Role of Folate Antagonists in the Treatment of Methicillin-Resistant *Staphylococcus aureus* Infection

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Methicillin-resistant *Staphylococcus aureus* (MRSA) infection has reached epidemic proportions, and therapeutic options are limited because these strains are often multidrug resistant. However, the new strains of community-acquired MRSA show decreased resistance to trimethoprim-sulfamethoxazole. Clinical and experimental reports show a mixture of successes and failures with trimethoprim-sulfamethoxazole treatment. A reason for failure might be the amount of thymidine released from damaged host tissues and bacteria, a concept strengthened by the fact that *S. aureus* thermonuclease releases thymidine from DNA. Thus, success or failure with trimethoprim-sulfamethoxazole may well depend on the amount of tissue damage and organism burden, rather than acquisition of a resistance gene. Clinical trials and experimental animal studies show high failure rates, perhaps because of released thymidine. The use of trimethoprim-sulfamethoxazole for community-acquired MRSA infection should not be endorsed without further research.

*S. aureus* resistance has been associated with increased mortality [1–7]. In the prepenicillin era, mortality from *S. aureus* bacteremia was 70% (in 1937), which decreased to 28% after the introduction of penicillin (in 1944), increased to 50% mortality as penicillinase-producing strains became widespread (in 1954), and again decreased to 30% after the introduction of methicillin (in 1962) [7]. Another study showed similar costs, both in terms of mortality and dollars, wherein hospital patients with *S. aureus* bacteremia had 3 times the length of hospital stay (14.3 vs. 4.5 days; *P* < .001), 3 times the total medical care cost ($48,824 vs. $14,141; *P* < .001), and 5 times the risk of in-hospital death (11.2% vs. 2.3%; *P* < .001) than did inpatients without this infection [8]. Over time, *S. aureus* has acquired even greater resistance to antimicrobials, which complicates therapy [9]. *S. aureus* is now the most common pathogen isolated in children in North America, comprising 27.4% of isolates [10].

The threat to the community from *S. aureus* infection has grown in recent years because of the emergence of methicillin-resistant *S. aureus* (MRSA) as community-acquired MRSA (CA-MRSA) [9, 11–18]. A problem in treatment of many CA-MRSA strains is the relatively limited options for oral therapy because many strains are multidrug resistant, and linezolid, some tetracyclines (e.g., minocycline and doxycycline), rifampin, clindamycin, fluoroquinolones, and trimethoprim-sulfamethoxazole (TMP-SMZ) are the only oral drugs that demonstrate activity [9, 12, 13, 18–24]. Linezolid is expensive and has notable toxicities, and bone marrow suppression and neuropathies occur with prolonged therapies [25]. Resistance and intolerance to the tetracyclines, clindamycin, fluoroquinolones, and rifampin further limit the choices for therapy [9, 12, 13, 18–24]. Thus, TMP-SMZ has been suggested for treatment of MRSA infection [13, 18, 19, 26, 27] because TMP-SMZ is inexpensive, is well tolerated, and penetrates well into tissues. This article examines the available information on the use of folate antagonists, including TMP-SMZ, for treatment of *S. aureus* infection. There have been a number of recent reviews highlighting clinical studies and pharmacological considerations for the use of other antibiotics in the treatment of MRSA infection [9, 11, 12, 28–38]; thus, these antibiotics will not be considered except as they bear on the comparisons made between the efficacies of these drugs and TMP-SMZ.

**MECHANISMS OF ACTION AND RESISTANCE**

Sulfonamides inhibit dihydropteroate synthase, which blocks folate biosynthesis [39]. This, in turn, leads to defective thymidine biosynthesis [39]. Sulfonamides are bacteriostatic
against *S. aureus* [40–42]. Some strains of *S. aureus* overproduce para-aminobenzoic acid (PABA), causing resistance to sulfonamides [41–43]. However, resistance to sulfonamides is more frequently caused by alterations in dihydropteroate synthase [44–46].

Trimethoprim is a tetrahydrofolate reductase inhibitor that, when added to sulfamethoxazole, provides a second step block in the folate biosynthetic pathway [46] (figure 1). TMP-SMZ proved to be bactericidal [9, 13, 47–49]. Blocking folate metabolism at 2 sites decreased the emergence of resistance [44, 46]. Nevertheless, resistance to TMP-SMZ has occurred because of amino acid substitutions in both enzymes [44, 46]. Plasmids (e.g., pSK41) carry the altered genes, which facilitate the spread of TMP-SMZ resistance [50]. Exogenous thymidine will render TMP-SMZ inactive, because it bypasses the double biosynthetic blockade.

**RATES OF RESISTANCE OF *S. AUREUS* TO TMP-SMZ**

Rates of TMP-SMZ resistance in *S. aureus* vary by location, as well as by time period, with a range of 0%–100%, with 16 of 23 reports showing resistance >30% [13]. In contrast, more-recent reports have shown high rates of susceptibility among MRSA strains: 73% [51], 85% [14], 91% [52], 92% [53], 95% [24], and 98% [54]. Three longitudinal studies revealed a substantial decrease in resistance over time: 16% [55], 59% [51], and 61% [53] decreases in resistance. This may be attributable to increased susceptibility of CA-MRSA strains [24, 54], changes limited to local susceptibility patterns, or decreased use of TMP-SMZ [53]. Support for this last idea comes from studies showing dramatically increased TMP-SMZ resistance in *S. aureus* in HIV-infected patients receiving TMP-SMZ prophylaxis [56]. Of note, >99% of methicillin-susceptible *S. aureus* (MSSA) strains were found to be susceptible to TMP-SMZ [57]. Thus, although MICs of vancomycin are increasing, and although high MICs are currently quite common (16.2% with ≥2 µg/mL), on the basis of a recent survey of >240,000 *S. aureus* isolates [28, 38], resistance to TMP-SMZ has decreased in most populations over time. Of concern is the recent linkage of the gene for high-level trimethoprim resistance with *SCCmecN1*, which was found in an epidemic of CA-MRSA strains [58].

**EFFECTS OF THYMIDINE ON TMP-SMZ**

Controversy concerning the value of sulfonamides for the treatment of staphylococcal infection dates from the earliest clinical reports. From 1938 to 1944, reports of successful and unsuccessful treatment of serious staphylococcal infections were published [40, 42, 59–74].

The cause of high failure rate for treatment with sulfonamides was first indicated in 1940 by Lockwood and Lynch [65], who reported that pus inhibited sulfonamides. MacLeod [40] found that inhibitory substances could be released from a large bacterial inoculum and from mammalian tissues by “autolysis.” A major component of pus is polymerized DNA, released from inflammatory cells and injured tissues [75]. *S. aureus* thermonuclease releases thymidine from DNA. Thymidine antagonizes the antistaphylococcal effects of both trimethoprim and sulfamethoxazole [76, 77] because it can be taken up via NupC and bypasses the metabolic blockades caused by TMP-SMZ (figure 1). Thermonuclease, encoded by *nuc* is present in virtually all *S. aureus* cells [78–81], although there was 1 outbreak of infection with *nuc*-negative MRSA strains [82]. Thus, thymidine release provides the theoretical underpinnings for concern about the use of antifolate drugs for *S. aureus* infection.

Concern for sulfonamide treatment failures was reduced by 1943–1944, when *β*-lactams were found to be superior to sulfonamides for the treatment of infection with gram-positive organisms [42, 70]. Penicillins were more effective, even when sulfonamide-susceptible strains were present. As penicillinase-producing strains became prevalent, penicillinase-resistant penicillins and cephalosporins were used to treat *S. aureus* infection. With the emergence of MRSA, vancomycin became the drug of first treatment choice, with TMP-SMZ as an oral alternative. The value of TMP-SMZ for MRSA infection takes on added dimensions because the use of vancomycin for treatment of MRSA is now a topic of spirited debate [28, 31].

**ANIMAL MODELS**

An overview of animal models supports the possibility that folate-antagonist failures result from the presence of pus, tissue damage, and relative load of *S. aureus*. In the murine peritonitis model, TMP-SMZ was successful when treatment started shortly after challenge (1, 3, and 5 h) and before the devel-
CLINICAL TRIALS OF SULFONAMIDES

Consideration of sulfonamide efficacy is pertinent when evaluating TMP-SMZ efficacy, because thymidine from pus or high bacterial burden would reduce the efficacy of both sulfonamides and TMP-SMZ. Data from 239 patients treated with sulfonamides are summarized in table 1. Torrey et al. [71] reviewed the data for 1933–1941 on the outcomes of patients with bacteremia. Of 128 patients, 45 (35%) died. This mortality rate is optimistic, because many cases were based on a single blood culture, suggesting that some of the successes may have been due to false-positive cultures. Considerably higher mortality rates were found in another 6 studies with more rigorous criteria (57 [51%] of 111 patients) (table 1). Overall, high rates of death (43%) and treatment failure (51%) were found in patients with serious S. aureus infection treated with sulfonamides.

The majority of favorable outcomes for serious infection occurred in children with bacteremic osteomyelitis [42, 66–68, 73, 74]. Clinical efficacy was also found with infections of the head, which would be best classified as severe cellulitis and/or abscesses that were drained [60–65]. Favorable outcomes also occurred with staphylococcal pneumonia [70]. In contrast, failures were seen when abscesses were present and with high-grade S. aureus bacteremia [71, 72, 90]. In a study of 8 patients with severe S. aureus infection treated with sulfapyridine [61], 6 of 8 patients died (4 of 6 patients with pneumonia, 1 patient with endocarditis, and 1 patient with bacteremia alone). Two survivors had pneumonia without bacteremia. One of the surviving patients with pneumonia developed a lung abscess despite 7 days of treatment with sulfapyridine. In another large series, overall mortality among patients infected with S. aureus strains that were susceptible to sulfonamides was 34%, whereas mortality for strains resistant to sulfonamides was 36% [42]. The failure to show efficacy on the basis of susceptibilities suggests that the drugs were not active in vivo. Finally, in a series of 38 cases, treatment with sulfamethoxazole was successful when the patients had cellulitis alone but failed when abscess was present [60].

CLINICAL TRIALS OF TMP-SMZ

Failure rates for TMP-SMZ (50%) are nearly identical to those for sulfonamides (51%), but the mortality rate is lower for TMP-SMZ (table 1). The efficacy of TMP-SMZ for serious infections was summarized by Markowitz et al. [47] for reports during 1972–1991 and represents 174 cases, but insufficient detail in these reports made evaluation of TMP-SMZ efficacy impossible. A total of 221 cases from 6 series [23, 47, 86–88] were included for analysis.

There is, to our knowledge, only 1 randomized, prospective trial comparing TMP-SMZ with another drug—specifically, vancomycin [47]. Patients were injection drug users, most of them had endovascular infection, and 47% of the S. aureus isolates from these patients were MRSA. Vancomycin was superior to TMP-SMZ in terms of the duration of bacteremia (6.7 vs. 4.3 days), sterilization of wound culture (5.8 vs. 3.8 days), duration of fever, and failure rates (6 of 43 with TMP-SMZ vs. 1 of 58 with vancomycin; P<.02). There was a strong trend toward better outcome of tricuspid valve endocarditis in favor of vancomycin (11 of 12 vs. 7 of 11 cures). Notably absent in this series of patients were patients with a large collection of pus and left-sided endocarditis, which is more severe and difficult to treat. There was a high failure rate in treatment of lung abscesses, which is of concern because most lung abscesses clear with appropriate therapy [91], and the comparator drug was vancomycin, which is less effective than cloxacillin in treat-
of patients with MSSA pneumonia and bacteremia (8 of 17 deaths with vancomycin vs. 0 of 10 deaths with cloxacillin) [29, 92]. Similarly, when examining vancomycin efficacy for staphylococcal endocarditis in patients and experimental models [, 83, 93–96], vancomycin is consistently and significantly less effective than β-lactam antibiotics. In anecdotal reports, 4 cases of MRSA endocarditis, including 1 with meningitis, were successfully treated with TMP-SMZ [97–100]. Although these single cases demonstrate that some infections can respond to TMP-SMZ, there is concern about the use of TMP-SMZ, because it is less rapidly bactericidal than is β-lactams or vancomycin [33], which is felt to be an important consideration in the treatment of endocarditis. Thus, even if TMP-SMZ were equivalent to vancomycin, these drugs are significantly less effective than β-lactams, thereby reducing enthusiasm for TMP-SMZ for treatment of S. aureus endovascular infection, unless extraordinary circumstances prevail. Another description of successful therapy with TMP-SMZ for a variety of S. aureus infections was reported in a letter [101]. The patients received multiple other drugs, such as aminoglycosides, β-lactams, and rifampin. Although 25 of 26 patients with MRSA recovered (1 death was caused by an TMP-SMZ-resistant strain), it was not reported which patients received which drugs, how many had surgery, how long the patients were treated, and what length of time patients were followed after the completion of therapy. The lack of detail does not allow one to define the efficacy of TMP-SMZ.

Other recent studies have reported treatment of skin and soft-tissue infection (SSTI) with TMP-SMZ. With a dramatic and significant (P < .001) increase of MRSA as a cause of SSTI in the ambulatory clinical setting in Boston from 1998 to 2005 (from 0% of cases in 1998 to 77% of cases in 2005), the success rate for TMP-SMZ was evaluated via a retrospective chart review [23]. Patients receiving treatment with TMP-SMZ, rather than with antibiotics without activity against CA-MRSA, had a better outcome. By 2005, the 60% clinical resolution rate was similar for MRSA and MSSA. Some of the success probably relates to high rate of incision and drainage, increasing from 0% in 1998 to 45.1% in 2005 for all infections and to 56.1% for MRSA infection, but the trend did not reach significance (OR, 1.28; 95% CI, 0.71–2.31). Others have emphasized the importance of abscess drainage for obtaining successful outcome for SSTI caused by CA-MRSA [16, 18, 21]. In contrast, the fact that treatment with antibiotics with no activity against MRSA (e.g., β-lactam antibiotics) resulted in cure rates >90% for mild SSTI [102] suggests that antibiotic treatment is not necessary for mild SSTI. Although the largest number of patients receiving TMP-SMZ treatment for S. aureus–related SSTI came from a single clinic and could bias the overall outcome data [23], the results may be most pertinent for the current outbreak of CA-MRSA. On the other hand, these less serious infections may underestimate the mortality rate for serious infection when TMP-SMZ treatment was used.

Stein et al. [88] treated orthopedic device–associated infection in 39 patients with TMP-SMZ and late device removal (for hips and knees, 6 months) unless joint instability occurred earlier. Patients then received another 3 months of therapy, for a total of 9 months of therapy. Twenty-four patients had MRSA infection. A deficiency in the study was that 15 (60%) of MRSA cases were isolated from fistula tracks, whereas only 9 had biopsy or puncture specimens. Three patients experienced treatment failure because of the emergence of resistance while undergoing therapy, suggesting that antibiotic resistance was a relatively minor reason for treatment failure among the 39 patients in the study. Of note, 8 (21%) of 39 patients stopped the treatment because of adverse effects from TMP-SMZ. With device removal and treatment with TMP-SMZ for MRSA, treatment for 1 of 7 patients failed, whereas treatment for 7 of 17 patients failed with TMP-SMZ treatment alone. A single case of vancomycin-resistant S. aureus peritoneal dialysis catheter infection responded to catheter removal and TMP-SMZ plus rifampin therapy [89].

THYMIDINE-AUXOTROPHIC SMALL COLONY VARIANTS (SCVS)

One of the complications of long-term use of TMP-SMZ is the development of thymidine-auxotrophic SCVs [103, 104]. Staphylococcal SCVs are more resistant to host cationic peptides [105, 106], other host defenses because of their intracellular location [104], and multiple antibiotics [104]. In addition, SCVs are better able to survive under starvation conditions [107]. In clinical studies of patients with cystic fibrosis, presence of staphylococcal SCVs are common [103, 108, 109] and are associated with more-progressive disease [103, 108, 109]. Because staphylococcal SCVs have been found in situations in which TMP-SMZ treatment has been recommended (e.g., osteomyelitis [110], prosthetic device infection [111], and SSTI [104, 112]), the development of SCVs would be an unwelcome adverse effect, because these organisms are difficult to treat [104].

CONCLUSIONS

There are many reports about the use of sulfonamides and TMP-SMZ for treatment of S. aureus infection, but there is only very limited information from controlled clinical trials. Although TMP-SMZ is bactericidal, the treatment failure rate with TMP-SMZ was equivalent to that with sulfonamides, which are bacteriostatic. Because thymidine release may limit the efficacy of folate antagonists, key issues involve what abscess size, what organism burden, and how much tissue damage will predict failure with TMP-SMZ treatment. A review by Adra and Lawrence [19] emphasizes TMP-SMZ efficacy with a low
Table 1. Summary of cases where folate antagonists alone were used to treat *Staphylococcus aureus* infection.

<table>
<thead>
<tr>
<th>Reference and no. of cases treated</th>
<th>Presence of pus or high numbers of organisms</th>
<th>Other therapy, including surgical drainage</th>
<th>No. of treatment failures (deaths)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>With sulfonamide</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Torrey et al. [71]a</td>
<td>Cases of bacteremia, 1933–1941</td>
<td>Many had surgery</td>
<td>45 (45)</td>
</tr>
<tr>
<td>128 Cases of bacteremia, 1933–1941</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>62 Cases of bacteremia, as well as deep infections</td>
<td>Not reported</td>
<td>42 (42)</td>
<td></td>
</tr>
<tr>
<td>Spink et al. [42]</td>
<td>13 Cases of bacteremia, 7 osteomyelitis, 13 with obstructive uropathy/renal abscess, 2 empyema, 1 meningitis, 1 pyoderma</td>
<td>32% Cured only after surgery; 8% cured after penicillin; 1 death after surgery</td>
<td>29 (12)</td>
</tr>
<tr>
<td>38</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Finland et al. [85]</td>
<td>Cases of bacteremia (1 pyonephrosis and 3 cases of osteomyelitis)</td>
<td>4 Cured only after surgery</td>
<td>4 (1)</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
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<tr>
<td>Norman [67]</td>
<td>Bacteremia with metastatic abscess developing while receiving therapy</td>
<td>Improved only after surgery</td>
<td>1 (0)</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Vivino and Spink [72]</td>
<td>Bacteremia</td>
<td>1 Patient cured only after surgery</td>
<td>1 (1)</td>
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<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grulee and Mason [63]</td>
<td>Scalp furuncles in children</td>
<td>Improved only after surgery</td>
<td>1 (1)</td>
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<tr>
<td>3</td>
<td></td>
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<tr>
<td><strong>Total, 239</strong></td>
<td></td>
<td></td>
<td>123 (102)b</td>
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<td></td>
</tr>
<tr>
<td><strong>With TMP-SMZ</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reviewed in Markowitz et al. [47]c</td>
<td>Multiple types of infections (1972–1991) with osteomyelitis, meningitis, and bacteremia; in addition to TMP-SMZ, patients received other antibiotics</td>
<td>Insufficient detail and treatment with multiple antibiotics make it impossible to define TMP-SMZ efficacy unambiguously</td>
<td>26 (2)</td>
</tr>
<tr>
<td>174 Infections with limited clinical information</td>
<td>...</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>Reference</td>
<td>Description</td>
<td>Outcome</td>
<td></td>
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<td>--------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------------------------</td>
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<tr>
<td>Markowitz et al. [47]</td>
<td>Mix of MRSA and MSSA in 101 patients (65% bacteremic), tricuspid valve endocarditis, as well as multiple other infections</td>
<td>Vancomycin superior to TMP-SMZ (P &lt; .02) 6; 4 of 11 with MSSA endocarditis*</td>
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</tr>
<tr>
<td>Yeldandi et al. [86]</td>
<td>Osteomyelitis</td>
<td>...</td>
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<tr>
<td>Honda et al. [87]</td>
<td>MRSA bronchiectasis with initial improvement</td>
<td>Initial treatment with other antibiotics 1 (1)</td>
<td></td>
</tr>
<tr>
<td>Stein et al. [88]</td>
<td>Prosthetic joint infections (24 MRSA; 15 coagulase-negative staphylococci)</td>
<td>No device removal in 17 of 28; TMP-SMZ alone cure in 10 of 17 with MRSA 1 With removal; 7 without removal</td>
<td></td>
</tr>
<tr>
<td>Smith et al. [89]</td>
<td>MRSA on a peritoneal dialysis catheter</td>
<td>Catheter removed 0</td>
<td></td>
</tr>
<tr>
<td>Moran et al. [18]</td>
<td>320 purulent infections (i.e., no cellulitis)</td>
<td>Good outcome was not associated with active drug, rather with surgery: 19% with surgery alone; 10% antibiotic treatment alone; 66% both; 5% neither Not able to calculate</td>
<td></td>
</tr>
<tr>
<td>Szumowski [23]</td>
<td>MRSA; skin and soft tissue infections; 12, 77, and 126 new and recurrent cases in 2003, 2004, and 2005, respectively (from figure 1)</td>
<td>45.1% Had surgery in 2005 94 60%, 50%, and 40% failure in 2003, 2004, and 2005, respectively^</td>
<td></td>
</tr>
</tbody>
</table>

Total, 221 Total from references 23, 47, and 87–89 ... 110^  

**NOTE.** MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *S. aureus*; TMP-SMZ, trimethoprim-sulfamethoxazole.

* In 1941, Torrey et al. [71] reviewed 128 cases of bacteremia treated with sulfonamides, and their summary of these cases is included here.

^ Treatment failure rate for all patients who received sulfonamide, 51%; death rate, 43%.

^ In 1992, Markowitz et al. [47] summarized the outcomes of 174 patients treated with TMP-SMZ, and these cases are included in the table.

^ No. of deaths, unknown.

^ From figure 3 in reference 23.

^ Treatment failure rate for all patients who received TMP-SMZ, 50%.
bacterial burden but failures with a high bacteria burden, which is very similar to the conclusions reached about sulfonamides [40, 42]. A confounding variable in assessing the literature also comes from the development of imaging technology that identifies small abscesses that were hitherto undiscovered. When pneumatoceles are found in the lung, a thickened wall that does not collapse in persistent pneumatoceles predicts antibotic failure [91]. In general, lung abscesses are much more likely to spontaneously drain, making surgery less important.

Clearly, additional clinical trials, as well as animal models, are needed to evaluate the effect of pus, tissue damage, organism burden, and the value of rifampin on the efficacy of TMP-SMZ. In addition, any clinical trial or animal model will need to consider the dosing of vancomycin if it is used as a comparator drug [113]. Entrance of cefotibprole, an anti-MRSA β-lactam [114], will bring another variable to the therapeutic equation, because β-lactams have consistently outperformed folate antagonists. Therefore, before any enthusiastic recommendation can be made for the use of TMP-SMZ for MRSA infection, we will need considerably more animal model and clinical trial data.

Acknowledgments

Potential conflicts of interest. R.A.P. has served on the speakers’ bureaus for Aventis, Bristol-Myers Squibb, and Pfizer; owns stocks in mutual funds that contain multiple pharmaceutical and biotech companies, with stock in all of the companies above, as well as Lily, Pfizer, and Wyeth-Arysta, to name but a few of the >50 companies; and has served as a consultant for Arrow, BioSynexus, Conjugon, and MicroPhage.

Financial support. American Heart Association, National Institutes of Health, and US Department of Agriculture; industrial support: BioSynexus, Kimberly-Clark, and Cubist.

References


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