The Rationale for Revising the Clinical and Laboratory Standards Institute Vancomycin Minimal Inhibitory Concentration Interpretive Criteria for *Staphylococcus aureus*

Fred C. Tenover1 and Robert C. Moellering, Jr.2

1Division of Healthcare Quality Promotion, Centers for Disease Control and Prevention, Atlanta, Georgia; and 2Beth Israel Deaconess Medical Center, Boston, Massachusetts

The Clinical and Laboratory Standards Institute (formerly, the NCCLS) established the susceptibility and resistance breakpoints for minimal inhibitory concentration (MIC) and disk diffusion testing of vancomycin against isolates of *Staphylococcus aureus* 20 years ago. The disk diffusion breakpoints were modified in 1998 when it was recognized that vancomycin-intermediate *S. aureus* strains were not detected by this method. In 2006, the vancomycin MIC breakpoints for *S. aureus* were lowered (from ≤4 μg/mL to ≤2 μg/mL for “susceptible,” from 8–16 μg/mL to 4–8 μg/mL for “intermediate,” and from ≥32 μg/mL to ≥16 μg/mL for “resistant”) to increase detection of heterogeneously resistant isolates of *S. aureus*. This decision reflected a growing amount of microbiological and clinical data indicating that isolates of *S. aureus* are less likely to respond to vancomycin therapy when the vancomycin MICs are ≥4μg/mL.

In January 2004, the Antimicrobial Susceptibility Testing (AST) Subcommittee of the Clinical and Laboratory Standards Institute (CLSI; formerly, the NCCLS) began consideration of a proposal to modify the MIC interpretive criteria (i.e., breakpoints) for testing of vancomycin against isolates of *Staphylococcus aureus*. Data in the literature regarding the failure of vancomycin therapy to cure *S. aureus* infection when the vancomycin MICs of the organisms were in the intermediate (8–16 μg/mL) or borderline-susceptible (4 μg/mL) range suggested that the susceptibility breakpoint used for MIC testing needed to be reconsidered. The AST Subcommittee reviewed the following: (1) in vitro susceptibility testing data from the United States and Europe, (2) clinical data on the efficacy of vancomycin therapy for treating *S. aureus* infection, (3) results of an Emerging Infections Network survey on treatment of persistent meticillin-resistant *S. aureus* (MRSA) bacteremia, (4) clinical trial data regarding vancomycin versus linezolid for treatment of pneumonia, and (5) data on vancomycin heteroresistance in *S. aureus* isolates. Several infectious diseases clinicians, serving as members and advisors of the AST Subcommittee, also presented their published and unpublished clinical experiences using vancomycin therapy to treat borderline vancomycin-susceptible MRSA infection. After considering the data presented at 4 meetings over a 2-year period, the AST Subcommittee lowered the vancomycin breakpoints from ≤4 μg/mL to ≤2 μg/mL for “susceptible,” from 8–16 μg/mL to 4–8 μg/mL for “intermediate,” and from ≥32 μg/mL to ≥16 μg/mL for “resistant” [1] (the vancomycin resistance breakpoint was also lowered because of the high likelihood of clinical failure at MICs of 16 μg/mL). The new CLSI vancomycin breakpoints for *S. aureus* were published in January 2006 [2]. The data considered by the AST Subcommittee and additional supporting information will be presented herein.

HISTORICAL PERSPECTIVE

Concern for the decreasing effectiveness of vancomycin for treating *S. aureus* infection did not begin with a report of resistance in staphylococci, but rather with 2 reports from France and England of vancomycin resistance in enterococci [3, 4]. The mechanism of vancomycin resistance in the French enterococcal isolate was encoded by a 5-gene operon that included the novel *vanA* ligase gene. It was assumed by many that the *vanA*-containing operon would eventually be transferred from enterococci to *S. aureus*, resulting in the loss of vancomycin for treating staphylococcal infections, particularly those caused by...
MRSA isolates [5, 6]. It was a surprise when the first reported case of vancomycin “resistance” in a clinical isolate of S. aureus was not because of acquisition of vanA by a strain of MRSA, but rather was the result of an unusually thickened staphylococcal cell wall containing numerous small dipeptides capable of binding vancomycin, as well as several other metabolic and structural changes in the organism [7–9]. The original vancomycin-intermediate S. aureus strain (VISA) was designated Mu50. Subsequently, VISA strains were reported in the United States [10, 11] and around the world [12–14]. None of the isolates contained the vanA gene. The isolates were classified as vancomycin-nonsusceptible by the broth microdilution method and by inoculating brain heart infusion (BHI) agar screening plates containing 4–6 μg of vancomycin per mL [15]. However, reduced susceptibility to vancomycin was not detected by disk diffusion [16].

Use of BHI screening agar identified groups of isolates that were susceptible to vancomycin by standard broth microdilution techniques but contained subpopulations of cells for which the vancomycin MICs were in the intermediate range (i.e., 8–16 μg/mL). These organisms were designated as vancomycin-heteroresistant S. aureus (hVISA) strains. The prototype hVISA strain was S. aureus Mu3, a clinical isolate recovered from a Japanese patient with staphylococcal pneumonia [15]. Hiramatsu et al. [15] reported that such heteroresistant strains, which could lead to vancomycin treatment failure, could be differentiated from Mu50-like strains of S. aureus (that were “homogeneously” resistant) by plating a large number of organisms on a BHI agar plate containing 4 μg/mL vancomycin and incubating the plate for 24 and 48 h. Heavy growth on the BHI agar screen plate after 24 h indicated a likely VISA strain, and growth that appeared only after 48 h was likely to represent an hVISA strain [15]. Population analysis curves using incremental dilutions (rather than doubling dilutions) of vancomycin were used to demonstrate the subpopulations of vancomycin-resistant cells [15]. This technique has been used by a number of investigators to study the incidence of hVISA among S. aureus isolates in hospitals [17–19]; however, no population-based studies of heteroresistance have been conducted in the United States.

In June 2002, the first naturally occurring vanA-containing S. aureus isolate was recovered from a patient in Michigan [20]. The vancomycin MIC for the vancomycin-resistant S. aureus (VRSA) isolate was 1024 μg/mL, which is 1000 times the normal vancomycin MIC for S. aureus (i.e., 1 μg/mL) [21–23]. Six additional VRSA isolates for which the vancomycin MICs ranged from 16 to 512 μg/mL [27] have been recovered from patients in Pennsylvania [24], New York [25], and Michigan [26].

**VANCOMYCIN HETERO RESISTANCE: A REASSESSMENT**

The advent of the “real” (i.e., vanA-containing) VRSA strain overshadowed the discussion of the clinical significance of hVISA strains (i.e., Mu3-like strains that contained subpopulations of cells that were resistant to vancomycin) [15]. Many physicians and microbiologists were skeptical that this difficult-to-detect heteroresistant phenotype signaled MRSA infections that would be refractory to vancomycin therapy, particularly because such strains were susceptible to vancomycin by reference MIC methods [28]. Surveillance studies for hVISA strains rarely commented on clinical outcomes of patients infected with such isolates. British and Japanese literature, which used 8 μg/mL as the breakpoint for vancomycin resistance, preferred the term “heteroresistant” (hVRSA) [15, 29]. Data from multiple laboratories demonstrated that the “resistant” subpopulations (i.e., vancomycin MICs of 8–16 μg/mL) typically represented ≤1 in 10^7 to 1 in 10^9 colony-forming units (CFUs) (figure 1), which is why the standard MIC tests—which use only 5 × 10^4 CFU/well (broth microdilution) or 1 × 10^4 CFU/spot (agar dilution)—failed to detect these subpopulations.
In a quest for better quantitation of hVRSA populations, Wootton et al. [17] used a spiral plating machine to generate population analysis profiles (PAP) of S. aureus isolates suspected of being hVRSA strains. They divided the PAP data by the area under the population curve (AUC) to produce PAP/AUC ratios. PAP/AUC values ≤0.9 were designated as vancomycin-susceptible S. aureus (VSSA), those with ratios of 1.0–1.3 were defined as hVISA, and those with ratios ≥1.3 were designated as VRSA (MICs ≥8 µg/mL) and not those containing vanA). This PAP-AUC procedure became the “gold standard” for investigating the clinical relevance of hVISA strains in several large surveillance studies. Using the PAP-AUC method, another study (A. Bolmström, unpublished data) demonstrated that, among a convenience sample of S. aureus isolates from around the world, heteroresistant strains could be detected, even among S. aureus isolates for which the broth microdilution MICs were 0.5 µg/mL (figure 2). Thus, there is a poor correlation between a vancomycin MIC result and the presence of heteroresistance. Additional studies from Turkey documented that the hVISA phenotype was not rare but was present in 46 (17.7%) of 256 MRSA isolates tested at Hacettepe University Hospital [30]. The vancomycin MICs of 45 of 46 isolates were 2.5–2.0 µg/mL, clearly in the susceptible range when tested by the CLSI broth microdilution method. A second study of vancomycin heteroresistant S. aureus isolates in France showed that 255 (11%) of 2300 isolates of S. aureus from Limoges Teaching Hospital were vancomycin heteroresistant, including 7 methicillin-susceptible S. aureus (MSSA) and 248 MRSA isolates [31]. Data on heteroresistance in the United States are lacking.

Unfortunately, there is no technique that is suitable for routine use in a clinical microbiology laboratory that can detect hVISA strains with the same degree of sensitivity and specificity as the PAP-AUC method [17, 29]. The inability of both automated and standard reference susceptibility testing methods to detect the hVISA phenotype makes it difficult to identify those infections that may not respond to vancomycin therapy. Thus, confirming the presence of a heterogeneously resistant strain of S. aureus remains a difficult challenge for both microbiologists and physicians.

Recent genetic studies of hVISA and VISA isolates revealed mutations in either structural or regulatory genes associated with the accessory gene regulator (agr) pathway of some, but not all, hVISA isolates [32–34]. These mutations result in decreased production of several virulence factors and toxins, such as δ-lysin, but lead to increased production of biofilm matrix, thus rendering them “tolerant” to the killing action of vancomycin. These changes may be the precursor for further genetic alterations that ultimately result in increases in the cell wall thickness and elevated vancomycin MICs, both of which have become the hallmarks of VISA strains. Heteroresistance has been observed only in isolates of agr types 1 and 2 but from all 5 major clonal complexes [35]. Strains with decreased susceptibility to vancomycin may also show decreased susceptibility to daptomycin [36, 37].

**CLINICAL RELEVANCE OF hVISA: THE BEGINNINGS OF OUTCOMES DATA**

In 2003, Fridkin et al. [38] reported a study of 19 infections caused by S. aureus isolates that showed reduced susceptibility to vancomycin (SA-RSV; i.e., vancomycin MICs of 4 or 8 µg/mL) conducted by the Centers for Disease Control and Prevention (Atlanta, GA). The vancomycin MICs for 4 isolates were 8 µg/mL, and the MICs for the remaining isolates were 4 µg/mL. Forty-two control subjects (with MRSA infections for which the vancomycin MICs of the isolates were ≤2 µg/mL) were also enrolled in the study. All SA-RSV cases (whether the vancomycin MICs for the isolates were 4 or 8 µg/mL) had similar outcomes. The attributable mortality rate of SA-RSV infection was 63%, compared with 12% for MRSA infection. The pri-
mary risk factors for being a case patient were exposure to vancomycin and having an MRSA infection during the previous 2–3 months. Predictors of inhospital death were bacteremia and SA-RSV infection. Thus, those patients with infections with S. aureus for which the vancomycin MICs were 4 μg/mL (susceptible, according to previous breakpoints) had similar outcomes to those with S. aureus infection for which the vancomycin MICs were 8 μg/mL (intermediate, according to previous breakpoints).

Several reports from Australia have focused on the clinical relevance of hVISA strains. Charles et al. [39] reviewed the clinical data for all 46 cases of MRSA bacteremia that occurred in their hospital during a single year. The MRSA isolates recovered from the bacteremic patients were tested blindly using the PAP-AUC method to identify hVISA and VISA isolates. No VISA isolates were identified, but 5 hVISA isolates were documented. The clinical data from chart reviews of the 5 patients infected with vancomycin-heteroresistant MRSA suggested that the patients had sustained fever and bacteremia, high-inoculum infections (i.e., greater bacterial loads), and inappropriately low vancomycin trough levels early in the course of their therapy (which may have selected for the emergence of strains with low-level resistance), compared with patients infected with non–vancomycin-heteroresistant MRSA. Although there were only 5 cases of hVISA, the study laid the groundwork for a second Australian study reported by Howden et al. [40]. This study reviewed the cases of 25 patients in which serious hVISA infections (8 cases of endocarditis, 9 cases of bacteremia with deep-seated infection, 6 cases of osteomyelitis/septic arthritis, and 2 cases of empyema) were confirmed by PAP-AUC analysis. The vancomycin MICs for the isolates ranged from 2 to 4 μg/mL. Of the 25 patients, 19 experienced vancomycin therapy failure (i.e., the patient was bacteremic for >7 days or had a positive sterile site culture result after 21 days of therapy with a glycopeptide). Strain typing studies demonstrated multiple PFGE patterns among the isolates, ruling out the spread of a single strain in the hospital. Four of the patients died. Of the 21 patients treated with agents other than vancomycin, 16 were cured (mostly with linezolid). Thirteen patients required surgical interventions. Aside from sustained bacteremia, there were no clinical features that differentiated cases of hVISA from other cases of MRSA.

A study conducted in the United States by Sakoulas et al. [41] among selected patients suggested that, when vancomycin MICs for MRSA isolates increase to ≥2 μg/mL, the number of clinical failures for patients treated with vancomycin increases. These investigators reported that the isolates from patients that experienced failure of vancomycin therapy typically showed a decrease in a bactericidal killing assay of <1.1 logs (i.e., <99.9% kill rate) after 72 h, compared with a killing assay of >7.1 logs for isolates from patients who benefited from vancomycin therapy. Moise-Broder et al. [42] reported the results of a study of 63 evaluable patients with MRSA bacteremia of whom 45 experienced failure or were intolerant of vancomycin therapy. When the vancomycin MICs of the isolates increased from 0.5 to 2 μg/mL, the rate of therapeutic failure increased, even though all of the isolates would have been classified as vancomycin susceptible. It should be noted, however, that the cases in both the studies by Sakoulas et al. [41] and Moise-Broder et al. [42] were enriched for vancomycin therapy failures, and therefore, likely overestimate failure rates. Nonetheless, the relationship of MICs to vancomycin clinical failure is striking. A third study of 95 patients by Hidayat et al. [43] confirms that high vancomycin MICs, defined as 1.5–2.0 μg/mL (as measured by the Etest method), is an independent predictor of poor response to vancomycin therapy for MRSA infection, even when vancomycin trough levels >15 μg/mL are achieved. Limited studies using the rabbit endocarditis model also lend support to the importance of hVISA isolates as a cause of vancomycin treatment failure [44].

**MICROBIAL POPULATION STUDIES**

Data from several surveillance studies demonstrate that the modal vancomycin MIC for S. aureus is typically 1 μg/mL [21–23] and that isolates for which the MICs are 4 μg/mL are rare. The Surveillance Network data from US laboratories confirm that vancomycin MICs of 4 μg/mL are rare (<1%) among over 240,000 S. aureus isolates (figure 3). Data from Europe that was made available via the European Union Committee on Antimicrobial Susceptibility Testing Web site [45] showed no S. aureus isolates for which the vancomycin MICs were 4 μg/mL among ∼85,000 organisms.

**DATA FROM CLINICAL TRIALS OF LINEZOLID AND VANCOMYCIN**

Two published studies compared the outcomes of patients with pneumonia who were treated with either linezolid or vancomycin [46, 47]. Both were retrospective subset analyses of previous trials in which vancomycin appeared to be less effective than linezolid for treatment of pneumonia due to MRSA infection (but not MSSA). These studies, although not without controversy, lend credence to the concept that at least some of the MRSA strains (all of which were “susceptible” to vancomycin by former CLSI criteria) respond less well to vancomycin than do MSSA strains. A similar observation was recently made in a clinical study of the effectiveness of linezolid and vancomycin for treatment of complicated skin and soft-tissue infections [48].

**SURVEY OF THE EMERGING INFECTIONS NETWORK**

In February 2005, representatives of the Emerging Infections Network surveyed (via email and facsimile) 891 infectious
The Surveillance Network data for 2005 showing the vancomycin MIC distribution for *Staphylococcus aureus* (top) and coagulase-negative staphylococci (bottom). These data demonstrate that *S. aureus* isolates for which the vancomycin MICs are $\geq 4$ μg/mL are rare in the United States.

### CLINICAL CASES

Table 1 provides a list of 14 cases—the extent of those in the medical literature plus several personal communications—in which vancomycin therapy failure was documented in the therapy of infections due to staphylococci (mostly MRSA) for which the MIC of vancomycin was 4 μg/mL. In the majority of these cases, there was persistent bacteremia (for as long as 42 days), despite administration of therapeutic doses of vancomycin. The diseases represented include endocarditis, osteomyelitis, and bacteremia, including a case of bacteremia from an infected catheter (which was removed). Without proper denominator data, it is impossible to state with assurance the frequency with which therapeutic failures occurred among patients infected with strains of *S. aureus* for which vancomycin MICs are 4 μg/mL, nor can we determine how many patients infected with such strains may have responded to vancomycin therapy. It is highly likely, however, that such failures are widely underreported, because most clinical microbiology laboratories in the past would have considered these isolates “susceptible” to vancomycin therapy. Irrespective of the above considerations, the fact that therapeutic failures with pro-

...
### Table 1. Clinical failures of vancomycin therapy among patients infected with *Staphylococcus aureus* for which the MICs for vancomycin were 4 μg/mL.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, years</th>
<th>Sex</th>
<th>Type of infection, source</th>
<th>Organism</th>
<th>Vancomycin MIC, μg/mL</th>
<th>Evidence of failure</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>80</td>
<td>Male</td>
<td>Endocarditis, native tricuspid value</td>
<td>MRSA</td>
<td>4</td>
<td>Positive blood culture result after receiving vancomycin for 29 days</td>
<td>[40]</td>
</tr>
<tr>
<td>2</td>
<td>73</td>
<td>Female</td>
<td>Endocarditis, native mitral valve</td>
<td>MRSA</td>
<td>4</td>
<td>Positive blood culture result after receiving vancomycin for 8 days</td>
<td>[40]</td>
</tr>
<tr>
<td>3</td>
<td>66</td>
<td>Female</td>
<td>Endocarditis, native mitral valve</td>
<td>MRSA</td>
<td>4</td>
<td>Positive blood culture result after receiving vancomycin for 13 days</td>
<td>[40]</td>
</tr>
<tr>
<td>4</td>
<td>45</td>
<td>Male</td>
<td>Bacteremia, vertebral osteomyelitis</td>
<td>MRSA</td>
<td>4</td>
<td>Positive blood culture result after receiving vancomycin for 12 days</td>
<td>[40]</td>
</tr>
<tr>
<td>5</td>
<td>63</td>
<td>Male</td>
<td>Bacteremia, vertebral osteomyelitis</td>
<td>MRSA</td>
<td>4</td>
<td>Positive blood culture result after receiving vancomycin for 33 days</td>
<td>[40]</td>
</tr>
<tr>
<td>6</td>
<td>83</td>
<td>Male</td>
<td>Bacteremia, source not specified</td>
<td>MRSA</td>
<td>4</td>
<td>Positive blood culture result after receiving vancomycin for 8 days</td>
<td>[40]</td>
</tr>
<tr>
<td>7</td>
<td>NR</td>
<td>NR</td>
<td>Bacteremia, source not specified</td>
<td>MRSA</td>
<td>4</td>
<td>Positive blood culture result after receiving vancomycin for ≥7 days</td>
<td>[39]</td>
</tr>
<tr>
<td>8</td>
<td>NR</td>
<td>NR</td>
<td>Bacteremia, source not specified</td>
<td>MRSA</td>
<td>4</td>
<td>Positive blood culture result after receiving vancomycin for 12 days</td>
<td>[50]</td>
</tr>
<tr>
<td>9</td>
<td>59</td>
<td>Male</td>
<td>Bacteremia, infected dialysis catheter (subsequently removed)</td>
<td>MRSA</td>
<td>4</td>
<td>Positive blood culture result after receiving vancomycin for 39 days</td>
<td>F. Nolette (personal communication)</td>
</tr>
<tr>
<td>10</td>
<td>60</td>
<td>Male</td>
<td>Endocarditis, native mitral valve</td>
<td>MRSA</td>
<td>4</td>
<td>Positive blood culture result after receiving vancomycin for 42 days</td>
<td>M. Weinstein (personal communication)</td>
</tr>
<tr>
<td>11</td>
<td>81</td>
<td>Male</td>
<td>Endocarditis, prosthetic valve (responded to linezolid therapy without valve replacement)</td>
<td>MRSA</td>
<td>4</td>
<td>Positive blood culture result after receiving vancomycin for 22 days</td>
<td>R. C. Moellering, Jr. (personal communication)</td>
</tr>
<tr>
<td>12</td>
<td>57</td>
<td>Female</td>
<td>Bacteremia, vertebral osteomyelitis</td>
<td>MSSA</td>
<td>4</td>
<td>Positive blood culture result after receiving vancomycin for &gt;10 weeks</td>
<td>S. Pillai and R. C. Moellering, Jr. (personal communication)</td>
</tr>
<tr>
<td>13</td>
<td>45</td>
<td>Female</td>
<td>Endocarditis, native valve (not specified)</td>
<td>MSSA</td>
<td>4</td>
<td>Positive blood culture result after receiving vancomycin for 36 days</td>
<td>[51]</td>
</tr>
<tr>
<td>14</td>
<td>44</td>
<td>Male</td>
<td>Infected transjugular intrahepatic portosystemic shunt</td>
<td>MRSA</td>
<td>4</td>
<td>Positive blood culture result after receiving vancomycin for 42 days</td>
<td>J. S. Lewis (personal communication)</td>
</tr>
</tbody>
</table>

**NOTE.** MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*; NR, not reported.

Longed bacteremia have already been documented among 14 patients would argue strongly for lowering the susceptibility breakpoints for vancomycin.

### SUMMARY

Surveillance data of >300,000 *S. aureus* isolates from the United States and Europe show that vancomycin MICs of 4 μg/mL are very unusual and represent <0.3% of all vancomycin MIC values for that species. Thus, lowering the vancomycin breakpoint by 1 doubling dilution would not significantly impact the distribution of “susceptible,” “intermediate,” and “resistant” results for *S. aureus* infection collected over the past decade but will likely improve the predictive value of a vancomycin-susceptible result. The resistance breakpoint was also lowered, reflecting the clinical judgment that a vancomycin MIC of 16 μg/mL was fully resistant. Data from PAP-AUC studies from England, Australia, and Sweden, and from additional studies using similar methods from Turkey and France, indicate that up to 17.7% of *S. aureus* isolates for which the vancomycin MICs are 0.5–4 μg/mL are heteroresistant (i.e., contain vancomycin-resistant subpopulations) and, thus, are more likely to result in clinical failure in patients treated with vancomycin. Currently, there is no practical, validated laboratory test that accurately and reproducibly detects heteroresistant *S. aureus* isolates and that could alert clinicians to patients at greater risk.
of experiencing vancomycin therapy failure. Lowering the intermediate breakpoint to 4 μg/mL flags those *S. aureus* isolates that are likely to be heteroresistant and cause vancomycin therapy failure, while retaining high specificity. Although pharmacokinetic data may suggest a susceptible breakpoint of ≤1 μg/mL, such a breakpoint would encompass 16.2% of *S. aureus* isolates from the United States (figure 3). This breakpoint was considered but rejected by the CLSI AST Committee. Ultimately, the identification of heteroresistant VISA isolates for which the standard broth microdilution vancomycin MICs are ≤2 μg/mL will require the development of more sophisticated tests.

On the basis of these data and the clinical experiences of the AST Subcommittee members who are infectious diseases clinicians, the AST Subcommittee lowered the susceptible breakpoint for vancomycin therapy for *S. aureus* isolates from ≤4 μg/mL to ≤2 μg/mL, redefined the intermediate category as 4–8 μg/mL, and lowered the resistance breakpoint to ≥16 μg/mL. There was no change to the disk diffusion breakpoint for *S. aureus* isolates. There was also no change in the breakpoints for coagulase-negative staphylococci because of lack of clinical data supporting such as change.

**Acknowledgments**

We are grateful to Dr. Daniel Sahm for sharing the Surveillance Network data and Dr. Anne Bolmstrom for sharing the vancomycin heteroresistance data.

**Financial support.** The Centers for Disease Control and Prevention.

**Potential conflicts of interest.** R.C.M. has served as a consultant to Pfizer Laboratories, Cubist Pharmaceuticals, Astellas Pharmaceuticals, Tar- ganta Pharmaceuticals, and Sanofi-Aventis. F.C.T.: no conflicts.

**References**


30. Sancak B, Erics S, Menemenlioglu D, Colak-


