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Perspective

Quo Vadis, Pulmonary Tuberculosis?

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Abstract

Tuberculosis (TB), is mainly caused by the ancient stealth pathogen, *Mycobacterium tuberculosis*, and remains among the top ten leading causes of death worldwide. Downward disease trajectories in the US and Europe, have been juxtaposed with high disease burdens in other countries. Current diagnostic tests are inadequate to differentiate between a third of the global population infected with latent TB and those destined to develop active TB i.e. who may be asymptomatic or have subclinical forms of the disease. In addition to a seemingly endless repertoire of antibiotic resistance mechanisms, transiently tolerant *M. tuberculosis* subpopulations called persisters can survive treatment. In this article, I synthesize relevant preclinical and clinicals studies, as well as review articles, detailing the past, present, and future of TB research. A proposal is made to tailor management and prevention strategies, taking into account knowledge regarding treatment limitations and the availability of new management options.

Keywords: *Tuberculosis; Persister; Directly Observed Short Course Treatment; Antibiotics; Vaccines*

Abbreviations

BCG: Bacillus Calmette–Guérin; CDC: Centers for Disease Control and Prevention; HIV: Human Immunodeficiency Virus; MDR: Multidrug Resistant; MMP: Matrix Metalloproteinase; MNK: MAP Kinase-Interacting Kinase; MTB/RIF: *Mycobacterium Tuberculosis*/Rifampicin; PI3K/AKT/mTORC1: Phosphatidylinositol-3-Kinase/Serine/Threonine Protein Kinase/Mammalian Target of Rapamycin Complex 1; TB: Tuberculosis; US: United States; XDR: Extensively Drug Resistant; USPSTF: United States Preventive Services Task Force

Introduction

Tuberculosis (TB)–mainly a fatal disease of antiquity–persists today largely because the predominant causative pathogen, *Mycobacterium tuberculosis*, has coevolved with modern humans to outwit their best defenses. This important contagious disease continued to make more than 10 million people ill worldwide and killed almost 2 million people in 2015 (including at least 400,000 with HIV and TB) [1], primarily in low- and middle-income countries. While one-third of individuals across the globe are infected with latent tubercle bacilli, six countries account for 60% of the total reported number of active cases (India, Indonesia, China, Nigeria, Pakistan and South Africa) [2]. Moreover, since the sputa of young children are usually smear-negative, these approximations may under-estimate the true burden of the disease in these countries [3].

The story of TB in the United States (US) and Europe is a far rosier one, but that does not mean that the number one infectious killer has been eliminated from this hemisphere. Known by various names e.g., "white plague, galloping consumption, phthisis," TB has been described by a "Who's Who" list of philosophers, poets, and scientists across the centuries ranging from Hippocrates to Selman Waksman. In the era of Hippocrates, fresh air, good food, and exercise formed the main components of treatment. Morbidity and mortality rates continued to soar over the ensuing centuries (one in seven human beings were estimated to have succumbed due to the disease during the 1800s and 1900s) [4], until Robert Koch's discovery of tubercle bacilli paved the way for successful chemotherapy [5], notably Selman Waksman's streptomycin and other antimycobacterial agents. Today, the declining TB trends (< 20 cases per 100 000) in low-burden,

high-income countries such the United States and United Kingdom reflect improved TB control practices [6]. Although one-third of the world's population is infected with TB, the USA reported only 9,557 cases in 2015 (a slight increase compared to 2014). Foreign-born individuals, members of ethnic minority groups, and patients with HIV were disproportionately affected by the illness [7].

Lack of access to affordable and effective TB control measures, treatment non-adherence, overuse and under-use of antibiotics are among the reasons most often cited for the geographically skewed epidemiology of the disease and the rise in drug-resistant bacteria.

According to the World Health Organization (WHO), multidrug-resistant bacilli (MDR-TB) are defined by growth in the presence of isoniazid (INH) and rifampin (RIF), with or without the presence of other first-line drugs. Based on 2004 estimates, 3% of newly diagnosed patients were estimated to have MDR-TB. Incident cases of MDR-TB were forecasted to rise even further in the high-burden countries without adequate interventions (India, the Philippines, Russia, and South Africa) [8].

Resistance to at least isoniazid and rifampicin, and to any fluoroquinolone, and to any of the three second-line injectables (amikacin, capreomycin, and kanamycin) are features of extensively drug-resistant TB (XDR-TB) [9]. Despite improvements in the management of drug resistance, the percentage of XDR-TB among incident MDR-These TB cases will likely also increase in the high-burden countries [8]. Based on 2009 estimates, a cohort of 15 patients in Iran was reported to be resistant to all anti-TB agents [9].

Diagnosis

Most of the bacteria are killed or inhibited by alveolar macrophages following inhalation, but a few bacilli may survive to multiply intracellularly and can be disseminated to distant organs and tissues. The fact is that the stealth pathogen can lurk in any organ of the body, including the lymph nodes, peritoneum, urinary tract, brain, heart, bones and joints. Infections of the inner lining of the abdomen and the sac around the heart are rare, but can be lethal. Bacteria can infiltrate the meninges, especially in babies and young children, while bone infections have been observed in the elderly [10]; however, the disease is primarily spread as pulmonary TB i.e., bacilli are spread in the air from person to person following a cough or sneeze.

Pulmonary symptoms (coughing up blood, and chest pain) that accompany clinical signs such as unexplained weight loss, loss of appetite, night sweats, fever, and fatigue are reasons to suspect a case of active TB. Demographic factors (e.g., race, nationality, occupation), a positive response to the Mantoux tuberculin skin test, and a chest radiograph showing variable lesions may also be suggestive of the disease. According to Gadkowski., *et al*. [11], "*M. tuberculosis* generally has the highest prevalence of cavities among persons with pulmonary disease of any infection, probably because this pathogen causes extensive caseous necrosis." This disease subtype can be visualized as enlarged air pockets in the upper, highly oxygenated lobes of the lung. When present in excess, tissue-remodeling enzymes known as matrix metalloproteinases (MMPs), particularly MMP-1, can destroy lung tissue. A recent study showed that *M. tuberculosis* could globally suppress signaling that normally limits MMP-1 production e.g., the MAP kinase-interacting kinase (MNK) and PI3K/AKT/mTORC1 pathways, to release too much MMP-1, resulting in lung tissue destruction [12]. Cavities formed via aberrant pathways typically contain large numbers of bacteria that can be efficiently propagated to another susceptible host.

The destructive and contagious nature of cavitary pulmonary TB, which represents a later stage of the disease, adds to the imperative to facilitate early and accurate diagnosis in order to engage in curative therapy. To aid this strategy, definitive tests are needed in select individuals to rule out other diseases as sarcoidosis and community-acquired pneumonia [13]. Pathologic, molecular, or cytological findings consistent with the presence of acid-fast bacilli in the sputa or other aspirates from the patient will usually prompt the initiation of treatment. Clinicians continue to rely on the identification of *M. tuberculosis* in the sputum of an infected patient as a definitive diagnostic test. Because of its viscous nature, such samples inevitably require further processing, adding to the cost of TB management. Other tests based on blood, urine, or breath, lack the sensitivity and specificity of standard-of-care tests. Moreover, the detection of a large load of undiagnosed tuberculosis, subclinical tuberculosis, and tuberculosis comorbidity with HIV, among other diseases in autopsies has underscored the urgent need for better, rapid, point-of-care tests. Molecular tools such as nucleic acid amplification and cheaper next-

generation technologies can potentially fill this void. Examples of molecular tests include the MTB/RIF and Xpert MTB/RIF assays as well as the Genexpert system for the detection of *M. tuberculosis* DNA and RIF resistance in samples [14] however, in practice, it may be difficult to detect to make an accurate radiological, clinical, or molecular diagnoses in children. Children accounted for 1 million of the 10.4 million cases of active TB identified in 2015 [2].

The CDC recommends that drug susceptibility testing should be done on the initial isolate and on patients who do not respond adequately to therapy or have a positive culture despite three months of therapy [13]. It is worth noting that TB can reside within the body in a latent state, mainly as a result of being kept under control by the immune system of asymptomatic individuals in granulomas [15] Since the United States Preventive Services Task Force (USPSTF) issued a recommendation to screen high-risk individuals for latent TB (examples include individuals with diabetes or co-infected with HIV who have a 30% risk or a risk of up to 10% per year, respectively, of contracting active TB without appropriate interventions) [15], there has been a growing interest in the development of appropriate diagnostic tools; however this recommendation has to be weighed against the lifetime risk of developing TB following an infection i.e., 5% to 10%, in an otherwise healthy individual [16]. Moreover, infected individuals may exhibit a range of immune responses e.g., subclinical disease, asymptomatic persistent infections, or clearance as a result of mounting an extremely effective immune response. Therefore, immune signatures may need to be identified to categorize individuals as being at "low" or "high" risk for reactivation of latent TB or disease recurrence.

Treatment

Sustained political and financial commitment, diagnosis by quality ensured sputum-smear microscopy, standardized short-course anti-TB treatment given under direct and supportive observation (DOT), a regular, an uninterrupted supply of high quality anti-TB drugs, and standardized recording and reporting form the five cornerstones of the successful Directly Observed Treatment, Short Course (DOTS) strategy spearheaded by the WHO [17]. Between 1995 and 2009 the case identification increased from 46% to 63% in 127 national DOTS programs. Concomitant rates of treatment success for cases of smear-positive TB increased from increased from 57% in 1995 to 86% in 2008; [18] however, the global cost of more than $12 billion to treat this leading infectious cause of death has sparked a debate as to the merits of this strategy [19]. Up to 75% of the provider costs of TB are attributed to DOT (patients are required to complete treatment under supervision of an accredited healthcare provider). Moreover, the emergence of drug-resistant strains, and the worldwide case fatality rate of 17% have led to a call for more research and rigorous evaluation of approaches to guide tuberculosis public health policy.

The American Thoracic Society/Centers for Disease Control and Prevention/Infectious Diseases Society of America has attempted to partially fill this void with the 2016 clinical practice guidelines for the management of drug-susceptible tuberculosis. This set of recommendations is of value in regions with access to mycobacterial cultures, molecular and phenotypic drug susceptibility tests, and radiographic studies, among other diagnostic tools [20].

First line treatment of drug-sensitive TB owes much to history. In 1952, the discovery of isoniazid was described "the most potent drug introduced thus far and was inexpensive, well tolerated, and safe" [4]. Patients inevitably relapsed, necessitating the development of a 18- to 24-month long combination regimen comprised of isoniazid, "triple therapy," which included oral INH, para-amino-salicylic acid, and streptomycin. Although this was the standard of care for more than a decade, adverse effects and drug resistance, caused researchers to explore other agents. Isoniazid-based regimens containing the bacteriostatic drug, ethambutol (EMB; minimum inhibitory concentration [MIC] = 1 - 5 μg/mL) and RIF were found to lead to improved cure rates and shortened the treatment duration to 9 months. Pyrazinamide (PZA) was found to further reduce the treatment time to 6 months when combined with isoniazid and rifampin [4].

The ideal goals of modern anti-TB treatment remains curing the patient, eradicating persisting bacilli, and prevention of the acquisition of drug resistance [21]. Eleven drugs have been approved to date by the FDA for the treatment of TB. Initial or "intensive phase" treatment of drug-sensitive bacteria typically consists of mainly of 2-month regimen of the historically validated 4 drugs i.e., INH, RIF, EMB, and PZA. INH exerts early bactericidal activity against actively growing drug-sensitive strains (MIC = 0.02 - 0.20 μg/mL), but may

not have the same effect against slow-growing or dormant bacteria. This hepatotoxic agent should be monitored closely in older patients or those with liver disease. The same cautionary note holds true as the complexity of the regimen increases e.g., combination of isoniazid/rifampin with hydantoins, imidazoles, carbamazepine, azathioprine, or cyclosporine warrant close monitoring for the early detection of drug-induced liver injuries [22].

Rifampin (MIC = 0.05 - 0.50 μg/mL) kills metabolically active and stationary phase *M. tuberculosis* (reduced growth rate). Rifamycins such as rifampin taken together with PZA are thought to prevent post-treatment relapses [22]. The continuation phase may consist of 4 months on INH and RIF and other agents [21]. Mild adverse effects may be controlled with appropriate treatment, while severe adverse effects may require replacement of one or more drugs in the regimen with suitable alternatives.

Tailored drug combinations are also suggested for special situations e.g., HIV infection, extrapulmonary tuberculosis, culture-negative pulmonary tuberculosis, advanced age, children, tuberculosis during pregnancy and breastfeeding, renal disease, and hepatic disease. The continuation phase may be extended for HIV co-infections, in cases with a high risk for relapse, selected extrapulmonary sites of disease, namely tuberculous meningitis, and bone, joint, and spinal TB. Treatment of patients co-infected with HIV are particularly challenging because of interactions between rifamycins and antiretroviral therapies, potential for rifamycin resistance when following an intermittent strategy, and paradoxical reactions that may manifest as clinical decline. With rare exceptions, Nahib., *et al*. recommends that ART should usually be started within the first 2 weeks of TB management for patients with CD4 counts < 50 cells/μL and by 8 - 12 weeks of the initiation of TB management for patients with CD4 counts ≥ 50 cells/μL [21]. Because cotrimoxazole (trimethoprim-sulfamethoxazole) prophylaxis reduces morbidities and fatalities in patients infected with newly diagnosed TB and HIV, the WHO has recommended this agent for all HIV-infected people with active tuberculosis disease regardless of the CD4 cell count; however, this agent is primarily used in HIV-infected patients with CD4 cell counts < 200 cells/μL in high-income countries [21].

Apart from second-line drug resistance, extrapulmonary disease and pregnancy, the WHO has recently issued guidelines for the treatment of MDR-TB or TB resistant to rifampin. Treatment involves a standardized seven-drug regimen taken for a period of 9 to 12 months. This regimen can be taken by any patient irrespective of age or HIV status and is coupled with the need for patient-centered care and social support to ensure adherence. Authors of the guidelines anticipate that the regimen would be feasible in most settings and that costs would be lowered to < $1,000 per patient [23].

Bacterial Persistence: A Challenge to TB control

The genome size of the best-characterized strain of *M. tuberculosis*, H37Rv, is 4,411,529 base pairs [24]. Based on a novel program, approximately 570 of the 4,000 genes are considered essential for the growth of the bacterium in rich media. Some of these druggable protein domains, were chosen because of the uniqueness of their roles in the *M. tuberculosis* 'metabolome' (i.e., function of the target could not be compensated for by another target) [25]. By adopting large-scale comparative genomic methods, a broader picture can be obtained of drug resistance sites among different isolates. A description of drug targeting studies currently under way is beyond the scope of this article. Rather, the focus will be placed on bacterial subpopulations thought to be the major cause of chronic infections i.e. persisters.

Bacterial Persistence

Antibiotic-sensitive and -resistant bacteria can coexist in natural environments in the absence of or at low concentrations of the drugs. Bacteria have typically been thought to acquire resistance through mutations or acquisition of "resistance" genes from other microbes, enabling pathogens to thrive in the presence of therapeutic levels of antibiotics. Ubiquitous subpopulations cause antibiotics to fail and may be selected for following repeated doses of antibiotics. According to Fisher., *et al*. these "slow-growing or growth-arrested bacterial cells have a decreased susceptibility to killing by bactericidal antibiotics within an otherwise susceptible clonal population" [26]. In 1944, Joseph Bigger first coined the term "persisters" for 1% of the bacterial population not killed by penicillin, but able to regrow under appropriate conditions and remain sensitive to the same antibiotic.

Today, more than seven decades later, persisters are recognized to exist in a metabolic continuum (in the presence of stresses such as antibiotics and even in the absence of the drugs), ranging from viable, but non-culturable bacteria to slow-growing and non-growing cells that can interconvert *in vitro* and *in vivo* [27]. One example of an unstable persister cell is the "cell-wall-deficient" bacterium first cultivated by mycoplasma pioneer, Emmy Klieneberger-Nobel in 1935 [28]. Mycoplasma-like, osmosensitive variants of the Gram-negative bacterium, *Escherichia coli* (Doubling time[DT] ~ 20 minutes in rich media), have been purified *in vitro*, using, cell wall constituents and sucrose as an osmotic stabilizer [29], among other laboratory techniques.

L-form bacteria have also been induced experimentally in cryogenically stressed *M. tuberculosis* bacilli (DT of parental strain ~15 to 20 hours) treated with ethambutol (EMB), which targets the mycobacterial cell wall through interaction with arabinosyl transferases. Slavchev., et al. were able to show that these variants could form additional structures, including biofilm-like formations [30]. In addition, oxidative stress or anaerobic conditions are known to promote L-form growth in laboratory strains. The presence of these variants in macrophages and other cells in animal models and tissue culture [31], suggests that L-forms are also present in humans, but the roles in virulence and persistence await further exploration.

Alternative mechanisms for persister formation may involve the activation of toxin–antitoxin modules through (p)ppGpp alarmone signaling. How this pathway or any other putative mechanism facilitated by switching environmental conditions e.g., a diauxic shift, would facilitate persister formation in a Gram-positive bacterium such as *M. tuberculosis* is currently unknown. Target inactivity, lower metabolic flux, higher afflux of antibiotics, and low uptake of drugs are some of the properties associated with persisters. All bacteria are not created equal in terms of the ability to form persisters e.g., *Streptococcus pneumoniae* can readily be treated with a single antibiotic in a week or two, with usually no relapse. In contract, the typical 6-month treatment regimen required for *M. tuberculosis* to treat a chronic infection, renders the immune system incapable of ridding the body of residual persisters following treatment [27].

Regardless of the molecular mechanisms underlying persister formation, these variants are known to be drug-tolerant and therefore represent major challenges to TB control. How can persisters be eradicated? The development of novel small molecules validated in the clinic that can degrade essential proteins is one option. Another strategy is to coerce persisters into reinitiating growth so that the variants can then be killed by the existing repertoire of antibiotics. Interestingly, one preclinical study showed that the addition of reactive oxygen species scavengers enhanced the sterilizing activity of INH against *M. tuberculosis*. Additionally, enhancing the respiration of *M. tuberculosis in vitro*, prevented the formation of both drug resistance and persister cells, thereby causing bacterial cell death [32].

New treatments kill TB

About 60% of current and candidate anti-TB drugs target cell wall biosynthesis [25]. Because these drugs are likely to be more active against actively growing bacteria, there is a need to find other drug targets that can shorten TB treatment and eradicate persisters.

Microbial consortia from soils and oceans continue to provide a treasure trove of antibiotics against actively replicating bacteria, and sansamycin analogues are no exception. Analogs of sansanmycin uridylpeptide natural products i.e. soil bacteria compounds, were found to exert antimycobacterial activities by inhibiting an enzyme involved in making lipid 1, a building block of the cell wall [33]. Another bacterium known to have swapped one ecological niche i.e., soil, for another i.e. the lungs, is *Burkholderia gladioli*. One species isolated from the sputum of a child with cystic fibrosis, was shown to produce an antibiotic, gladiolin, which killed four drug-resistant TB strains *in vitro* [34]. In a separate study, a research group from the University of Central Florida has recently identified cytotoxic metabolites known as puupehenone-like molecules from marine natural sources that selectively killed dormant *M. tuberculosis* in the laboratory [35].

While these results suggest that judicious biomining of nature can extend the antibiotic pipeline, the long road to the clinic still involves establishment of pharmacokinetic and pharmacodynamic parameters that would result in a safe and effective dose-exposure-response relationship in children and adults infected only with TB, or with more pathogens such as HIV. One drug that has successfully navigated clinical and regulatory hurdles is bedaquiline, an inhibitor of the proton pump of mycobacterial ATP synthase, which became

the first drug in more than four decades to be approved by the US Food and Drug Administration against resistant TB [36]. Another drug, delamanid, has also shown encouraging clinical trials against MDR-TB and XDR-TB in clinical trials [37,38].

Alternative strategies include repurposing drugs such as linezolid and meropenem for the treatment of drug-sensitive and resistant pulmonary TB [39]. Linezolid, an oxazolidinone antibiotics, is typically used to manage XDR-TB, but is associated with a high frequency of side effects (up to 59%) [4]. In addition, The Rapid Evaluation of Moxifloxacin in Tuberculosis (REMoxTB) consortium and other groups have conducted studies on the merits of shortening the TB regimen. The trials designed to test whether a reduced treatment duration was feasible, mostly demonstrated that the experimental intervention was non-inferior to the standard of care and highlighted the need to incorporate a triage process to enable accelerated assessments of short-term treatment in terms of risks and benefits [39].

Are there new vaccines on the horizon?

Bacillus Calmette–Guérin (BCG), discovered in 1908 [40], is a TB vaccine not widely used in the USA. Instead, it is used to prevent disseminated forms of the disease in infants from high-burden countries, where it provides only variable protection in later life [41]. Because the response is not sustained, lasting only up to a decade based on one estimate, efforts have been underway to improve, boost, or replace this shot. Next-generation recombinant BCG vaccines have been developed to induce a strong T-cell response, in keeping with the knowledge that the BCG vaccine induces CD4 T cells or "memory T cells." Initially, it was thought that invoking a strong expression of Th1 cytokines produced by CD4 or CD8 T cells e.g., Interferon γ, Tumor Necrosis Factor α, and Interleukin-2 would be sufficient for new vaccine candidates to outperform the BCG vaccine, but it is likely that other biomarkers would be needed for this purpose in clinical trials.

The rationale for the development of the current roster of more than 15 vaccine candidates is simple i.e., improve, boost, or replace the BCG vaccine; however, the fact that the bacterium can "hide" from T-cell mediated immune responses and that the disease manifests in different forms at different ages, complicate the search for preventive or therapeutic inoculations. Currently, several vaccines are undergoing Phase 1, 2, or 3 evaluations, with disappointing results reported from individual studies. Many reasons have been cited for the failure of a modified Vaccinia virus Ankara expressing the Ag85A antigen to provide significant additional protection to children or HIV-positive adults who had received BCG shots as children; one reason offered was that the BCG vaccine had already been effective in eliminating *M. tuberculosis* in the study population, thus obviating the need for a second shot.

Thus far, SRL172, an inactivated whole-cell booster derived from a non-tuberculous mycobacterium, is the only new TB shot to have demonstrated efficacy in a Phase 3 trial. Recently, a DAR-109 inactivated vaccine derived from the non-pathogenic SRL172 cells, was shown to be safe and effective in healthy volunteers. In a preclinical study, a DAR-109 booster provided better protection against a TB challenge compared with a BCG booster. A larger "prevention of infection" Phase 2b randomized, controlled, trial is currently underway to among adolescents in Tanzania.

Other strategies have been proposed to completely eradicate TB in low-burden countries by developing vaccines against latent TB infections and to accelerate a drug-treatment-induced cure. The last option would be of value in patients infected with drug-resistant organisms [41]. According to Dockrell, ultimately pre-exposure, post-exposure, and therapeutic immunizations may be needed, either alone, or in combination with antibiotics or immunotherapy to address the multiple facets of a complex organism and manifestations of the disease in different geographical regions over human lifespans [40].

Discussion

Tracing the co-evolution of the primary causative agent of tuberculosis, *M. tuberculosis*, and humans has revealed the way the disease spreads from person to person. The successful contagious nature of the disease relies on the establishment of pulmonary TB i.e., phagocytosis and intracellular growth of the bacteria, the latent contained phase of infection and finally the active lung infection. Immunocompromised patients are among the high-risk groups likely to be prone to the disease, with a curative therapies of drug-susceptible TB

relying on at least 6 months of daily treatment with multiple antibiotics. The resurgence of this disease in certain geographical regions, particularly, in MDR-TB and XDR-TB variants, show that initial successes in the management of this disease cannot be taken for granted.

While increasing diagnostic and treatment rates with more efficacious tools and public health strategies are important goals, it may also be worth thinking about ways to prevent or control the infections in the first place. Lifestyle alterations like staying at home during the first few weeks of treatment for active TB, adequately ventilating the room (rather than using recirculated air), covering the face when coughing or sneezing, or wearing a surgical mask may lessen the risk of transmission. When tailoring a TB strategy to any region, particularly in countries with high burdens of MDR-TB and XDR-TB, it is worth considering the approach within the context of better antibiotic stewardship at each institution, to avoid adding *M. tuberculosis* to the list of hospital- or community-acquired infections.

Conclusion

This qualitative exploration of the past, present, and future of TB management has highlighted the positive impact of multifaceted strategies centered around a standardized drug regimen for reducing the burden of the contagion, *M. tuberculosis*, around the world. By breaking down the silos that exist among various healthcare stakeholders, the perspective outlined in this article could be harnessed in multifaceted strategies against an ever-evolving stealth pathogen.

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Conflicts of Interest

The author has no conflicts of interest to declare.

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