A Phase I Trial of Mushroom Powder in Patients With Biochemically Recurrent Prostate Cancer: Roles of Cytokines and Myeloid-Derived Suppressor Cells for *Agaricus bisporus*-Induced Prostate-Specific Antigen Responses

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**BACKGROUND:** Each year in the United States, nearly 50,000 prostate cancer patients exhibit a rise in prostate-specific antigen (PSA) levels, which can indicate disease recurrence. For patients with biochemically recurrent prostate cancer, we evaluated the effects of white button mushroom (WBM) powder on serum PSA levels and determined the tolerability and biological activity of WBM. **METHODS:** Patients with continuously rising PSA levels were enrolled in the study. Dose escalation was conducted in cohorts of 6; this ensured that no more than 1 patient per cohort experienced dose-limiting toxicity (DLT). The primary objective was to evaluate treatment feasibility and associated toxicity. The secondary objectives were to determine WBM’s effect on serum PSA/androgen levels; myeloid-derived suppressor cells (MDSCs); and cytokine levels. **RESULTS:** Thirty-six patients were treated; no DLTs were encountered. The overall PSA response rate was 11%. Two patients receiving 8 and 14 g/d demonstrated complete response (CR); their PSA declined to undetectable levels that continued for 49 and 30 months. Two patients who received 8 and 12 g/d experienced partial response (PR). After 3 months of therapy, 13 (36%) patients experienced some PSA decrease below baseline. Patients with CR and PR demonstrated higher levels of baseline interleukin-15 than nonresponders; for this group, we observed therapy-associated declines in MDSCs. **CONCLUSIONS:** Therapy with WBM appears to both impact PSA levels and modulate the biology of biochemically recurrent prostate cancer by decreasing immunosuppressive factors.

**KEYWORDS:** cytokines, myeloid-derived suppressor cells (MDSCs), prostate cancer, PSA recurrence, *Agaricus bisporus*, mushroom.

INTRODUCTION

Adenocarcinoma of the prostate causes a significant number of cancer-related deaths in men. This year, an estimated 238,590 men will be diagnosed with prostate cancer, and 29,720 men will die from the disease.1 In the past 2 decades, testing of serum prostate-specific antigen (PSA) has markedly increased diagnoses of organ-confined prostate cancer.2 As a consequence, many men with newly diagnosed disease are treated (with a curative intent) using either radical prostatectomy or radiation therapy. The initial disease stage, PSA level, and Gleason score all influence the rates of recurrence. Within 10 to 15 years of radical prostatectomy or radiation therapy, approximately 25% to 30% of men will demonstrate disease recurrence.3 Clinical disease recurrence is usually presaged by elevated PSA levels; the vast majority of patients who relapse initially have no radiographic evidence of metastatic disease. The definition of PSA relapse varies depending on specific local therapy. Following prostatectomy, PSA is expected to be undetectable within 30 days of surgery.
Consequently, even a small elevation in PSA level (≥0.2 ng/mL) is considered a sign of persistent disease, and small levels are associated with subsequent disease progression. Conversely, after primary radiation therapy, PSA frequently remains detectable, reflecting the presence of residual prostatic tissue. Thus, recurrence after radiation is frequently defined as an increase of PSA by >2.0 ng/mL above nadir. While the natural history of this cohort of patients is variable, it is generally characterized by a protracted clinical course. In these patients, the risk of developing metastatic disease depends on several factors: time to initial biochemical recurrence (2 vs ≥ 2 years), PSA doubling time (<10 or ≥10 months), and pathologic Gleason’s score. Patients who have received only 1 type of local therapy can be cured occasionally with another local modality (radiation or surgery). However, patients who received both types of local therapies yet continue to experience an increase in PSA levels are considered incurable.

In this setting, the primary treatment option is androgen deprivation therapy. This approach is highly effective in suppressing prostate cancer; however, the side effects are potentially significant and include fatigue, weight gain, muscle weakness, hot flashes, erectile dysfunction, loss of libido, increased risk of diabetes, and cardiovascular problems. In the context of an otherwise asymptomatic individual, such effects are especially concerning, because treatment is applied frequently for many years. Furthermore, androgen deprivation therapy is not curative. In PSA-recurrent disease, the ability of androgen deprivation to enhance patient outcomes (especially overall survival) is unclear. Thus, in biochemically recurrent prostate cancer, the optimal treatment approach remains undefined, and new therapies with minimal toxicities need to be evaluated for this population. An ideal therapy would be outpatient, orally administered, and have minimal systemic toxicity; these properties facilitate long-term, repeated administration.

According to epidemiologic and basic science evidence, plant-derived phytochemicals may play a significant role in prostate cancer prevention, risk of recurrence, and therapy. For cancer patients with limited therapeutic options, such phytochemicals may represent a source of alternative treatment. Reports on pomegranate juice, modified citrus pectin, lycopene, and isoflavone have demonstrated that these materials have a modest, favorable effect on PSA kinetics. These studies should be interpreted with caution, however; a control arm was absent in each study, hence the clinical significance of the endpoints is uncertain. Nevertheless, for centuries in Asia, a wide variety of plants have been used for medicinal purposes. In particular, several mushroom species have been shown to exhibit anticancer effects; these include inhibiting cell proliferation in prostate, colon, and breast cancer cell lines. As suggested by these and other studies, further investigation is warranted into both specific mushroom species and pharmacologically active components of mushroom species.

White button mushroom (WBM, Agaricus bisporus) is the most common edible mushroom in the United States. Accumulating evidence demonstrates that WBM has beneficial effects on various kinds of cancers. Lectins isolated from WBM increase the sensitivity of lung, colon, and glioblastoma cancer cells to chemotherapeutic drugs. In addition, WBM lectins inhibit colon cancer cell proliferation and enhance cellular antioxidant defense mechanisms. As we have demonstrated previously, by inhibiting aromatase activity, both total WBM extract and certain isolated fractions effectively decrease breast cancer cell proliferation. In these active WBM fractions, conjugated linoleic acid was an important component; it was an inhibitor of both breast cell proliferation and aromatase activity. In addition to breast cancer cells, we evaluated the effect of WBM on prostate cancer cell lines in vitro and in vivo. According to our results, in all prostate cancer cell lines, WBM extract significantly inhibits cell proliferation; this inhibition occurs through induction of apoptosis of cancer cells. In mice gavaged with mushroom extract, tumor size and cell proliferation decreased, whereas apoptosis increased. Similarly, for mushroom-fed mice, microarray analysis of tumors identified significant changes in gene expression. Particularly altered were the gene networks involved in cell death; growth and proliferation; lipid metabolism; the tricarboxylic acid cycle; and immune response.

Based on these preclinical data and a clinical unmet need, we conducted a single-institution phase 1 trial of WBM powder in patients with biochemically recurrent prostate cancer. The primary objective of the trial was to evaluate the feasibility, toxicity, and biological activity of prolonged therapy with WBM powder. All patients enrolled for this trial had (before enrollment) continuously rising PSA levels after previously undergoing definitive local therapy with prostatectomy, radiation therapy, or both. Following the intake of WBM by biochemically recurrent prostate cancer patients, correlative serum samples were collected to attempt to answer the question, “What are the biological differences between PSA responders and nonresponders?”
MATERIALS AND METHODS

Patient Eligibility
A histologically or cytologically confirmed history of adenocarcinoma of the prostate was required for all patients. Any number of local therapies was allowed. Patients had to experience the following PSA failure: a PSA level of $\geq 0.2$ ng/mL that increased above nadir after prostatectomy. If no prostatectomy was performed, and radiation or other local therapies were used as a primary therapy, patients had to experience a PSA increase of 2.0 ng/mL above post-therapy nadir. The increase in PSA values had to be established by at least 2 consecutive measurements (each separated by at least 2 weeks). No clinical or radiographic evidence of metastatic disease was allowed. In conjunction with their prior primary definitive therapy, patients were permitted to receive up to 9 months of neoadjuvant or adjuvant hormone ablation. Androgen deprivation had to be completed at least 6 months prior to registration, and testosterone levels had to be in the noncastrated level defined as $>50$ ng/dL. No complementary or alternative therapy (eg, St. John’s Wort, PC-SPES, or other herbal remedies taken for the purpose of treating prostate cancer) could be given during protocol treatment. For patients who received neoadjuvant and/or adjuvant chemotherapy, such treatments must have been completed at least 6 months prior to protocol registration.

Patients were enrolled between January 2009 and September 2011. The trial was registered with ClinicalTrials.gov (NCT00779168) and was conducted according to the Declaration of Helsinki and its amendments. The study protocol was approved by the City of Hope Institutional Review Board and the Institutional Scientific Peer Review Committee of the City of Hope Comprehensive Cancer Center. All patients provided written informed consent before participation.

Patient Characteristics
Thirty-six patients were enrolled in the study; the demographic characteristics are summarized in Table 1. The median patient age was 68 years (range, 53-80 years). The median PSA level at the onset of therapy was 1.9 ng/mL (range, 0.2-22.2 ng/mL). For all patients, the median baseline testosterone level was 295.3 ng/dL (range, 86.0-833.7 ng/dL). All patients had prior radiation therapy, the majority of which took place in the postprostatectomy salvage setting. Thirty-three patients (92%) underwent prior prostatectomy. Eleven patients (30%) underwent earlier hormonal therapy in the neoadjuvant or adjuvant setting.

### Table 1. Patient Characteristics (n = 36)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (range), y</td>
<td>68 (53-80)</td>
</tr>
<tr>
<td>Place, n (%)</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>27 (75)</td>
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<tr>
<td>African American</td>
<td>7 (20)</td>
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<tr>
<td>Asian</td>
<td>2 (5)</td>
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<tr>
<td>Gleason score, n (%)</td>
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<tr>
<td>$\leq 6$</td>
<td>6 (18)</td>
</tr>
<tr>
<td>$7$</td>
<td>14 (44)</td>
</tr>
<tr>
<td>$\geq 8$</td>
<td>12 (33)</td>
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<td>Baseline PSA, median (range), ng/mL</td>
<td>1.9 (0.2-22.2)</td>
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<tr>
<td>Baseline testosterone, median (range), ng/dL</td>
<td>295.3 (86.0-833.7)</td>
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<td>Prior radiation, n (%)</td>
<td>36 (100)</td>
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<tr>
<td>Prior prostatectomy, n (%)</td>
<td>33 (92)</td>
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<tr>
<td>Duration on treatment, median (range), months</td>
<td>10.2 (0.9-57.9)</td>
</tr>
</tbody>
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Abbreviation: PSA, prostate-specific antigen

Preparation of Mushroom Tablets
See Supporting Information for details regarding the preparation of the mushroom tablets.

Mushroom Tablet Treatment
The mushroom tablets were taken twice daily until PSA progression, clinical progression, or toxicity. Twenty-eight days constituted 1 treatment cycle. Dose escalation was conducted in cohorts of 6 patients each at 6 dosages: 4, 6, 8, 10, 12, and 14 g/d. If no dose-limiting toxicities (DLT) were encountered for a cohort of patients during the first 28 days of treatment, the next highest dosage was tested (up to 14 g/d). Approximately 90% of fresh WBM weight consists of water; therefore, 4- to 14-g mushroom tablets are equivalent to 40 to 140 g of fresh WBM.

Rationale for Dose Selection
Our trial examined 6 dosages beginning with 4 g/d. The maximum dosage was capped at 14 g/d (ie, 28 tablets daily). For chronic ingestion, this was thought to be the highest practical dosage. We did not attempt to reach the maximum tolerated dosage (MTD) because our preclinical data did not indicate a dose-dependent effect.

Efficacy and Safety Evaluation
Patients were evaluated clinically and with laboratory tests every 28 days while on treatment. At every visit, medication lists including vitamins and supplements were updated. A mushroom tablet pill count was performed at every visit to verify compliance. Toxicity assessment was performed in all patients who began therapy using the NCI Common Terminology Criteria for Adverse Events (CTCAE version 3.0). DLT applied only to cycle 1 and had to be drug-related (possible, probable, or definite). Any grade 2 or greater toxicity (excluding allergic rhinitis,
fatigue, sweating, weight gain or loss, alopecia, dry skin, nail or pigmentation changes, pruritus, hot flashes, flatulence, mouth dryness, sense of smell or taste disturbance, erectile impotence, decreased libido, oligospermia, and/or insomnia) was considered a DLT. Unless a patient stopped treatment because of toxicity, patients were evaluated for response if they were on therapy for at least 28 days; otherwise, they were replaced. PSA progression was defined as a 100% increase over either the baseline (pretreatment, within 4 weeks of start of treatment) or nadir. To further define PSA progression, the minimum change in PSA needed was an absolute increase of 1 ng/mL, which was confirmed by second value 3 to 4 weeks later.

**Hormone Measurement**
The ADVIA Centaur Testosterone assay kit (Siemens, NY/USA) was used to measure testosterone in patients’ serum. Dihydrotestosterone (DHT), dehydroepiandrosterone (DHEA), and estrogen were measured by ARUP laboratories.

**Cytokine Multiplex Analysis**
Per the manufacturer’s protocol, plasma samples were analyzed for 30 cytokines using the Human Cytokine Thirty-Plex Antibody Bead Kit (Invitrogen). See Supporting Information for details regarding the procedure.

**Analysis of Immunosuppressive Cell Subset in Patients’ Blood**
See Supporting Information for details regarding the analysis of immunosuppressive cell subset in patients’ blood.

**Statistical Analysis and Data Management**
Of the 6 different dosage levels tested in this study, no MTD was reached. No signal was detected related to dosage, resulting in pooled analysis across dosages for response rate and serum biomarkers. For binomial rates, confidence intervals presented were calculated by the Clopper-Pearson (exact) method. Due to the small number of samples available for serum biomarkers, nonparametric test (Wilcoxon rank sum) was used to compare responders with nonresponders. Because these biomarker evaluations were exploratory, there was no attempt to adjust for multiple comparisons; thus significant results require a confirmatory study.

**RESULTS**

**Clinical Efficacy**
A PSA complete response (CR), defined as a PSA decline to ≤0.04 ng/mL (the threshold limit of detection at the City of Hope National Medical Center), was confirmed at

Figure 1. Prostate-specific antigen (PSA) levels of 4 patients with significant PSA responses beginning 12 months prior to treatment initiation and followed until end of treatment (Pt 4) or last PSA assessment.

least 4 weeks later. The overall PSA response rate (the sum of partial and complete responders vs nonresponders) was 4/36, or 11% (95% confidence interval, 4%-26%). Two patients (at dosages of 8 and 14 g/d) experienced a prolonged CR that continues to date. At the time of this report, the lengths of these CRs have been 49 and 30 months (Fig. 1). In another 2 patients, we observed a PSA partial response (PR), which was defined as a 50% decline from baseline PSA. One patient with PR demonstrated durable response and has remained on the study for 39 months since beginning therapy. Another partial responder eventually had disease progression and was taken off treatment after 7 months on therapy. At the time this paper was prepared, an additional 5 patients remained on protocol treatment because of their stable PSA values. Altogether, 8 patients remain on the study. Of 4 responders, 3 had a prior prostatectomy and salvage RT that was completed 4, 13, or 16 months prior to starting WBM powder treatment. One patient with a PR had prior brachytherapy followed by salvage prostatectomy. The characteristics of the responders are summarized in Table 2. In addition to the partial and complete responses, 36% of patients demonstrated some decline in PSA within 3 months after beginning therapy (Fig. 2). The median duration of therapy for all patients was 10.2 months (range, 0.9-57.9+ months). The patient with the longest treatment duration has exhibited a stable PSA level.
Correlation of PSA and Testosterone in Patients with CR

Two patients experienced a decrease in PSA to undetectable levels (≤ 0.04 ng/mL) during mushroom therapy; this was maintained for 49 and 30 months. In both patients, total serum testosterone levels fluctuated over the study period; however, there was no noticeable trend. Testosterone levels remained in the noncastrated range (Fig. 3). PSA responses did not correlate with serum testosterone, DHT, or DHEA levels (data not shown).

Toxicity

Minimal side effects were noted and mostly limited to grade 1 abdominal bloating (Table 3). The mean compliance with protocol-defined mushroom powder treatment was 98.6%. One patient at dose level 3 (8 g/d) experienced grade 3 hyponatremia, possibly related to therapy, and was taken off the protocol for toxicity. This occurred during the second month (cycle 2) of therapy and therefore was not classified as a DLT.

Cytokine Multiplex Analysis

There were no definitive patterns for cytokine levels except for interleukin-15 (IL-15). Both at baseline and post-treatment, IL-15 levels of complete responders were significantly higher than levels of nonresponders. Compared with baseline, IL-15 levels were not significantly changed after 13 weeks of mushroom intake in both complete-responders and nonresponders (Fig. 4A).

Analysis of Immunosuppressive Cell Subset in Patients’ Blood

Compared with the percentage of myeloid-derived suppressor cells (MDSCs) in peripheral blood mononuclear cells (PBMCs) sampled at enrollment, at week 13, the
percentage of MDSCs (CD33+ HLA-DR−) decreased in the 4 CR and PR patients; specifically, the levels dropped by 78%, 45%, 94%, and 65%. In contrast, for the 9 non-responders at week 13, the percentage did not decrease significantly from enrollment (mean decrease of 25%). In all groups after mushroom intake, no changes were apparent for CTLA4 T cells (CD4+ CD152+), T-regulatory FoxP3 cells (CD4+ CD25+ FoxP3+), or PD1+ T cells (CD4+ CD279+) (Fig. 4B).

DISCUSSION

In this study of 36 patients with biochemical recurrence of prostate cancer, WBM powder therapy was associated with declining PSA levels in 13 (36%) patients. Two patients responded completely, and 2 other patients responded partially. An additional 5 patients remain on protocol treatment to date because of their stable PSA values. These results indicate that mushroom intake can modulate PSA levels in biochemically recurrent prostate cancer. When comparing responders with nonresponders, the following factors did not correlate to response: daily mushroom dose, Gleason score, baseline PSA level, weight, age, baseline testosterone levels, and type of prior therapy. We did not detect any effect of mushroom powder therapy on testosterone, DHT, or DHEA levels.

As such, patients had exhausted a chance for a cure via traditional localized treatment. Compliance with the daily ingestion of up to 28 large mushroom powder tablets was excellent, reflecting a lack of significant, chronic toxicity. The most common adverse events were grade 1 abdominal bloating and grade 1 SGPT/SGOT elevation. One patient sustained asymptomatic grade 3 hyponatremia.

We were aware of potential confounding effects of other medications (statins, thiazide diuretics and nonsteroidal anti-inflammatory drugs) on PSA levels. As such, on a monthly basis, we verified concurrent medications and supplements. For 1 patient with prolonged stable PSA, changes in PSA reflected both his cholesterol level and compliance (or lack thereof) with simvastatin. For the rest of the group, PSA responders did not change their baseline medications or supplements. Importantly, they did not ingest any compounds known to affect PSA levels. In addition, we have not discovered any correlation between weight changes and PSA; this was true for patients demonstrating significant PSA responses. According to the literature, WMB not only lowers blood glucose and cholesterol levels in diabetic and hypercholesterolemic rats, but it also prevents hepatic steatosis in...
WBM extract enhances NK cell activity in mice. We have not observed significant changes in blood glucose and lipid levels in patients treated with WBM.

Our trial examined 6 dosages beginning with 4 g/d. The maximum dosage was capped at 14 g/d, because this was thought to be the highest dosage that was practical to ingest over a long period based on the number of tablets required. Because there were no DLTs, dose determination is challenging. We observed CR and PR in the 8 g/d group (Table 2); consequently, in future studies—especially single product studies on the mushroom powder/tablets in asymptomatic patients—we will start patients on 8 g/d, the minimum dose where we observed responses, and permit the patient to escalate up to 14 g/d if well tolerated.

For decades, the capacity of various mushrooms to both inhibit tumor growth and modulate immune functions has been studied. Tumor growth was primarily inhibited by stimulating the immune system. In particular, macrophages and natural killer (NK) cells were stimulated, as were T cells and their cytokine production. Using the Luminex platform, we assayed a variety of serum cytokines involved in immune function, angiogenesis, and lipid and bone metabolism; however, we did not observe a correlation between serum cytokine levels and PSA responses. Despite this result, the analysis produced an interesting outcome: for 3 patients, with either CR or PR, particularly high levels of plasma IL-15 were detected (compared with nonresponsive patients). As it has been suggested, IL-15 may both promote NK cell differentiation and optimize NK cell function. Congruent with this, in prostate tumor models, IL-15 has been evaluated as an anticancer immunotherapeutic agent because it can effectively stimulate lymphocyte subsets, including CD8 T cells, natural killer cells, and natural killer T cells. Consequently, IL-15 can promote tumor suppression. As evidenced in the literature, WBM extract enhances NK cell activity in mice.

Several clinical trials of IL-15 in cancer immunotherapy have been started recently; however, IL-15 may not be optimal when it is used as a single treatment. According to Yu et al., combination therapies involving both suppression of the immune negative feedback systems and IL-15 therapy abrogated prostate tumor growth in mice models. For the responding patients described herein with high IL-15 levels, we hypothesize that WBM influences immune inhibition. In tumor-bearing mice, β-glucans, which are extracted from mushrooms, can decrease the levels of tumor-associated immunosuppressive cells, such as T-regulatory T cells. Thus, patients with high IL-15 levels may benefit from WBM treatment via suppression of immune-negative feedback effects. To both protect the host from pernicious immune stimulation (excessive stimulation during infections) and to control autoimmune responses, there are multiple mechanisms for immune response attenuation. Through these inhibitory mechanisms, cancer cells can escape immune surveillance and expand their niche. There are multiple inhibitory mechanisms to mediate cancer-related immune suppression. Ligands that are expressed by antigen presenting cells or tumor cells can bind to T cells’ inhibitory receptors (CTLA4 and PD1). Furthermore, inhibitory cells (such as T-regulatory cells and MDSCs) can play critical roles in influencing immune cells.

DHT, which is an active androgen, upregulates PSA levels. WBM was also found to inhibit 5α-reductase, the enzyme that produces DHT from testosterone. The WBM extract inhibits both 5α-reductase 1 and 2 (Supporting Fig. 1). Although we did not detect changes in testosterone and DHT that were freely circulating, in tissues we cannot rule out inhibitory effects of WBM on 5α-reductase (and thus tissue production of DHT).

The trial was performed with 2 lots of lyophilized WBM powder. Both lots were obtained from Monterey Mushrooms (Watsonville, CA). The standard assay in our laboratory to define the activity of mushrooms or their extract is to determine their anti-aromatase activity. The assay procedure was reported by Kanaya et al. The water extract generated from these lots inhibited aromatase in a dose-dependent manner. Our laboratory first reported the ability of WBMs to suppress the activity of aromatase, an enzyme that catalyzes the biosynthesis of estrogen.

The assay is robust and has been the method of choice for us to compare the activity of different mushroom preparations. It is not clear whether aromatase activity is a good surrogate for activity related to the effect observed in biochemically recurrent prostate cancer, and efforts will be made to develop assays more relevant to prostate cancer.
The lack of placebo control in our analysis raises the following question: In this population of patients, do the PSA responses simply represent natural variability of relatively low PSA levels? Based on both the literature and experience, this is unlikely. Specifically, consecutive rises in PSA to ≥0.2 ng/mL (as required per eligibility criteria) in post-prostatectomy patients almost always leads to eventual progression.2-4

In conclusion, WBM therapy appears to impact PSA levels and potentially modulates the biology of prostate cancer in some patients, especially in the setting of low tumor burden. Our future efforts will focus on both the isolation and characterization of WBM compounds with anti–prostate cancer activity and the development of a placebo-controlled clinical trial in patients with early biochemical recurrence of prostate cancer.

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CONFLICT OF INTEREST DISCLOSURES
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