaroline with respect to antimicrobial stewardship programmes. Similarly, in the context of increasing incidence of resistance of *Pseudomonas* and *Acinetobacter* species to carbapenems, the lack of activity of ceftaroline against these pathogens may be considered a positive characteristic. Use of ceftaroline fosamil for infections in which coverage of these pathogens is not necessary is an attractive alternative and may reduce the burden of selective pressure.

**Summary and conclusions**

CAP continues to be a problematic infection in our society. Antibiotic resistance, our ageing population and concomitant co-morbidities will continue to impact the outcomes associated with treatment. The pattern of antibiotic use for CAP has significantly changed over the past 10 years, with more patients receiving combination therapy and coverage for resistant pathogens.\textsuperscript{68} These data suggest the need for newer antimicrobials offering safe, effective treatment with a spectrum of activity relevant to today’s array of respiratory pathogens. The approval of ceftaroline fosamil for CABP provides a new empirical option that addresses the need to cover today’s pneumonia pathogens, while preserving the well-recognized tolerability profile of the cephalosporin class. Furthermore, the pharmacokinetic and pharmacodynamic profiles along with uncomplicated dosing and administration should foster utility of ceftaroline fosamil in the hospital and outpatient parenteral antimicrobial therapy settings.

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**Transparency declarations**

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J. B. L. is an employee of the Forest Research Institute (Jersey City, NJ, USA), and holds stock and stock options in Forest Laboratories, Inc. Jennifer Darby, PharmD, of Scientific Therapeutics Information, Inc. (Springfield, NJ, USA) provided editorial assistance on this manuscript.

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