MAJOR ARTICLE

A Systematic Review of *Borrelia burgdorferi* Morphologic Variants Does Not Support a Role in Chronic Lyme Disease

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Background. Much of the controversy that surrounds Lyme disease pertains to whether it produces prolonged, treatment-refractory infection, usually referred to as chronic Lyme disease. Some have proposed that round morphologic variants of *Borrelia burgdorferi*, known variably as "cyst forms" and "L-forms," are responsible for the pathogenesis of chronic Lyme disease. We have undertaken a systematic review of the literature to determine if there is a documented role of these variants in Lyme disease pathogenesis or in syndromes compatible with chronic Lyme disease.

Methods. Two systematic literature searches were performed to identify studies in which round morphologic variants of *B. burgdorferi* have been described in situ in human specimens.

Results. Our primary literature search identified 6 studies that reported round morphologic variants of *B. burgdorferi* in specimens obtained from 32 total patients. No study described these forms in patients who had purely subjective symptom complexes (eg, fatigue or pain). No study investigated a causal relationship between morphologic variants and clinical disease or evaluated treatment of morphologic variants in vivo. Of 29 additional studies that described the morphology of *B. burgdorferi* from patients with Lyme disease, the organism was invariably described as having spirochetal morphology.

Conclusions. In the context of the broader medical literature, it is not currently possible to ascribe a pathogenic role to morphologic variants of *B. burgdorferi* in either typical manifestations of Lyme disease or in other chronic disease states that are often labeled chronic Lyme disease. There is no clinical literature to justify specific treatment of *B. burgdorferi* morphologic variants.

Keywords. *Borrelia*; Lyme disease; cyst; L-form; spheroplast.

Lyme disease, which is caused by the tick-borne spirochete *Borrelia burgdorferi* sensu lato, is by far the most common vector-borne infectious disease in the temperate northern hemisphere. Many aspects of the pathogenesis, clinical manifestations, appropriate treatment, and outcomes of Lyme disease are well-accepted by the mainstream medical and scientific communities. There is considerable controversy, at least in the public discourse, about "chronic Lyme disease." This is a largely undefined term that is applied by a small minority of practicing clinicians to patients with a wide variety of presenting symptoms. Moreover, the diagnosis is not contingent upon laboratory evidence of *B. burgdorferi* infection. Most often such patients lack the objective clinical findings that are most closely associated with Lyme disease [1–10]. In contrast to authentic infection with *B. burgdorferi*, a diagnosis of chronic Lyme disease is often given to patients who either have alternative medical diagnoses or who have syndromes of prolonged, unexplained subjective complaints such as fatigue, pain, and/or cognitive dysfunction [11, 12]. Two central assumptions accompany this diagnosis: Such syndromes are caused by chronic, cryptic infection with *B. burgdorferi*; and *B. burgdorferi* assumes a

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fastidious biology in these infections that necessitates prolonged antibiotic therapy.

Advocates for greater recognition of chronic Lyme disease have presented a number of arguments meant to validate the biological plausibility of this concept. Perhaps the most commonly voiced theory contends that morphologic variants of the *B. burgdorferi* spirochete, known variably in the medical literature and lay Internet content as "L-forms," "cyst forms," "spheroplasts," "morphologic variants," "propagules," "round bodies," and "cell wall–deficient forms," are responsible for chronic Lyme disease [13–16]. In fact, articles about morphologic variants of *B. burgdorferi* constituted more than 10% of 176 publications submitted to contest practice guidelines for Lyme disease from the Infectious Diseases Society of America [17, 18]. In some cases, patients with a diagnosis of chronic Lyme disease have been treated with antibiotics believed to be selectively active against these morphologic forms, such as metronidazole and tinidazole [19, 20].

The terminology around morphologic variants of *B. burgdorferi* has proved confusing (Table 1). The commonly used terms "cyst" and "cystic" are often used colloquially to describe round morphologies of *B. burgdorferi* when seen microscopically. In microbiologically strict terms, there is no true encystment performed by this organism as is the case among a few bacterial genera, such as *Azotobacter*, *Azospirillium*, and *Rhodospirillium*. As this has become recognized, less specific descriptors such as "round bodies" have come into more common use regarding chronic Lyme disease.

We have undertaken a systematic review of the medical and the scientific literature to evaluate whether these morphologic variants of *B. burgdorferi* play a role in human Lyme disease, whether they have been associated with illnesses compatible with "chronic Lyme disease," and whether there is evidence to support antibiotic choices meant to eradicate these morphologic variants.

METHODS

Searches of the medical literature were designed to examine the evidence that "cystic" morphologic variants of *Borrelia burgdorferi* are associated with any specific form of human disease.

We performed a Boolean search of Medline (via PubMed), Embase (via OvidSP), and Thomson Reuters (formerly ISI) Web of Knowledge for studies of *B. burgdorferi* morphologic variants and their role in the microbial pathogenesis or natural history of Lyme disease. Two searches were performed. The first was intended to identify articles specifically reporting the presence of morphologic variants of *B. burgdorferi* identified in situ in human specimens. The second search was intended to evaluate more generally the description of *B. burgdorferi* in specimens from human patients with established Lyme disease.

Table 1. Terminology That Has Been Used to Describe Morphologic Variants of *Borrelia burgdorferi* and Conventional Definitions of the Terms Used

Term	Description
L-form	Bacteria with phenotypic deficiency of the rigid cell wall, usually described in the context of antibiotic exposure, noxious growth conditions, or genetic alteration. L-forms have been observed in many bacterial species, including *Borrelia burgdorferi* [21].
Alternative nomenclature	
Cell wall–deficient form	
L-variant	
L-phase	
L-organism	
Subtypes	
Stable L-forms	Cell wall alterations are permanent (ie, genetic). Stable L-forms cannot revert to parental "N-form."
Unstable L-forms	Cell wall alterations are temporarily induced by exposure to certain conditions. These include drugs (eg, penicillin). May revert to parental "N-form" once noxious conditions are removed.
Spheroplast	L-form in which some cell wall structure is retained. May be stable or unstable.
Protoplast	L-form in which no cell wall structure is retained. May be stable or unstable.
Cyst	In bacteriology, a differentiated structure that is resistant to desiccation or other noxious conditions. Cysts are characterized by a central body surrounded by a membrane-derived capsule [22, 23]. *Borrelia burgdorferi* is not known to produce cysts.
Propagule	Propagules refer to infectious "units" of material that transmit disease. These may be composed of a mix of microbial and host material.
Round, coccoid, globular, or spherical	Descriptive morphologic terms, not biologically defined.
Bleb	An irregular membrane bulge.

For the first search, our medical subject heading terms (for Medline), EMTREE terms (for Embase), and text (for others) were [(Borrelia OR Lyme) AND (cyst OR spheroplast OR "morphologic variant" OR "L-form" OR "cell wall-deficient" OR "cell wall-free" OR pleomorphic OR "round body" OR propagule)].

In addition, we reviewed the references contained in a bibliography of *B. burgdorferi* "round forms" maintained by a Lyme disease advocacy website [24]. This bibliography contained 63 references about *B. burgdorferi* and 199 references about other microorganisms, such as *Treponema pallidum*. We restricted our review to references specific for *B. burgdorferi*. A number of studies showing subcellular membrane structures, that is, "blebs," were listed in this bibliography but not retrieved in our database searches. Perusal of these articles showed that the term was mainly restricted to subcellular membrane defects observed on spirochetes, rather than ultrastructural changes in bacterial morphology. We excluded these articles because these were felt to not be synonymous with the bacterial morphologies relevant to this study.

An additional literature search was performed in Medline to identify studies describing the morphology of *B. burgdorferi* as seen in vivo in human infection. This search was performed because articles reporting morphologic variants might not actually be identified by morphology-based search terms. The additional search terms were [(Lyme OR borrelia) AND ("electron microscopy" OR "electron micrograph" OR autopsy OR histopathology OR biopsy)].

Articles were only included if they reported direct morphologic characterization of *B. burgdorferi* within a human tissue specimen. Articles (and results within articles) were excluded if they characterized morphology only after culture.

We searched the databases between inception and 10 May 2013. We also searched the reference list of each study, as well as those of relevant reviews, editorials, and correspondence that were returned in our database search. Case reports, case series, and scientific studies were included provided we could access full text in English. We excluded reviews, correspondences, expert opinions, editorials, meeting abstracts, poster presentations, and proceeding papers, as these sources lacked independent data or sufficient detail to assess the observations.

RESULTS

Search Results

Our first search yielded 57 results from Medline, 90 results from Embase, and 54 results from Thompson Reuters Web of Knowledge. From these databases 23, 26, and 20 references were selected, respectively, for further review based upon the parameters described above. After adding additional studies from the LymeInfo.net bibliography [24] and eliminating duplicates, a total of 41 studies were ultimately included in our review.

Among these 41 references were 9 relevant articles involving human subjects [15, 25–32]. In addition, there were 3 mouse studies, 28 studies done in vitro only, and 1 tick study. None of the mouse studies reported the identification of round morphologic forms of *B. burgdorferi* in vivo [33–35]. Two studies describing the effects of spirochete cultivation in ex vivo human tissue (cerebrospinal fluid and tonsillar tissue) were considered to be culture experiments rather than direct demonstration of the disease process in vivo [36, 37].

Round morphologic variants were reported in the findings of 6 of these 9 studies (Table 2) [25–28, 30, 38]. Three studies did not report morphologic results in their findings [15, 29, 32]. Altogether, these 6 "positive" studies had specimens from approximately 63 total subjects (the exact number is not possible to determine). Round *Borrelia* morphologies were described microscopically in up to 32 total patients. With the exception of a single case report from the United States, these studies and all of their subjects were from Europe.

Table 2. Characteristics of Studies Reporting Round Morphologic Variants of *Borrelia burgdorferi* in Specimens From Human Subjects

Reference	Study Subjects	Countries	No. of Subjects	Source	No. Positive[a]
[25]	Cutaneous Lyme disease[b]	Austria and Germany	43[b]	Skin biopsy	15
[26]	Erythema migrans	Bulgaria	1	Skin biopsy	1
[28]	Erythema migrans	Czech Republic	5	Skin biopsy	4
[27]	Multiple sclerosis	Norway	10	Cerebrospinal fluid sediment	8[c]
[30]	Alzheimer disease	United States (Arizona)	1	Brain	1
[38]	Alzheimer disease	Switzerland	3	Brain	3

[a] The total number of positive subjects was not made clear in 2 of the references; thus, this column represents the maximum number of positive specimens.

[b] Conditions included erythema migrans (19), prior erythema migrans (3), and acrodermatitis chronicum atrophicans (21). Subjects with a variety of other skin conditions were included in this study, making a total of 103 clinical subjects and 7 controls.

[c] This study reported 8 specimens that were positive on examination of cerebrospinal fluid sediment. Other methods performed after 4–7 months of culture were positive in all 10 subjects. These were not considered in vivo demonstrations of *B. burgdorferi* morphology.

Study Descriptions

The following are summaries of the reports describing morphologic variants of *B. burgdorferi* from human specimens.

Cutaneous Lyme Disease

A case report described a single untreated patient from Bulgaria who had presented with erythema migrans [26]. A biopsy was obtained from the skin lesion. The following findings were reported: "In the sections from the deeper strata of the dermis (str. reticulare) *Bb* [*Borrelia burgdorferi*] was observed in two different structural forms: (a) cylindrical bodies (protoplasm cylinder) with circular ends, covered with a three-layered membrane which undulated in places (Figure 2); (b) in most of the sections another structural form of the spirochete was found: granules, situated among the collagenous fibres in places closely adhered to them, sometimes covered with a membrane." The authors did not examine negative control specimens.

Another European study presented microscopic findings from 4 patients with erythema migrans [28]. Both spirochetal and "cystic" morphology were observed by light and electron microscopy. Round forms were seen primarily in dermis obtained from the central part of erythema migrans lesions; 2 healthy control specimens were negative.

A larger study reported findings from 4-mm biopsies of 103 patients with a variety of skin conditions as well as 7 control subjects [25]. The study patients included 19 patients with erythema migrans, 3 with former erythema migrans, and 21 with acrodermatitis chronicum atrophicans. Positive control slides were prepared from a *Borrelia*-injected skin model. Negative controls included normal skin sections; additionally, negative labeling controls were prepared by incubating specimens with swine serum rather than the primary antibody. *Borrelia* was immunolabeled in biopsy specimens using the antibody H9724 and visualized using videomicroscopy. Organisms were visualized in 25% of specimens. The investigators described a number of morphologic features including tangles, rope ladder–like structures, intertwined borreliae, filamentous, granules, rods, vibrio-like, a "gemma"-like body, and spheroplasts. Larger "granules" up to 3 μm were detected in areas of inflammatory infiltrates. A seronegative patient who ultimately had neuralgias 6 months later reportedly had "perineural rod-like structures," and "agglutinated intertwined spirochetes" were seen in specimens from acrodermatitis chronicum atrophicans.

Alzheimer Disease and Multiple Sclerosis

One study reported the brain pathology of a deceased patient from Arizona who had died suddenly after a short illness characterized by cognitive dysfunction [30]. The authors reported that a comprehensive workup had been done to evaluate medical causes of her syndrome, but the results of Lyme disease serologic testing and spinal fluid examination were not provided. A provisional diagnosis of Alzheimer disease was made before the patient's death, and postmortem examination of the brain was consistent with this diagnosis. The actual or presumed cause of death was not reported. According to the report, "an unexpected observation was the identification of cystic forms of the *Borrelia* spirochete in dark-field preparations of cultured hippocampus, and in imprints of hippocampus using the monoclonal antibody H9724 . . . Oil immersion examination of sections from the hippocampus impregnated with silver disclosed a rare cystic structure." Positive and negative tissue controls were stained and examined using the same methodology.

Three deceased European patients with pathologically confirmed Alzheimer disease were found to have brain tissue cultures positive for *B. burgdorferi* [31, 39]. Histopathologic examination using OspA monoclonal antibody labeling revealed a variety of structures, described as spherules, loops, rings, and cysts [38]. These varied from 4 μm to >30 μm in diameter. No antemortem clinical information was provided. The investigators also examined brains from 3 patients without neurologic disease or neuropathology as negative controls. They did not report whether blinded observations were made by additional investigators.

In a study of 10 patients with multiple sclerosis (MS), cerebrospinal fluid (CSF) sediment was examined by dark-field microscopy [27]. *Borrelia burgdorferi* "cysts" were described in 8 of these 10 specimens. No immunolabeling was performed for this preculture microscopic analysis. Polymerase chain reaction (PCR) for *B. burgdorferi* was negative in all 10 cases. Transmission electron microscopy, performed after 4–7 months' incubation, revealed "cyst-like" structures in all 10 cases. These structures were "intensely labeled" using antiborrelial serum and the monoclonal antibody H5332. The authors also looked at CSF from 5 control patients who did not have MS who had been admitted for "ischialgia." One of these subjects had also had erythema migrans, and this individual was also found to have cyst-like CSF structures.

None of the studies reported blinded observations by multiple investigators. Clinical responses to therapy and/or patient follow-up were not reported in any of the above-mentioned studies.

Descriptions of Morphologic Variants In Vivo

Table 3 summarizes the characteristics used to describe morphologic variants from each of the pertinent studies and the methods used to specifically identify these forms as *B. burgdorferi*. Immunolabeling was performed in 3 studies. In 2 cases the monoclonal antibody H9724 was used; in 1 case a polyclonal anti-*Borrelia* rabbit immunoglobulin was used in addition to the monoclonal antibody H5332. Two studies did not use any specific labeling method for the forms visualized in vivo.

Table 3. Characterization of Round Morphologic Variants of *Borrelia burgdorferi* Observed in Human Specimens

Reference	*Borrelia burgdorferi* Immunolabeling	Dimensions	Morphologic Description[a]
[25]	H9724 mAb	0.2–0.4 μm	Granules
		1–3 μm	Large granules or spherical bodies ("gemmae")
		NR	Vibrio-like forms, short rods
[26]	NR	NR	(a) Cylindrical bodies with circular ends
			(b) Granules
[28]	Polyclonal rabbit anti-*Borrelia* Ig	~0.8 μm[b]	Cyst-like
	mouse mAb H5332[c]		
[27]	NR	1–5 μm	Single cysts, cysts in clusters
[30]	H9724 mAb	NR	Rare cystic structure
[38]	OspA mAb	~4–30 μm[c]	Spherules, cysts, spirochetal loops, rings

Abbreviations: Ig, immunoglobulin; mAb, monoclonal antibody; NR, not reported or not performed.

[a] Only descriptions of round morphologies are included in this table.

[b] This was estimated based on the figures provided in the studies.

[c] Immunolabeling was performed after 4–7 months of culture, not on the primary cerebrospinal fluid sediment.

Three studies either reported or allowed estimation of cyst diameter, which ranged 25-fold from 0.2 μm to 5 μm in diameter. Investigators used a number of qualitative descriptors, including "cysts," "granules," "gemmae," "cylindrical bodies," "vibrio-like" forms, and "short rods."

Reports of *Borrelia* Morphologic Variants Using Other Search Terms

Our second literature search yielded 1917 articles. Of these, 29 reported morphologic descriptions of *B. burgdorferi* seen in situ in tissues of infected humans. Tissues reported included skin from erythema migrans and acrodermatitis chronicum atrophicans [29, 40–49]; synovial fluid, synovial tissue, or ligamentous tissue [29, 43, 50–54]; cardiac tissue [55–61]; muscle tissue [62–64]; splenic and lymphatic tissue [43, 65]; brain [66, 67]; and ocular tissue [68, 69]. In all cases the bacteria had the morphology of spirochetes. Round morphologic variants were not described in any of these studies.

Systematic Studies

No study in humans or animals systematically investigated whether a defined clinical syndrome correlates with the presence or absence of morphologic variants of *B. burgdorferi.* No study in humans or animals reported a relationship between morphologic variants of *B. burgdorferi* and either objective or subjective clinical severity. No study in humans or animals evaluated whether the long-term outcome of appropriately treated Lyme disease was related to the presence or absence of morphologic variants of *B. burgdorferi*. No study in humans or animals evaluated whether alternative treatments directed at these variants would (1) result in quantitative reduction in these organisms in vivo or (2) result in improved clinical outcomes.

DISCUSSION

One of the inherent challenges facing any scientific discussion of chronic Lyme disease is that the term itself is essentially undefined, even by its staunchest advocates [70], and most individuals who have received this label either have medically unexplained symptoms (such as chronic fatigue and/or pain) or alternative medical diagnoses [11, 12]. Several lines of argument have been offered by chronic Lyme disease advocates to support the biologic plausibility of this diagnosis: (1) Antibiotics are not effective against *B. burgdorferi* when the organism is intracellular—an untenable argument as a wide variety of intracellular infections are readily treated with the major antibiotics available for Lyme disease; (2) there is animal evidence of bacterial persistence following antibiotic treatment—yet these animals are not said to have syndromes compatible with "chronic Lyme disease," and these studies are further belied by human clinical trials showing favorable outcomes; and (3) *B. burgdorferi* assumes a fastidious, treatment-refractory "cystic" or "L-form" morphology.

Many bacterial species can assume L-form properties [21]. Their clinical significance has been debated for decades [71, 72]. L-forms of *B. burgdorferi* have been observed under laboratory conditions, and advocates for chronic Lyme disease have proposed that these forms are responsible for clinical chronicity and refractoriness to treatment. In some cases antibiotics are given specifically to eradicate these forms. In this systematic review, we investigated literature describing the presence and clinical significance of *B. burgdorferi* morphologic variants specifically obtained from human patients.

We identified a small number of studies reporting morphologic variants of *B. burgdorferi* in human tissue specimens. This

body of literature consists entirely of case reports and small case series from patients with 1 of 4 clinical conditions: erythema migrans, acrodermatitis chronicum atrophicans, Alzheimer disease, and multiple sclerosis. Round morphologic variants were specifically immunolabeled in only 3 studies, ranged greatly in size, and were described using a variety of terms. Due to discrepancies in size, terminology, and labeling, it is not clear when comparing across studies that each investigative team was actually describing the same biological phenomenon. Two of the studies used the monoclonal antibody H9724, which is known to cross-react with human antigens [73–75]. This calls into question the specificity of structures identified in this way.

Approximately 21 patients from 3 studies had round morphologic variants seen in association with erythema migrans or acrodermatitis chronicum atrophicans, well-recognized cutaneous manifestations of Lyme disease [25, 26, 28]. In the broader literature, however, organisms visualized in situ from patients with active Lyme disease (including both cutaneous and extracutaneous disease) are almost invariably described as having normal spirochetal morphology—round variants compatible with L-forms are not described [29, 40–58, 62–69]. In the end, one can do little more than acknowledge that round morphologic variants have been on rare occasion described in vivo.

Round morphologic variants were also reported in 12 patients with chronic medical conditions that are not typically attributed to Lyme disease. These comprised 4 patients with Alzheimer disease and 8 patients with MS. The information provided did not allow us to determine whether these patients had active Lyme disease or had been treated for it. Undiagnosed Lyme disease and Alzheimer disease or MS may have been coincident in these subjects, but causality cannot be concluded from these studies. Further systematic investigations of patients with Alzheimer disease have failed to demonstrate evidence of neuroborreliosis by either culture or microscopy [76–79].

As for the report of "cyst-like" structures in the CSF of MS patients, it must be noted that these subjects all tested negative by PCR for *B. burgdorferi* and that no immunolabeling was performed on the uncultivated CSF sediment. An older electron microscopy study of CSF sediment did not identify structures similar to those described by Brorson et al [80]. MS and Alzheimer disease do not share the highly specific geographic distribution of Lyme disease. Even MS, which is generally distributed in more northerly latitudes of the temperate northern hemisphere, occurs in areas where Lyme disease is either rare or nonendemic [81]. One would expect a high degree of geographic concordance if Lyme disease were responsible for a significant fraction of MS. The rarity of seroreactivity to *B. burgdorferi* despite intrathecal antibody production (oligoclonal bands) in MS makes a causal relationship with Lyme disease doubtful [82, 83].

We were unable to find even a single case report associating morphologic variants of *B. burgdorferi* with syndromes commonly diagnosed as chronic Lyme disease, such as chronic fatigue, neurocognitive dysfunction, chronic pain, or behavioral disease. Nor did we find published evidence of morphologic variants in patients with "post–Lyme disease syndromes," individuals with symptoms persisting for months after initial treatment of Lyme disease. In fact, studies of patients with post–Lyme disease syndromes have consistently failed to demonstrate the continued presence of viable *B. burgdorferi* [84–86].

The vast majority of research about *B. burgdorferi* morphologic variants has been conducted only in laboratory settings. Most of these studies are limited to describing morphology of *B. burgdorferi* in culture [36–38, 87–96]. Round morphologic variants have been shown to arise in a variety of laboratory culture conditions, including cultivation in ex vivo human tonsillar tissue and human cerebrospinal fluid. The latter 2 examples, however, cannot be assumed to approximate growth characteristics in vivo, in which the organism would face the evolving biological conditions of tissue injury and inflammation with innate and adaptive immune responses. A number of additional in vitro studies have reported that such forms arise after exposure to antibiotics or (more generally) that antibiotics induce pathologic effects on cell morphology; still others have evaluated their susceptibility to a variety of antibiotics and other compounds [97–109]. Tested compounds have included vancomycin, tigecycline, telithromycin, tinidazole, metronidazole, ranitidine bismuth sulfate, hydroxychloroquine, and grapefruit seed extract. It must be emphasized that these studies have never been performed clinically or even in animal models of Lyme disease. One can only conclude that published evidence does not justify extending such laboratory-based findings to clinical decisions for human patients.

In conclusion, there is little evidence that supports a role of *B. burgdorferi* morphologic variants in the pathogenesis of Lyme disease and no evidence that they influence treatment outcomes. The presence of round morphologic variants in vivo has been described only in a small number of case reports and case series. As different terminology and laboratory methods were used in these studies, it is difficult to be sure that in aggregate they describe similar structures. We found no convincing scientific evidence that these morphologic variants are associated with chronic *B. burgdorferi* infection, or with the sometimes disabling and protracted symptoms that are often the pretext for a chronic Lyme disease diagnosis.

Note

Potential conflicts of interest. P. G. A. has served as an expert witness in malpractice cases involving Lyme disease. G. P. W. has received research grants from the Centers for Disease Control and Prevention, the National Institutes of Health, Immunetics Inc, Bio-Rad, DiaSorin Inc, and

bioMérieux; holds equity in Abbott; has been an expert witness regarding Lyme disease in a disciplinary action for the Missouri Board of Registration for the Healing Arts and in malpractice cases involving Lyme disease; is an unpaid board member of the American Lyme Disease Foundation; and has served as a consultant to Baxter for Lyme vaccine development. P. M. L. reports no potential conflicts.

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