Workshop on the Safety of Black Cohosh in Clinical Studies

WORKSHOP SPONSORS:

National Center for Complementary and Alternative Medicine
NIH Office of Dietary Supplements

National Institutes of Health
Bethesda, Maryland
November 22, 2004

Acronyms

ADRAC	Adverse Drug Reactions Advisory Committee, Department of Health and Ageing, Therapeutic Goods Administration, Australia
AE	adverse events
AhR	aryl hydrocarbon receptor
AIH	autoimmune hepatitis
ALP	alkaline phosphatase
ALT	alanine animotransferase
ANA	antinuclear antibodies
AP	alkaline phosphatase
APTT	activated partial thromboplastin time
AST	aspartate aminotransferase
BC	breast cancer
BCE	black cohosh extract
BfArM	German Federal Institute for Drugs and Medical Devices
BMI	body mass index
BPH	benign prostatic hyperplasia
CSM	Committee on Safety of Medicine (independent committee advising the U.K. Licensing Authority, Government Health Ministers)
DES	diethylstilbestrol
DHT	dihydrotestosterone
DILIN	Drug Induced Liver Injury Network, NIDDK, NIH
DMBA	dimethylbenzanthracene
DSMB	data safety monitoring board
E2	estradiol
ER	estrogen receptor
EROD	7-ethoxyresorufin O-deethylase
FDA	Food and Drug Administration, DHHS
FSH	follicle-stimulating hormone
GnRH	gonadotropin-releasing hormone
GSH	glutathione
HGPIN	high grade prostatic intraepithelial neoplasia
HPLC	high performance liquid chromatography
HRT	hormone replacement therapy
ILIAD	Idiosyncratic Liver Injury Associated with Drugs, NIDDK, NIH
IM	intramuscular
IVDA	intravenous drug abuse

LBA	ligand-binding assays
LC	liquid chromatography
LH	leutinizing hormone
MMTV	mouse mammary tumor virus
MS	mass spectrometry
NAF	nipple aspirate fluid
NBJ	Nutrition Business Journal
NCCAM	National Center for Complementary and Alternative Medicine
NFM	Natural Foods Merchandiser
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
NIH	National Institutes of Health
PC	prostate cancer
PCPT	prostate cancer prevention trial
PCR	polymerase chain reaction
PIN	prostatic intraepithelial neoplasia
PR	progesterone receptor
PSA	prostate-specific antigen
QR	quinone reductase
RAPD	random amplification of polymorphic DNA
SERM	selective estrogen receptor modulator
SSRI	serotonin selective reuptake inhibitors
TGA	Therapeutic Good Administration (Canberra, Australia)
TLC	thin-layer chromatography
VEGF	vascular endothelial growth factor
WHI	Women's Health Initiative, NIH
WHO	World Health Organization

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Introduction

On November 22, 2004, the National Center for Complementary and Alternative Medicine (NCCAM), National Institutes of Health (NIH), in collaboration with the NIH Office of Dietary Supplements (ODS), convened a small workshop to discuss issues related to the safety of black cohosh. Also participating in this meeting were representatives from the Office of Research on Women's Health, NIH; the National Cancer Institute; the National Institute on Aging; the Food and Drug Administration (CDER and CFSAN, FDA); American Herbal Products Association; and Center for Science in the Public Interest. (See Appendix 1 for the workshop agenda and Appendix 2 for the workshop participant list.) This meeting was called in response to several case reports of hepatotoxicity in the medical literature and an abstract referencing data from a murine model of breast cancer that raised questions about the safety of black cohosh. Because NCCAM and other Institutes and Centers at the NIH support clinical studies on black cohosh, we convened the workshop to reflect on what is currently known about risks associated with this dietary supplement ingredient. First and foremost is our concern for the safety of research subjects. Given ambiguity and uncertainty in the scientific information available when viewed in aggregate, it was not clear what additional actions if any NIH should take to protect human research subjects. Thus, we convened a meeting to:

- Gain a better understanding of the nature of the reported hepatotoxicity in humans;
- Understand what might be warranted to better understand the effects of black cohosh on metastatic processes reported in a mouse model; and
- Clarify steps that investigators might take to continue to protect participants in studies of black cohosh.

The desired outcomes for this meeting were focused on issues related to the mandate of the NIH and its role in supporting biomedical research. We did not expect the meeting to produce a definitive statement on the safety of this botanical. That would certainly be beyond the scope of a one-day workshop. Similarly, we did not expect that it would address questions concerning efficacy, which await the findings of several ongoing studies supported by the NIH. Finally, we did not expect participants to develop an extensive research agenda. Rather, we hoped to gain from this interactive forum a better understanding of:

- the nature of the reported hepatotoxicity in humans;
- what approaches might be warranted to better understand the effects of black cohosh on metastatic processes reported in a mouse model; and
- what investigators and sponsors could do to further protect participants in studies of black cohosh if warranted.

Participation and ideas were solicited from a group of scientists representing a broad range of disciplines (e.g., pharmacology, pharmacognosy, toxicology, endocrinology, hepatology, oncology, gastroenterology, and medicine) that reflect the multidisciplinary nature of the topic.

This report summarizes the presentations and discussions from the November 22 workshop and is organized into three general areas: 1) background information on safety and efficacy of black

cohosh; 2) data from a murine model of breast cancer and metastases to lung associated with black cohosh; and 3) case information on suspected toxicity associated with use of black cohosh products.

We are very grateful for the hard work and enthusiasm of the workshop participants.

Overview of Black Cohosh

Heather Miller, Ph.D. and Qi Ying Liu, M.D., NCCAM

Black Cohosh, a member of the buttercup family, has been known at different times by different names, such as *Cimicifuga racemosa* (L.) Nutt., *Actaea racemosa* L., macrotys, bugbane, bugwort, rattleroot, black snakeroot, and many others. Historically, it has been used for a range of conditions. U.S. colonialists used it for amenorrhea, bronchitis, chorea, dropsy, fever, hysteria, itch, lumbago, nervous disorders, snakebite, yellow fever, and uterine disorders. Interest in black cohosh and other dietary supplements has increased recently in the wake of findings from the Women's Health Initiative (WHI), which pointed to a shift in the risk/benefit balance of hormone therapy due to cardiovascular events, stroke, breast cancer, blood clots, and other conditions. Women are seeking alternative treatments for symptoms associated with the menopausal transition, including hot flashes and night sweats, anxiety and depression, and vaginal dryness. Dr. Bolton will discuss what is known about estrogenic activity of black cohosh. There is a growing literature on the efficacy of black cohosh for several menopausal symptoms, but when viewed in aggregate, the literature is equivocal. Some studies have reported a positive effect on specific symptoms while others have not. NCCAM and other NIH Institutes and Centers are supporting ongoing research on the efficacy of black cohosh for a range of symptoms and health problems. We hope to learn more about efficacy from those activities.

Several reasons might account for such variability in the scientific literature. There are a variety of black cohosh preparations available today, including isopropanolic and ethanolic extracts made from the rhizome and roots of the plant. Possible adulteration and contamination are concerns as some preparations include a combination of herbal substances. Dr. Farnsworth will provide additional information on the process of preparation and problems associated with product variability and adulteration. Black cohosh preparations come in different doses. The usual recommended daily dose is 40-80 mg, though some studies have used other doses. Moreover, it is often unclear if the weight is based on extract or amount of extract equivalent to weight of starting plant material. It is often unclear if the dose refers to the amount of root and rhizome used to manufacture an extract or the amount of extract itself. Moreover, some preparations control for the quantity of triterpene glycosides while others do not. In reading the literature, it is often difficult to determine exactly what was used in a given study. Information on the preparation used can be scant, and often information on quality assurance measures taken to ascertain characterization and standardization of the substance is absent. Studies tend to be short term but with variable treatment durations, generally from several weeks to six months. Not only do studies include different outcomes (depression, anxiety, vaginal dryness, hot flashes, etc.), but they also can use different measures of the same outcomes. Few studies have been randomized clinical trials with double blinding and placebo control. Placebo effects for any treatment of hot flashes have been well documented, and self-reported measures of this endpoint are problematic. When these design limitations are considered along with Hawthorne effects,

small sample sizes, and heterogeneous samples, it is not surprising that the literature in aggregate yields equivocal results concerning efficacy.

In terms of risks, several presenters at this workshop will be providing detailed information on specific tissues and case reports. Drs. Sauter and Wuttke will discuss the effect of black cohosh on breast tissue while Dr. Dobs will address the prostate. Dr. Davis will present data on a murine model that raise questions concerning increased risk of metastatic lesions in lung from breast tumors among animals given black cohosh. Later, Drs. Kerlin and Cohen will present several cases of hepatotoxicity associated with black cohosh use.

In addition to the scientific literature, important information on risk can come from surveillance systems. Two systematic reviews of the safety of black cohosh have been conducted, and both began with extensive reviews of the published literature from various countries and included pre-clinical and clinical studies (Dog et al., 2003; Huntley and Ernst, 2003). Both looked at adverse event reporting systems that included the Food and Drug Administration (FDA) and the World Health Organization (WHO), and both sought additional information on adverse events from manufacturers of black cohosh preparations. (See Table 1.)

Table 1. Adverse Event Reports

	Number of adverse events reported on black cohosh (salts/esters and root) to the WHO cumulative through July 31, 2000	**Number of adverse events reported on black cohosh, excluding combination preparations to similar systems in Australia, Germany, UK, US, and WHO**
Abdominal pain	1	1
Arthralgia/back pain	2	2
Breast enlargement/pain	2	4
Depression	1	1
Endometrial hyperplasia	2	2
Decreased fertility	1 (female)	1
Genital neoplasm malignant	1	1
Hemorrhage rectum/vagina	2	3
Hepatic failure	1	3
Hepatitis	1	2
Hypertension	1	3
Intermenstrual bleeding	1	1
Jaundice	1	2
Malaise	1	2
Menstrual disorder	2	1
Nausea	1	2
Rash/Rash erythematous	3	9
Source:	Dog et al. (2003)	Huntley and Ernst (2003)

These surveillance systems rely on spontaneous reporting, which often does not provide much detail. Similarly, there is no way to know how complete the reports are or how valid the information provided regarding the preparation taken or the medical outcome. These data also

lack a precise denominator, precluding the calculation of rates of occurrence. However, one could conceive of the basis of reporting as the population of black cohosh users in countries reporting to these systems. With these caveats in mind, surveillance data reported in these studies indicate that adverse events are relatively few and do not appear to be concentrated in any particular organ system.

Black Cohosh: What Do We Know About This Botanical?

Preparation and Possible Adulteration and Contamination

Norman Farnsworth, Ph.D., University of Illinois at Chicago (UIC)

Dr. Farnsworth underscored the importance of knowing exactly what is being used in clinical studies, highlighting the need for adequate proof of identity of the source material. Minimally, the source material should undergo taxonomic verification by one of the following methods to compare with an authentic root/rhizome: thin-layer chromatography (TLC), high performance liquid chromatography (HPLC) mass spectrometry fingerprinting,[1] polymerase chain reaction (PCR) analysis (which is not useful for extracts), and possibly biological assays. Investigators should retain a rhizome/root specimen that is representative of the bulk collection. Dr. Farnsworth noted that few companies adhere to verification procedures, despite the fact that commercial collections of black cohosh are not homogenous. Rather, they are usually mixtures of plant material from different areas. For example, a vendor trying to fill an order for 500 kilos often buys material from many sources, and some sources might add extraneous substances. Additional requirements for more rigorous testing would include heavy metal analysis, pesticide/herbicide analysis, assays for microbial load, and perhaps a stability study to determine changes in strength with increased shelf life or under different storage conditions.

In comparing two capsules (40 percent i-PrOH [Remifemin® @ 200 mg/ml] and 75 percent ethyl alcohol (EtOH) capsules made for the UIC clinical trial [10 mg/ml]), Dr. Farnsworth reported distinct differences in mass spectra produced by the two products. Comparison of products is hampered by some manufacturers' reluctance to provide information about their product.

Random amplification of polymorphic DNA (RAPD)-PCR profile comparisons of six common *Cimicifuga* species found clear differences across products (Xu et al., 2002).[2] Such variability across closely related species underscores the need to proceed cautiously in interpreting findings from studies of black cohosh since in general substances used are not well specified and adulteration with the other cohosh species may occur sometimes. Dr. Farnsworth noted that it is not unlikely that a small amount of *C. simplex* gets mixed in with black cohosh during collection. There is increasing concern among importers that some of the products marketed in the U.S. may not be genuine black cohosh (*C. racemosa*) but one of the Chinese species instead. Regardless of the product used, it is important to ascertain exactly what the product contains, and it is certainly possible to differentiate among species at the raw material stage and in single ingredient products.

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[1] For the HPLC analysis, a USP/NF standardized assay is available that is based on an evaporative light scattering detector (ELSD). Black Cohosh USP Monograph 28 (5), 2002: 1455-66.

[2] The six species compared were: 1) *C. racemosa*; 2) *C. foetida*; 3) C. *heraclefolia*; 4) C. *acerina*; 5) C. *dahurica*; and 6) C. *simplex*. Species 2, 3, and 5 are commonly known as Chinese cimicifuga.

Another potential source of product confusion relates to blue cohosh (*Caulophyllum thalictroides*). This plant grows in the same places as black cohosh but is not related. Blue cohosh can be mistaken for black cohosh by inexperienced wild crafters who have received little or no training in what to look for. It should be noted that blue cohosh contains toxic quinolizidine alkaloids (Woldemariam et al., 1997).

Dr. Farnsworth then reviewed eleven different commercial products that have been used in clinical studies. Black cohosh products come in a variety of forms, including capsules, solution, drops, tincture, and tablets. Remifemin® is the most widely studied product, at least of those sold in the United States. Over time, the manufacturer has switched between isopropanolic and ethanolic preparation, and between fluid extracts and dried fluid extracts.[3] While no clinical trials have been reported using powdered plant material, a range of different formulations has been used in clinical research. The changes in preparation method of Remifemin® make comparisons of this one product complicated when comparing across different studies. And the variety of commercial preparations of black cohosh administered in clinical trials complicates comparisons across studies in terms of safety, toxicity, and efficacy.

Black cohosh can be taken as part of a mixture of botanicals rather than a single ingredient dietary supplement. Multi-component mixtures are becoming more prevalent in the marketplace, but their content is not always clear. For example, Lydia Pinkham's liquid compound initially contained black cohosh. Then the manufacturer removed it, and now it has been added again. Analyses of multi-component mixtures present certain challenges. Dr. Farnsworth noted that there has been little discussion about how to handle Chinese multi-component mixtures. While the analytic process to characterize multi-component mixtures may be challenging, it is not impossible. Nevertheless, studying such multi-component mixtures requires considerable expertise.

Dr. Farnsworth reported that only two patients have been reported to suffer hepatotoxic episodes following ingestion of a single plant product. There have been over 2,000 subjects in clinical trials that have ingested a black cohosh extract (BCE), with no reported cases of hepatotoxicity reported from these studies. In the UIC Center for Botanical Dietary Supplements Research, a four-arm Phase II study is in progress that is comparing red clover, black cohosh, Prempro, and placebo. To date, more than thirty subjects have enrolled in the study, with at least seven taking black cohosh. No adverse events have been observed.

Meeting participants raised questions about the number of people using black cohosh. There was an interest in trying to provide a rough estimate of the denominator associated with adverse events reported in surveillance systems. Participants noted that the use of black cohosh increased dramatically after the WHI study results were published. It is believed that statistics on the use of Remifemin® in Germany could be obtained as Remifemin® is considered a regulated drug and monitored as part of a post-marketing surveillance system. Thus, it might be

[3] It is not clear why Remifemin changed their preparation and packaging. It was marketed originally as a liquid (ethanol) extract and now is sold in the form of pills with isopropanol extract only. The label on Remifemin has changed at least twice. Remifemin is labeled to contain extract equivalent to 20 or 40 mg root and rhizome. However, it is unclear if this is 20 or 40 mg of extract or an amount of extract equivalent to 20 or 40 mg of starting plant material. This situation can confuse comparisons and data interpretation.

helpful to look at both adverse events reported under the German surveillance system and use of Remifemin® and other black cohosh products in that country.

Dr. Dentali provided additional information and estimates of use of black cohosh in the U.S. Black cohosh was the fourteenth most popular herbal product in the U.S. Natural Foods Merchandiser (NFM) Market Overview. For 2003, black cohosh sales were $15.7 million dollars (up 26.2 percent from the previous year) with 1.435 million units sold. These data might be considered a conservative estimate since they come from sales reported by supermarkets, drug stores, and mass merchandisers but do not includes sales from Wal-Mart and many health food stores, internet sales, or multilevel marketing. It should be noted that according to NFM estimates of black cohosh sales increased by 27.4 percent in 2002 while sales of 25 other products (of 37 herbal products tracked by this system) declined in 2002. Similarly, the Nutrition Business Journal (NBJ) estimated from a variety of sources that 2002 black cohosh sales totaled $42 million for all channels. That estimate seems plausible as the mass market, multilevel marketing, and health foods segments are believed to account for approximately equal market portions. The NBJ data showed only a 3 percent growth for 2002 sales of black cohosh over those of 2001.

Mechanism of Action, Estrogenic Activity, and In Vitro Toxicology Screening Assays

Judy Bolton, Ph.D., University of Illinois at Chicago (UIC)

Black cohosh has been used in several clinical trials, some of which have shown a positive outcome for the relief of menopausal hot flashes. It is not known how black cohosh works to alleviate menopausal symptoms. Dr. Bolton described the ongoing clinical trial being conducted by UIC. This study uses a double blind, placebo-controlled design and is comparing: 1) placebo; 2) Prempro; 3) black cohosh; and 4) red clover. The study will continue for the next year.

Despite being referred to as a phytoestrogen, studies of black cohosh have shown that it is not estrogenic (Liu, Burdette, et al., 2001; Lupu et al., 2003; Borrelli et al., 2003). Estrogenic activity was not demonstrated in *in vivo* experiments in ovariectomized mice. That murine model found no estrogen receptor ligands or the ability to induce alkaline phosphatase, pS2, or progesterone receptor.

Dr. Bolton hypothesized that black cohosh may be acting through serotonin and serotonin receptors. Serotonin selective reuptake inhibitors (SSRI) have been used successfully to treat hot flashes in women with breast cancer, and there appears to be a link between estrogen and the regulation of serotonin receptors in the brain and regulation of tryptophan hydroxylase (Pecins-Thompson et al., 1996; Loprinzi et al., 1998; Loprinzi et al., 2002; Liao et al., 1995). Looking at various serotonin receptor subtypes, Dr. Bolton and colleagues have found black cohosh to inhibit binding in two serotonin receptor subtypes ($5HT_{1A}$ and $5HT_7$). Both receptors are associated with the hypothalamus, which is involved in thermoregulation. In looking at receptor binding kinetics, black cohosh acts as a mixed competitive agonist of the $5\text{-}HT_7$ receptor. In addition to receptor binding assays, investigators at UIC also conducted functional assays to determine agonist or antagonist properties. The 5-HT7 receptor is positively coupled to G protein and promotes adenylyl cyclase activity. Adenylyl cyclase catalyzes the formation of cAMP. Thus, receptor activity can be determined by measuring cAMP concentration with a radioreceptor binding assay (Amersham kit) or an enzyme fragment complementation assay.

Activation of 5-HT7 was seen as an increase in camp production. Investigators at UIC have also conducted several preliminary studies using bioassay directed fractionation on a number of black cohosh constituents that could be responsible for inhibition activity, which further suggests that black cohosh is acting as a serotonin receptor agonist.

Black cohosh may have an effect on other systems as well. Researchers have found black cohosh to have antioxidant activity. DNA damage induced by reactive oxygen species contributes to the development of cancer. Reduction of these reactive oxygen species decreases the risk of cancer development via free radical scavenging and induction of quinone reductase (QR). Menadione, a derivative of vitamin E, is a cytotoxic quinone that is known to cause DNA damage. Black cohosh was found to protect cellular DNA from menadione-induced damage (Burdette et al., 2002).

To examine whether black cohosh causes hepatotoxicity, UIC researchers screened an ultrafiltrate containing liver microsomal metabolites of BCE using a liquid chromatography-mass spectrometry-mass spectrometry (LC-MS-MS) process that allows for the selective detection of glutathione (GSH) conjugates (Johnson and van Breemen, 2003). Indication of potential toxicity stemmed from the finding that catechols from black cohosh were activated to quinoid metabolites. However, it should be noted that there appears to be no absorption of these catechols across the intestinal epithelium. Only triterpenoids were found to have been absorbed. A completed Phase I study to measure the pharmacokinetics of black cohosh did not detect any catechol metabolites in the blood or urine of ten women following oral administration of BCE at three dosages: 40, 80, and 120 milligrams (Liang, 2004). Liver enzyme analyses from at least six women who have been taking black cohosh during the course of this one week study did not detect any change.

In summary, Dr. Bolton restated that black cohosh lacks estrogenic properties, as evidenced from both in vitro and in vivo experiments. It appears to act through serotonin receptors, with compounds acting as mixed competitive agonists of the 5-HT_7 receptor. Black cohosh has antioxidant activity and lacks toxicity, as demonstrated in Phase I and II clinical studies and in vitro assays.

In discussing their ongoing clinical research, it was noted that the UIC research team uses plant material collected from the east coast. The company that manufactured the black cohosh powdered extract initially had been employing a filler material that was inhibiting activity. This problem was solved when the filler was switched to rice bran powder. A company on the west coast encapsulates the powder, conducts accelerated stability studies, and then turns the finished product over to the UIC research team to assay. UIC researchers standardize the preparation on four active triterpenes. To date, the research team has seen no adverse events, even at higher doses. Dr. Farnsworth reiterated the need to standardize, both biologically and chemically, any substance administered as part of a clinical trial involving human subjects.

The UIC research team has considerable experience in black cohosh preparation. They have conducted over 50 collections in various regions of Appalachia where *C. racemosa* is found. They have made extracts using different solvents at different raw material to solvent ratios and have HPLC profiles on all the resultant preparations. Dr. Farnsworth noted that the concentration of triterpenes varies by plant source. For example, one plant found growing on rock had virtually no triterpenes. Since plants come from different sources, it is important to

keep root or rhizome samples from each source for identification and analytic purposes whenever possible.

Experiments in Rats and Clinical Studies

Wolfgang Wuttke, M.D., University of Göttingen (Germany)

Dr. Wuttke began his presentation by defining herbal drugs and extracts, important concepts when considering dosage. An herbal drug is a plant or part of a plant (generally in dried form) that is used to prepare herbal extracts. An herbal extract is the dried residue after extraction and removal of solvent from the extract. In the case of black cohosh, for most European preparations, the ratio of plant or root to native extract (before fillers are added) is approximately 10:1 (e.g., 100 g of plant or root will lead to 10 g of extract.) The choice of extraction solvent can affect the characteristics of the final product. While there are chemical differences between isopropanolic and aqueous ethanolic (60 percent) extracts, pharmacological or clinical differences are not apparent.

In Europe, many companies conduct research on black cohosh in in-house laboratories as well as through contract-supported research with scientists working in academia, who may also receive support for such research from other sources. Dr. Wutte mentioned three companies in Europe that provide black cohosh extract (BCE). Schaper and Brümmer in Germany produce an isopropanolic extract, Remifemin®, which is marketed in the U.S. by GlaxoSmithKline. Bionorica Company (Germany) produces an aqueous ethanol (60 percent) extract that is the source of material for researchers, such as Dr. Wuttke and other European Union-funded university researchers. Zeller is a Swiss company that also produces an aqueous ethanol (60 percent) extract and contracts with university researchers. Clinical studies have been conducted primarily with Remifemin® (Stoll, 1987) although there have been a number of placebo-controlled trials of aqueous/ethanolic extracts as well (Klimadynon®, Menofem®, ZE 450).

When Remifemin® came on the market more than 40 years ago, many plant extracts in Germany were registered as drugs, and their use was therefore reimbursed by insurance. As of January 1, 2004, use of plant extracts is no longer reimbursed. As in many European countries, patients cannot buy the product over the counter without requesting it at a pharmacy. Since prescriptions must be written in Germany for black cohosh, there should be fairly good records of black cohosh use in that country, unlike in the U.S. and many other European countries, where botanical products are available over the counter without having to ask for them.

Dr. Wutte reviewed information available on estrogenic activity of black cohosh (specifically, *C. racemosa* extract BNO 1055). A prerequisite of any estrogenic activity is the binding of the ligand to the estrogen receptor (ER). Currently two subtypes of the estrogen receptor are known: ERα and ERβ, which are encoded by two distinct genes. To examine whether any component of black cohosh binds to either receptor, ER ligand-binding assays (ER-LBA) were performed with preparations obtained from uterine tissue or human recombinant ERα and ERβ. In the initial stage of the investigations, there was evidence of binding of BCE to a cytosolic ER preparation, prepared from porcine uteri. BCE was found to compete with radioactive-labeled estradiol, indicating that the extract contained phytoestrogens. However, investigators were surprised to

find that these phytoestrogens were not able to displace radioactive-labeled estradiol from either ERα or ERβ, suggesting that some other protein or ER is involved (Jarry et al., 2003).

Investigations suggest that the aryl hydrocarbon receptor (AhR) binds compounds in extracts of black cohosh (Jeuken, et al., 2003). Stimulation of AhR causes increased production of CYP1A1, which is a detoxification enzyme that is distributed widely in the body. CYP1A1 bioactivity can be tested by a 7-ethoxyresorufin O-deethylase (EROD) bioassay, a cell bioassay that is used for detecting dioxin-like compounds (Whyte et al., 2000). In the EROD binding assay, 7-ethoxyresorufin is converted by CYP1A1 to resorufin, and resorufin can be detected and measured. The results of experiments on black cohosh show that BCE stimulates CYP1A1 protein expression (unpublished findings).

Black cohosh has been used for symptoms associated with menopause for many years. BCE have been associated with a reduction in the number of hot flashes in clinical studies and hot flash equivalents in a model of ovariectomized rats. In a recent double-blind randomized study that compared placebo, conjugated estrogens, and black cohosh (preparation BNO 1055), the therapeutic effects of black cohosh were equally potent to conjugated estrogens in diminishing vasomotor symptoms (Wuttke, Seidlova-Wuttke, and Gorkow, 2003). Moreover, treatment with black cohosh had no effect on endometrial thickness, which was significantly increased among women receiving estrogen. The lack of effect of black cohosh on endometrium is confirmed by animal studies. Dr. Wuttke and his colleagues recently completed a one-year study (MEN-SES-1 Study) of BCE. This study did not include a placebo control because the primary endpoint was an objective measure of endometrial thickness. (Secondary end points included climacteric complaints as well as changes in mammary tissue, bone, and clinical chemistry, including liver enzymes and hemostasis endpoints.) This study found no significant effect on endometrial hyperplasia or endometrial thickness as measured by sonography. Similarly, Osmers and colleagues (2005), utilizing the isopropanolic extract present in Remifemin®, and Saller et al. (2005), using another ethanolic extract, reported significant beneficial effects on climacteric complaints. In the latter study, this effect was dose dependent. Dr. Wuttke noted that it would be interesting to learn more about the effect of black cohosh on the brain, and more specifically the hypothalamus, to understand its relationship to endocrine functions, including leutenizing hormone levels, as well as to other tissues affected by menopause and aging, such as bone, breast and endometrial tissue.

Dr. Wuttke next discussed the effect of black cohosh on bone. In a rat model, researchers measure bone mineral density of the tibia, which is sensitive to levels of estrogen. Within three months of an ovariectomy, up to 50 percent of bone density is lost in rats. This loss in bone density can be prevented with E2, and to a lesser degree by black cohosh and soy. Ovariectomized rats experienced significant improvement in tibia when treated with E2 or black cohosh (Wuttke, Jarry, Westphalen, et al., 2003). In his recently completed double blind, placebo controlled study that compared conjugated estrogen and black cohosh (CR BNO 1055) in a sample of postmenopausal women, Dr. Wuttke and colleagues found the black cohosh preparation to have comparable effects on serum markers of bone metabolism as estrogen (Wuttke, Seidlova-Wuttke, and Gorkow, 2003). Data on alkaline phosphatase (a marker for bone formation) or CrossLaps (a serum marker of bone degradation) indicate that black cohosh may have an osteoprotective effect due to increased bone formation.

Findings of increased risk for breast cancer in women receiving estrogen and progesterone in the Women's Health Initiative (WHI) has raised question about the safety of phytoestrogens. While black cohosh does not appear to have estrogenic activity, as noted above, questions remain about the effect of this botanical on breast tissue. Unlike the effect of E2, black cohosh does not seem to stimulate mammary gland tissue (Wuttke, Jarry, Westphalen, et al., 2003). Recent evidence argues against a mammary cancer-stimulating effect (Wuttke, Jarry, Becker, et al., 2003). In other studies, as little as 10 nanograms of purified BCE inhibited proliferation to MCF7 cells (derived from human Caucasian breast cancer cell line) and inhibited proliferation of E2-stimulated MCF7 cells. In only one study did BCE stimulate MCF7 cells (Kruse, et al., 1999). Dr. Wuttke therefore concluded that, in E2-sensitive MCF7 cells, BCE (CR BNO 1055) or fractions thereof had no stimulatory effects on proliferation of MCF7 cells, with most fractions inhibiting proliferation.

In summarizing data from his research on BCE BNO 1055 in the ovariectomized rat model, Dr. Wuttke and colleagues found that this black cohosh extract:

- inhibits pulsatile LH release, perhaps accounting for decrease in hot flashes;
- has anti-osteoporotic effects;
- has a mild vaginotropic effect;
- has tonus-stabilizing effects in the urinary bladder;
- has no uterotropic effect nor does it affect E2-regulated genes;
- has no mammotropic effects; and
- has no effect on serum lipids.

Regarding effect of black cohosh on the liver, Dr. Wuttke's review of the clinical literature found few if any problems. Neither in a subsequent publication of the study by Wuttke and colleagues in 2003 (Wuttke and Seidlova-Wuttke, 2005) nor the studies of Saller et al. (2005) and Osmer and coworkers work (2005) were there any effects on liver or hemostasis parameters.

Hepatotoxicity is a serious health concern. Dr. Wuttke routinely screens for liver function in studies that he has conducted on black cohosh, and he has never identified such an adverse effect from this dietary supplement ingredient. He expressed some surprise at the level of concern about black cohosh in the U.S. at this time.

Dr. Wuttke recommended a broad research agenda for black cohosh. That research should build on existing research findings, looking at potential benefits for bone, lipids, and menopausal symptoms, as well as its possible hepatotoxicity and adverse effects on mammary tissue, the endometrium, and hemostasis. This research should utilize a range of tools from areas such as cell biology, animal models, and clinical research.

Effect on Breast

Edward Sauter, M.D., Ph.D., University of Missouri –Columbia

Dr. Sauter briefly reviewed *in vitro* data on the effects of BCE on the breast, which were consistent with what had been presented by Dr. Wuttke. Liu and colleagues (2001) found that BCE decreased the doubling time of MCF7 cells, as did estradiol, when compared with control. In another study, the proliferation of EMT6 mouse breast cancer cells was not altered by three preparations of BCE (Rockwell et al., 2003). BCE also did not alter the response of EMT6 cells

to radiation or cisplatin but increased the toxicity of adriamycin and taxotere. These findings have potential implications for breast cancer patients. The preponderance of scientific data, particularly from more recent studies, indicates that BCE does not increase the proliferation of breast cancer cells (Hostanska et al., 2004; Bodinet et al., 2002; Zierau et al., 2002; Zava et al., 1998; Liu, Yu et al., 2001; Burdette et al., 2002; and Liu, Burdette et al., 2001). Animal studies demonstrate that BCE does not affect mouse mammary tumors or breast cancer cell growth at BCE doses below, at, or significantly above equivalent human doses (Freudenstein, et al., 2002; Davis, et al., 2003; and Rockwell, et al., 2003).

Dr. Sauter then reported on two studies of breast cancer survivors. In the first study, tamoxifen with and without BCE was administered for 12 months to 136 breast cancer survivors in a randomized open label trial. When compared with those who did not receive BCE, women in the BCE group reported fewer severe hot flashes (24 percent versus 74 percent of women) (Hernandez and Pluchino., 2003). In the second randomized, double blind, placebo controlled study, 85 breast cancer survivors, most of whom were post-menopausal, were given either BCE or placebo for 2 months. Analyses were stratified on tamoxifen use. There was no significant effect of BCE on hot flashes, follicle-stimulating hormone (FSH), or LH, although a reduction in sweating in the BCE group was observed (Jacobson et al., 2001).

Dr. Wuttke noted that tamoxifen increases the severity of hot flashes, so studies of BCE in tamoxifen users can be confounded. Dr. Sauter clarified that only a portion of women (68 percent) in the Jacobson study received tamoxifen, and some of the women were post-menopausal, thus raising concerns that the results must be interpreted with caution as the study groups were not ideally matched.

In 1996, Petrakis and colleagues reported on a 12-month study of 24 healthy pre- and postmenopausal white females from whom nipple aspirate fluid (NAF) and blood specimens had been collected for three months for baseline data. The investigators then collected both fluids for six months while women were ingesting a soy protein isolate containing 38 mg genistein. Finally, fluids were collected for a final three-month washout period. Each subject served as her own control and as well as a point of comparison with other subjects. There was a two to six fold increase in NAF volume (a surrogate endpoint of secretory or proliferation activity in the breast) and plasma estradiol in premenopausal women and the development of cytologic hyperplasia in 29 percent of pre-menopausal women and 30 percent of postmenopausal women on soy. This was one of the earlier clinical studies showing a potential breast-specific effect of soy.

Another marker of estrogenic activity, pS2 protein, has also been examined. pS2 levels in NAF rise after treatment with HRT and fall with administration of tamoxifen (Harding et al., 2000). A study involving 84 pre-menopausal healthy women who took soy for two weeks found increased pS2 levels in NAF ($p<0.002$) in response to soy supplementation (Hargreaves et al., 1999). However, there was no effect of soy on breast epithelial cell proliferation. This suggests that pS2 is an early indicator of estrogenic activity, and short-term supplementation may not be sufficient to detect changes in cell number or morphology.

The previous two studies were important starting points for the study that Dr. Sauter and his colleagues are undertaking to learn whether BCE alters a woman's breast cancer risk. Dr. Sauter's team, which has been looking at systemic and organ-specific effects of specific food

substances, such as soy, modified citrus pectin, and resveratrol, has examined nipple aspirate fluid (NAF) from the breast, a collection of secreted proteins and shed ductal cells, which are organ specific. Frequently the levels of proteins in the breast are not reflected well in systemic circulation. Results from that study are pending completion. Dr. Sauter noted that his study is not a placebo-controlled trial but compares changes in NAF biomarkers compared with baseline in samples from each woman. The study seeks to determine if ingestion of BCE for 12 weeks by postmenopausal women experiencing menopausal symptoms results in estrogenic stimulation of breast tissue as measured by changes in NAF levels of estradiol, pS2, FSH, and LH, as well as NAP cytology. The researchers also intend to evaluate if BCE saponins are detectable in the NAF of women receiving BCE and if this treatment leads to a reduction in postmenopausal symptoms. The study has accrued 60 percent of the 73 subjects it seeks to enroll. Preliminary interim data show that BCE significantly relieves menopausal symptoms. However, data suggest that symptoms return after the washout period. To date, they found no significant effect of BCE on NAF cytology. For a minority of women (13 percent), cytology went from normal to hyperplasia at 4 weeks. However, when cytology from baseline was compared to cytology at 12 weeks, the women exhibiting hyperplasia at 4 weeks had returned to normal. Overall, there was no significant effect of BSE on NAF cytology or pS2 level.

Effect on Prostate

Adrian Dobs, M.D., M.H.S., Johns Hopkins University

The existing literature on the effects of black cohosh on the prostate disease raises more questions than answers. Dr. Dobs provided some background on the epidemiology of prostate cancer (PC), prostate growth and factors regulating it, complications of treatment, and the possible role of black cohosh in the management of prostate disease.

U.S. age-specific incidence rates of prostate cancer rise around age 55, with higher rates for black males than white for unknown reasons. Autopsy data from 249 cases in Wayne County, Michigan, where access to health care did not differ significantly by race, suggest that factors other than health care access may account for increased mortality rates among African Americans, with perhaps genetic or other factors producing more aggressive disease in some groups (Sakr et al., 1994).

The United States and Japan have comparable prevalence rates of PC based on histological evidence, although mortality rates for PC are not as high among Japanese men when compared to American men. Several people have speculated about the role of soy in the diet with respect to PC (Carter, 1990).

An important problem in the study of PC is the issue of occult disease. The prevalence of abnormal cells, or high-grade prostatic intraepithelial neoplasia (HGPIN), and occult PC increase with age (Sakr, 1994). Important questions remain concerning the relationship of HGPIN and the risk of progression to PC. Some investigators have considered the possible use of botanicals to prevent progression of disease. However, this area remains controversial and requires further investigation.

It is known that serum prostate specific antigen (PSA) levels have poor predictive value for PC. Biopsies done as part of the PC Prevention Trial (PCPT) found that 15 percent of healthy men over age 55 years with negligible serum PSA levels (0.0-1.0 ng/mL) at entry into the study in

fact had PC (Thompson et al., 2003). There is a significant incidence of PC at what is considered "normal" PSA levels (< 4.0 ng/mL). In the PCPT, for example, 16.3% of the 2,196 men with PSA levels of 0 – 1.0 ng/mL were found to have PC on biopsy. That rate rose to almost 30% among men with PSA levels of 2.1-3.0 ng/mL. Although tumor mass is highly correlated with circulating serum PSA, PSA levels are difficult to interpret. For example, PSA levels can be higher in the prostate gland of normal men when compared to men with PC, but blood vessel leakage also is higher. So there is a "leakage" factor that affects systemic PSA levels. There is also a relationship between PSA levels and the size of the prostate gland, suggesting that levels should be standardized to prostate mass. Velocity of change in PSA levels may be a better measure for PC than absolute levels. For example, a change of 2 ng over the course of one year may be significant. In the PCPT, subjects underwent a biopsy whenever there was a change in velocity of PSA change that clinically suggested a need for a biopsy.

With respect to prostate histology, Dr. Dobs described how normal prostate tissue changes. Normal tissue undergoes proliferative inflammatory atrophy; prostatic intraepithelial neoplasia (PIN) precedes full-blown prostate cancer (Nelson, 2003). The mechanism of conversion from PIN to neoplasia is not clear. Some have speculated about the role of antioxidants in the prevention of a conversion of inflammation to PIN and cancer (Nelson, 2004; Russo et al., 2004; Nelson et al., 2004), but little is known about this.

Prostate growth is hormone dependent, mediated by testosterone, dihydrotestosterone (DHT), and estrogen. DHT is often implicated in proliferation of prostatic tissue, but estrogen may be the more active hormone in inducing prostate growth. It is unclear if estrogen is a modulator or direct stimulant of PC, but ironically synthetic estrogen (diethylstilbestrol or DES) is used to treat PC. Most PC tumors are treated with medical castration (GnRH agonists, anti-androgens). Of considerable debate is whether men are being over-treated in the process of making them hypogonadal. Androgen receptor signaling in PC can occur in androgen-dependent PC and androgen-independent PC. Often men with hormone-dependent tumors are given a cycle of treatment with GnRH agonists to shrink the tumor; after stopping treatment, the tumor grows back, and it is again treated with another round of GnRH.

It is known that testosterone therapy can produce change in the prostate. Several studies have shown that testosterone therapy grants important physical and psychological benefits to hypogonadal men, but some concerns remain about the possible risks associated with this therapy. There are unanswered questions regarding the relationship of benign prostatic hyperplasia (BPH) and testosterone treatment. This is of particular importance for hypogonadal older men, who may already be at risk for BPH. However, one study has shown that elderly hypogonadal patients receiving intramuscular (IM) testosterone therapy experienced fewer bladder outlet obstruction symptoms than younger hypogonadal men receiving IM testosterone (Haijer, et al. 1997). A few studies have looked at PSA levels in prospective and controlled studies of testosterone replacement therapy. (See for example Meikle et al., 1997.) However, more research is needed to fully understand the effects of testosterone therapy and the various forms in which it can be delivered upon PSA levels and prostate volume. Although it is known that testosterone accelerates the clinical course of prostate cancer and may stimulate the growth of previously undiagnosed prostate tumors, there is no evidence from short-term studies that testosterone therapy increases the incidence of prostate cancer. However, these short-term studies may be too limited to make inferences with respect to long-term safety (Basaria and Dobs, 1999).

The state of the science points to a number of dilemmas in the detection and treatment of PC. Should PSA screens be routinely provided and, if so, for which age groups? Given uncertainties with respect to the natural history of PIN and the progression of disease as well as the lack of specificity of PSA, are men being over-diagnosed and over-treated? Is medical castration the best treatment option given complications associated with hypogonadism?

Growth factor signaling in PC reveals a very complex process. If there is a role for black cohosh or estrogen to play in the modification of disease progression, it could happen at any number of steps during the development of cancer. For example, animal studies have found black cohosh compounds to have inhibitory effects on mouse skin tumor production (Sakurai et al., 2003) and on prostate tumor xenografts (Ng and Figg, 2003). The precise mechanism through which it might operate is not clear, perhaps reducing microvascular density around the tumor, inducing an antioxidant effect, or inducing some dopaminergic activity with the tumor cell. At this time it is unclear if black cohosh might be effective in the prevention of induction of prostate disease or the treatment of disease or the treatment of complications associated with PC therapy. For example, men undergoing androgen ablation therapy suffer from vasomotor symptoms. Black cohosh may be able to ameliorate those symptoms. Medical induction of hypogonadism is also associated with osteopenia and osteoporosis, as well as feminization and increased body fat, increased insulin insensitivity, and decreased sexual function. All of these side effects could have a profound effect on the quality of life for this population. Dietary supplements, including black cohosh, red clover, and soy, represent possible treatments to deal with a number of symptoms (Spetz et al., 2003).

In summary, there are several plausible mechanisms by which black cohosh may be useful in prostate disease. It can inhibit prostate cancer xenografts via an anti-angiogenic mechanism; it has an anti-oxidant effect; and it appears to have dopminergic D2 activity (Ng and Figg, 2003). While animal data suggest a reduction in tumor growth associated with BCE, one must be cautious about inferences drawn from these limited studies and the different preparations of black cohosh available. Dr. Dobs hypothesized that black cohosh is unlikely to have a role in the pathogenesis or direct treatment of PC, but it may be useful for symptoms associated with medically induced hypogonadism, treatment of PC, including vasomotor symptoms. Indeed, many men with PC already take black cohosh to deal with symptoms associated with treatment. However, meeting participants added that one should not overlook the possible beneficial role that black cohosh could have on tumor growth. Everyone agreed that more research was needed in this area. And as was the case with research on black cohosh in women, existing studies have been relative short term. The group noted the need for longer studies and the need to include a wash out period for studies using cross over designs.

Black Cohosh, Breast Cancer, and Metastases to Lung: Data from the Mouse Model

Vicki Davis, Ph.D., Duquesne University

The goal of Dr. Davis' study is to examine the effects of black cohosh on different stages of mammary tumorigenesis, including tumor development, growth, and progression, using the mouse mammary tumor virus (MMTV)-neu transgenic mouse model. This model was selected because of characteristics shared with human breast cancer and detectable endpoints. All stages

of the disease can be examined in this model, including primary mammary tumor development; latency or age of onset, which is variable; multiplicity of tumors; tumor growth and survival of animals; incidence of metastastic diseases, which includes necessary steps from invasion to colonization in lung. Dr. Davis has used this MMTV-neu model for other investigations, which have demonstrated decreased incidence of tumors with tamoxifen preventive treatment and ovariectomy.

Dr. Davis' study was conducted using one batch of Remifemin®, which was an isopropanolic extract in liquid formulation. The only information on the quality and characteristics of the product was that provided by the manufacturer.

Black cohosh was provided in the diet. This was done for a number of reasons: it is a non-traumatic method for continuous long-term treatment; it accounts for metabolism versus body weight in mice; and, according to Dr. Davis, it mimics oral ingestion in women. The lowest recommended dose for humans is 40 mg, and women on average consume 1800 calories a day. Because mice do not consume 1800 calories a day, their dose was adjusted to approximately 0.4 mg a day based on typical caloric intake. The control diet was isoflavone-free. Breeders were maintained on isoflavone-free diets, and study females were maintained on the control diet until treatment was initiated. Treatment began after sexual maturity was achieved, and only intact females were used in the study to more closely mimic the hormone environment found in perimenopausal women. In general, black cohosh therapy was well tolerated by the mice.

The study design included two groups of two month old MMTV-neu females: (1) a control group fed an isoflavone-free diet; and (2) a treatment group fed a variant on the control diet that included BCE in an amount proportional to 40 mg per 1800 calories of diet. Mice used in this study were derived from MMTV-neu dizygous (+/+) males, which were bred with FVB/N wild-type (-/-) mice, to generate hemizygous (+/-) study females. Two studies were conducted using these animals: a pre-tumor study (n=25/group) and a tumor study (n=150/group).

For the pre-tumor study, treatment with black cohosh was initiated at two months of age and lasted for one month. The mice in this group were euthanized in estrus to standardize the point in time in the estrus cycle for all mice. The endpoints for the pre-tumor study included: (1) serum estradiol levels; (2) serum progesterone levels; (3) body weight; (4) uterine weight; and (5) removal of mammary tissue for future histologic analysis. Compared with control animals, animals given the black cohosh diet showed no significant differences in steroid hormone levels (estradiol or progesterone levels), body weight, or uterine weight.

For the second tumor study, mice were randomized at 2 months to either the black cohosh diet or continuation of the control diet. Treatment was started at a point in time that should predate tumor initiation for most females but would include animals with mature mammary glands. Mammary gland exams were conducted every week with palpations and tumor measurements. The maximum age of animals included in this study 16 months. When animals developed a large tumor that restricted movement or affected their general health, they were euthanized. Most of the animals did not survive to 16 months of age.

The tumor study endpoints included: (1) incidence (number of females with primary tumors); (2) latency (age when tumor developed); (3) growth (survival time after detection); (4) multiplicity (number of mammary tumors); and (5) metastatic incidence (number of animals with lung

tumors). By 16 months of age, approximately 95 percent of animals would be expected to develop tumors. When comparing treatment and control groups, there was no significant difference in latency of tumor development, tumor multiplicity, body weight at necropsy (although there is variability in both groups), or uterine weight. Survival curves for both groups were very similar, suggesting that black cohosh has no effect on mammary tumor development. Both treatment and control groups averaged two tumors per animal, with the number of tumors per animal ranged from one to five.

One feature of the MMTV-neu model is that animals with mammary tumors will develop lung metastases. Lung metastases were detected by gross visualization and pathology. Dr. Davis compared the incidence of tumors in lungs of mammary tumor bearing animals receiving black cohosh to those receiving the control diet. On gross visual examination, the percent of animals in the black cohosh group with lung tumor was significantly higher than control animals (27% versus 10%). Similarly, among animals the lung tumors, the percentage with multiple lung lesions ($\geq$ 3) was greater in animals given black cohosh than control animals (70% versus 25%). The survival time for animals with lung tumors was not different across groups. Blinded histological examination of lung tissue found a statistically significant increase in the incidence of metastatic lesions in the black cohosh-treated mice with primary tumors compared with the control group. However, there were no statistically significant differences detected between the animals fed black cohosh and those fed the control diet for intra- or extra-vascular lesions or number of total lung foci.

In summary, the only significant differences detected in animals receiving black cohosh when compared with those receiving the control diet was an increased incidence of lung metastases as detected by gross examination of the lung as well as by histopathology. Data on development of primary mammary tumors suggest that black cohosh would not alter breast cancer risk. However, data with the increased incidence of metastatic cancer may suggest that black cohosh has the potential to increase the aggressiveness of existing primary tumors.

Some of the limitations of this study are shared by all studies using animal models, which cannot mimic all aspects of human disease. In terms of the relevance of the MMTV-neu model to human breast cancer, the model is not an exact replica of breast cancer among postmenopausal women but perhaps is better than other models that rely on ovariectomized mice, which results in tumors in only a small minority of animals and occurs in mammary tissue in the absence of estrogen. A more specific limitation of the Davis study is the duration of treatment, which would correspond to continuous use of black cohosh from adolescence until death.

Discussion

Jeffrey E. Green, M.D., National Cancer Institute, Discussant

Dr. Green broadened the discussion to look at other animal models of breast cancer. He noted that it is not clear how good mouse models are in predicting human disease and then identified a number of limitations with the MMTV-neu mouse model. Perhaps the most notable limitation relates to the promoter region that directs expression to mammary epithelial cells. It is a hormone-responsive promoter that can be activated by pregnancy, glucocorticoids, and progestins. Investigators using the MMTV-neu model may be modulating activation of the promoter and need to assess this possibility to avoid misinterpreting the meaning of a therapeutic response.

Dr. Davis noted that MMTV is regulated by glucocorticoids, progestins, and androgens. Therefore, in females, progestins may regulate expression levels of the neu transgene via this promoter. But male MMTV-neu animals with high levels of testosterone do not react similarly, so this issue it is not simply stimulation. In addition, the c-neu itself is upregulated by progestins in breast cancer and the normal breast, so the transgene may mimic the progestin-regulation of the normal gene (Taverna et al., 1994; Gompel et al., 1996). The issue does not appear to be an increase in expression but rather some conversion that occurs. Progestins are going to influence ER levels and thereby estrogen estrogen responsiveness. Mice have the normal c-neu gene and transgene, but neither are oncogenes initially. When looking at mutational analysis, it appears that the mutation is occurring in the transgene. In humans, mutations rarely occur in the gene itself. Instead, tumor incidence is driven by either amplification or over-expression through a different mechanism. In mice, a mutation may affect activity of the neu receptor instead of just its over expression.

Dr. Green noted that in the transgenic mouse model, somatic activating mutations of neu occur in the tumors, and, therefore, selective advantage for the mutations seems to occur in the tumors, which may not result simply from over-expression. In humans, tumor incidence is driven by either amplification, multiple copies versus driven promoter, or over-expression through a different mechanism.

Dr. Davis indicated that in her studies and other published reports, tamoxifen is effective at inhibiting mammary tumor development in the MMTV-neu model. Tamoxifen inhibits the estrogen stimulation that can lead to breast tumor development and suppresses the growth of estrogen responsive tumors, and thus prevents the disease at its early stages. However, as in women, some tumors do develop in the presence of tamoxifen prophylaxis, suggesting that these tumors are ER negative or nonresponsive to estrogen, as is characteristic of neu (erbB2 HER+) tumors. This indicates that this model is similar to breast cancer in tumor prevention by tamoxifen since only ER negative tumors would develop.

Knockout models may represent a useful tool to examine problems associated with black cohosh and metastatic lesions from breast cancer to lung. One of the elements less prominent in tumors that develop using a transgene approach is genome instability, which may help explain some of the mutations during the tumor development process. Some of the knockout models that delete genes important for genome instability, like BRCA1 and p53, exhibit higher levels of genome instability than the transgenic models and therefore mimic human breast tumors more closely in this regard. A knockout model also eliminates the dependence on the promoter that may respond to therapeutic interventions. There are inducible models that can either turn on a trans-gene or lead to mutations of targeted genes at a particular time in a particular tissue. Inducing such changes following pregnancy, ovariectomy, or late in life may approximate certain human physiological conditions. Such manipulations may be a closer approximation to the stage of menopausal women, although mice don't go through menopause.

Dr. Davis cited a study that used an MMTV-neu model (with the neu oncogene) and impaired transforming growth factor (TGF) beta signaling (Siegel et al., 2003). In these animals, there was no difference in the time of tumor onset or tumor size and no effect on early stages of tumor development. But there was a decrease in tumor metastases. Metastases were found in the lung, the primary site of occurrence in mice. In addition, increasing TGF beta signaling has been

found to suppress the formation or growth of primary mammary tumors and to increase pulmonary metastases (Siegel et al., 2003; Muraoka et al., 2003). There has been only one model that has looked at tumor cell dormancy. A critical issue is whether seeding occurs that makes these cells emerge from dormancy and if compounds exist that can suppress the process. Similarly, there remain questions concerning immune surveillance effects that might allow more cells to survive and migrate to lungs and other areas.

Finally Dr. Green raised the possibility of using proteomics/microarray analyses to identify mechanisms of biologic responses resulting from the administration of preventive agents with gene expression. This could greatly accelerate progress in the field.

Black Cohosh and Hepatotoxicity: Case Reports

Hepatitis Associated with Black Cohosh

Stanley M. Cohen, M.D., Rush University Medical Center

Case studies are important because they can alert people to emerging problems. Acute liver failure is often idiopathic, but there are clinical risk factors known to be associated with toxicity, such as cumulative exposure, as has been seen with botanicals containing toxic pyrrolizidine alkaoids. Herbal hepatotoxicity is seen most frequently in older women, with more than 80 percent of cases in women with an average age of 45-60 years (Stedman, 2002). In establishing histories, it often is difficult to determine what dietary supplement has been used and for how long. The lack of standardization of products is also a major concern since it makes it difficult to know exactly what was actually consumed. The mechanisms of toxicity are generally unknown but there are several possibilities, including direct hepatotoxicity, toxicity from metabolites, induction of autoimmune hepatitis, interaction with other medications, allergic reactions, dosage-related, and idiosyncratic hepatotoxicity.

Black cohosh has been reported to decrease menopausal symptoms, but trials are extremely variable with respect to outcomes, and little is known about mechanism of action, active ingredient(s), efficacy for a range of purported uses, and safety. As of 2003, there have been 51 adverse events (AEs) associated with black cohosh reported to various monitoring bodies (Food and Drug Administration [FDA], WHO, Adverse Drug Reactions Advisory Committee [ADRAC], German Federal Institute for Drugs and Medical Devices [BfArM], the Committee on Safety of Medicines [CSM]). Dr. Cohen's review of the literature found five AEs associated with liver and black cohosh use, two of which he described in more detail.

<u>Case #1:</u>
A 57 year-old African American female complained of lethargy and fatigue for two weeks; she reported no other symptoms. Her prior medical history included diabetes and hypertension. She had been taking a variety drugs, including labetolol, fosinopril, verapamil, metformin, aminosalisylic acid (ASA), and insulin, all of which were taken for a period of more than two years, and a black cohosh product, which was taken for the previous three weeks. She had no history of liver or autoimmune diseases; alcohol, tobacco, intravenous drug abuse; transfusions; tattoos; or recent travel. She reported no abdominal pain, nausea, vomiting, fever, chills, pruritus, or contact with sick people. Her physical exam was unrevealing. Presenting labs included an ALT level of 1234 U/L, AST of 509 U/L, and AP of 170 U/L. Bilirubin, albumin, complete blood count and INR were all normal. The patient underwent further clinical

evaluation and was found negative for hepatitis A, B, and C and negative for hepatitis C virus RNA, although there was some evidence of prior hepatitis. The ANA titer was 1:640 with a homogeneous pattern. Right upper quadrant ultrasound was unremarkable, and smooth muscle antibody was negative. A liver biopsy showed piecemeal necrosis with plasma cells and eosinophils. Lobular infiltrates were also present. The clinical diagnosis was drug-induced autoimmune hepatitis, most likely due to black cohosh. Black cohosh was discontinued, and a tapering steroid course was instituted. The patient had complete resolution of symptoms within two weeks and complete resolution of liver function test within nine weeks. The subject was taken off steroids to rule out an allergic reaction. She did fine, but returned in approximately four months with jaundice and ultimately was placed on a maintenance regimen of azathioprine.

Dr. Cohen concluded that this is probably the first case of autoimmune hepatitis that was likely to have been induced by black cohosh. Evidence to support this diagnosis includes correct temporal relationship of botanical use to clinical signs and symptoms; conversion to positive antinuclear antibodies (ANA); biopsy with autoimmune and drug-induced features; and rapid response to steroid therapy.

Case #2

A 54 year-old white female presented with lethargy, nausea, and vomiting for the previous eight weeks, and she had grossly abnormal liver function tests. She had been on black cohosh for about three months. She had a history of hypothyroidism, fibromyalgia, osteoarthritis, and depression. She reported use of Synthroid, Prozac, and Darvocet. She had been taking a black cohosh product for the previous three months since her physician had told her not to go on hormone replacement therapy. The patient, who was an X-ray technician, had a very extensive workup at the hospital. She also got herself tested regularly at the hospital and was able to produce prior laboratory test results. She had no history of liver or autoimmune disease nor did the patient report any tattoos or intravenous drug use. She reported drinking one to two glasses of wine per day. She had traveled to Mexico seven months earlier. The initial laboratory data showed grossly elevated liver enzymes levels (AST of 1014 U/L, ALT of 1003 U/L, AP of 266 U/L). However, levels dropped unexpectedly in a few days and then increased again. Physical exam was unrevealing except for two cm hepatomegaly. Right upper quadrant ultrasound was normal. Further laboratory evaluation for infectious etiology found a positive test for hepatitis B surface antibody only (tests for HBsAG and HBclgM were negative) and a low positive test for Herpes simplex virus IgM. She was diagnosed with hepatitis of unknown etiology, which was generally interpreted as drug-induced as opposed to autoimmune hepatitis. She was told to discontinue black cohosh and alcohol. She was readmitted nine days later with nausea and vomiting, worsening jaundice, and mild confusion. She reported no alcohol use for the 14 days prior to admission and no use of black cohosh during the previous 14 days. The physical exam revealed asterixis and jaundice. Grossly elevated liver enzyme rates were found at re-admission (AST of 2083 U/L, ALT of 1324 U/L, AP of 296 U/L, and bilirubin of 16.1). The patient was put on steroids and the waiting list for a liver transplant. She died in the operating room during the liver transplant procedure. The pathology of the liver revealed extensive confluent necrosis and collapse, cholestasis, acidophil bodies, and minimal inflammation, a profile that is consistent with toxin-induced hepatitis.

Discussion

Discussion of the first case revealed that there is no certainty that the product taken by this patient included only black cohosh or any black cohosh. The patient recalled seeing the words

"black cohosh" on the bottle but could not remember any details about the label or the bottle, which she had discarded. Participants noted problems ascertaining precisely what product or material was taken in case reports and the importance of this step before an assignment of association can be properly made. The lack of authentication of material poses significant difficulties in the interpretation of case reports.

Questions were raised concerning the rate of spontaneous hepatatoxicity occurring in the general population. No estimates were known, which caused one participant to speculate that black cohosh might actually be protective if hepatotoxicity occurs less frequently among individuals taking black cohosh than in the general population.

Moreover, the group cautioned that there were problems establishing a cause and effect relationship on the basis of information presented. The hepatotoxicity might be coincidental or correlated with this adverse event but not necessarily be the cause.

Herbal Hepatitis – Black Cohosh

Paul Kerlin, M.D., Princess Alexandra Hospital (Australia)

In Australia, approximately half of the population report using CAM in some form. In 2000, Australians spent $2.3 billion on alternative therapies, a 62 percent increase since 1993 (MacLennan et al., 2002). Fifty-seven percent of CAM users did not tell their doctor about their CAM use. The CAM "craze" in Australia has been attributed to a number of factors, including a greater emphasis on chronic illness with an aging population; declining faith in the ability of science and technology (including medicine) to solve problems; social "green" movement preference for organic and non-chemical materials; individualism, which is associated with seeking greater levels of control over one's own life; more CAM providers, often paid by the state or insurance plans; and increased migration and transmission of established medicines from other lands, such as Chinese herbs (Coulter and Willis, 2004).

Dr. Kerlin presented two case studies of hepatotoxicity. In the first case, the patient took a product containing black cohosh alone. In the second case, the patient took a preparation that contained a mixture of herbals, including black cohosh.

Case 1

A 47-year-old women was admitted to the Princess Alexandra Hospital with acute liver failure. Three weeks before admission, she started taking black cohosh (Remifemin®) for menopausal symptoms at the manufacturer's recommended dose of one tablet twice daily. She ceased taking the product after noting the presence of dark urine on day 6 of use. Two weeks before admission, she developed lethargy and jaundice. During the 24 hours before admission, she was increasingly confused and incoherent. On admission, she was noted to be jaundiced and had grade 3-4 hepatic encephalopathy. She required intubation to protect her airway. An ultrasound examination demonstrated a patent portal vein and a small liver. The following liver tests were run with the following results: bilirubin 32 mg/100 ml; ALP 153 u/L; AST 1203 u/L; ALT 1276 u/L; γGT 74 u/L; and INR 5. The patient was negative for hepatitis A, B, and C (including negative tests for HBV DNA and HCV RNA). Autoantibodies (ANA, SMA, AMA) were negative. Serology for EB virus and CMV was negative. The patient required an urgent liver transplant, which was uneventful. The liver explant weighed 398 grams (median for normal

women is 1400 grams) and demonstrated massive hepatic necrosis. The patient remains well with normal liver tests four years later.

Case 2

A 52 year-old woman with acute liver failure had taken an herbal preparation for three months (for severe tinnitus) until four weeks before admission. The multi-herbal mixture, which was made and provided by a pharmacist, included black cohosh as well as ground ivy, golden seal, ginkgo, and oat seed. The patient was taking 7.5 ml of this herbal mixture twice a day for three months. She ceased use one month prior to admission. She experienced deep jaundice but no encephalopathy. One week later, she developed hepatic encephalopathy and hepatorenal failure. Laboratory tests found her bilirubin to be 20 mg/100ml, ALP of 230 U/L, ALT of 1380 U/L, and gamma-glutamyl transpepsidase of 134 IU/L, and INR of 3.0. Serology for hepatitis A, B, and C was negative. Extensive investigation excluded other recognized causes of acute liver failure. She underwent a liver transplantation and had an uneventful postoperative course. The explant liver showed massive hepatic necrosis. Analysis by the Therapeutic Goods Administration (TGA) in Canberra, Australia confirmed the presence of black cohosh, as well as goldenseal and ginkgo, and no undeclared pharmaceutical drug (Lontos et al., 2003).

The Committee on Safety of Medicine (CSM) in the UK recognizes that black cohosh is traditionally used to treat a range of conditions (e.g., rheumatism and rheumatoid arthritis; intercostal myalgia; sciatica; chorea; tinnitus; dysmenorrhoea; uterine colic; post menopausal symptoms) with some recognized adverse effects (e.g., gastrointestinal irritation, vomiting, headache, dizziness, hepatotoxicity). CSM has received seven reports of hepatotoxicity associated with black cohosh; all the patients recovered although one had experienced life-threatening hepatitis. The UK encourages the reporting of hepatotoxicity and for physicians to enquire whether herbal medications are being used by their patients.

Dr. Kerlin noted that medical practitioners should be aware of potential herb-drug interactions. Izzo and Ernst (2002) have drawn attention to interactions between St. John's wort and cyclosporin, digoxin, warfarin, and theophylline. Dr. Kerlin added that if St. John's wort lowers the blood level of cyclosporin, organ grafts might be lost. Moreover, he noted that gingko causes bleeding when used with warfarin, and both ginseng and garlic can lower the blood concentration of warfarin.

In Australia, the TGA is responsible for regulating medicines and medical devices, plants manufacturing, chemicals, as well as recalls, alerts, and reporting of problems. The medicines include prescription (registered) and non-prescription (over-the-counter/listed) medicines, as well as complementary medicines. The TGA requires that the distributors of complementary medicine products hold data on quality and safety of the product, but there is no requirement to substantiate efficacy. There is no compulsion to use the established system of reporting complications as is required with conventional medicines.

In conclusion, Dr. Kerlin observed that almost all physicians in clinical practice will at some point "share care" with a CAM practitioner, so there is a need to ask explicitly about complementary and alternative medicines. The efficacy and safety of CAM products is assumed and seldom questioned. CAM cannot exist in an "evidence-free zone" – the effectiveness and safety of CAM products needs to be determined just as rigorously as they are for prescription medicines.

Several participants questioned the strength of the link between black cohosh use and hepatotoxic effects. Dr. Kerlin stated that he believed that black cohosh-induced hepatitis is very real, although he acknowledged that there might be confounding issues. The case involving Remifemin® was not complicated by the presence of other herbal compounds. However, no independent testing of the material consumed by the first case was done. However, the TGA has samples of this patient's tablets, which will be tested. The preparation taken by the second case was tested and found to contain 40 percent ground ivy and 10 percent black cohosh. The preparation was tested by the TGA, and pulegone, a toxin purportedly found in ground ivy, was not detectable in the sample of extract provided by Lontos and colleagues (Cumming and Kelly, 2004). Some participants questioned whether black cohosh was considered the culprit in this case. While it remains unclear if the use of black cohosh induced the hepatotoxic reaction or if it precipitated a pre-existing hepatic problem, or if it co-occurred with the hepatic event, the association of use of Remifemin® and liver failure raised concern. Again, the weaknesses of a passive adverse event surveillance system were noted, as was the absence of baseline data on hepatotoxicity in the general population. With millions of women taking black cohosh, participants noted that very few adverse events have been reported.

Discussion

Leonard Seeff, M.D., National Institute of Diabetes and Digestive and Kidney Diseases

Dr. Seeff noted the skepticism about the role of black cohosh in hepatotoxicity as discussed at the meeting. If the use of this botanical were in fact as high as reported, then the incidence of hepatotoxicity would appear to be very low. He noted that most clinical studies are not powered to detect such rare events. Thus, it is often only after thousands of people take a specific product that a rare event, such as hepatotoxicity, can be detected. Nevertheless, the temporal relationship of use of black cohosh and hepatotoxic events is troubling and warrants serious consideration.

Dr. Seeff commented that case studies offer useful clues, and repeated occurrences become harder to dismiss. People die from hepatotoxicity, so it is important to track it. He cautioned that one must distinguish between reactions to specific products and allergic reactions. If it is truly a product reaction, then the level of a product's effectiveness must be balanced against the risks that it poses. Whether or not hepatotoxicity purported to be associated with black cohosh is real merits further investigation. Physicians must be trained to probe patients about use of herbal CAM products that are often not considered to be "drugs" or substances worth reporting by patients.

The NIDDK recently held a meeting to improve our understanding of the association between liver disease and ingestion of herbal products. Dr. Seeff reported that the results from a recent survey suggest that about 30 percent of respondents with liver disease were taking herbal products (Strader et al., 2002). The cause for all cases of chronic liver disease can be identified in about 90 percent of instances, but in approximately 10 percent of the cases, the cause is unknown. Hepatotoxicity is the most common basis for drugs not making it to market and for drugs being pulled from the market, so it is very important to try to understand this issue.

Hepatotoxicity covers a spectrum of liver disease. Making the connection between drug or botanical use and liver disease is very difficult and mostly relies on a diagnosis by exclusion. Dr. Seeff reminded participants that some forms of drug-induced liver injury depend on the dose

and total amount of drug taken. Others are referred to as idiosyncratic and immunologically based. The latter are rare events, even with conventional drugs. A major problem in assigning blame is that often multiple drugs or herbs are being taken. Today the most common cause of fulminant hepatitis is use of acetaminophen.

NIDDK has initiated a Drug-Induced Liver Injury Network (DILIN) that is comprised of a data coordinating center and five clinical research centers (University of Connecticut; Indiana University; University of Michigan, University of North Carolina; University of California, San Francisco). The network is undertaking a limited retrospective study (the Idiosyncratic Liver Injury Associated with Drugs [ILIAD]) and a prospective study (DILIN).

The ILIAD seeks to establish and maintain a clinical database on patients who have suffered severe idiosyncratic drug-induced liver injury, to establish a bank of biological specimens (e.g., serum, DNA, and immortalized lymphocytes) prepared from cases and suitable controls, and to maintain a registry. It will also include a yearly update of case contact information to make it possible to re-contact these individuals at a later date to offer participation in phenotype/genotype studies. The protocol specifies identifying subjects who (1) took one of the four target drugs (isoniazid, valproate, phenytoin, and amoxicillin/clavulanic acid) and (2) experienced hepatotoxicity. The protocol calls for drawing a blood sample and obtaining a detailed medical history. Then investigators will match these cases with subjects who took the same drug but did not develop hepatotoxicity. The study will also examine genetic polymorphisms and other factors to understand who is likely to develop hepatotoxicity. Drugs were considered for ILIAD if they had "signature" presentations, were given to relatively healthy people; and had sufficient usage to permit the collection of 50-100 cases with a similar number of controls.

In the prospective study, people around the country are asked to notify DILIN if they have *bona fide* cases of liver injury or other reaction that they believe is attributable to a drug or dietary supplement. Investigators will then match these cases with people who took the same drug or botanical but did not experience an adverse event involving the liver. The purpose of this study is to identify genetic, clinical, immunological, and environmental risk factors for liver injury.

NIDDK, in conjunction with FDA, is trying to develop its own causality instrument for assessing adverse reactions to drugs that would be an alternative to RUCAM. RUCAM is used primarily in Europe and has some limitations (Danan and Benichou, 1993). The important information to be collected in the new instrument include when a drug or herbal preparation was started, how long it was taken before toxicity was evident, whether jaundice developed while on the drug or afterwards, and whether the subject was screened for acute and chronic hepatitis, autoimmune hepatitis, or other named deficiencies. Ultimately, researchers hope to develop a reasonable scale that physicians will use.

Participants reiterated that a drug or herb should not be removed from the market place without having reasonable evidence of harm. Suspected hepatotoxicity should not be broadcast when toxicity has not been demonstrated. Dr. Seeff reported that he is working with the DILIN investigators to develop a causality instrument for grading the potential for toxicity according to a variety of steps of varying probabilities. A crisp terminology to categorize factors that fall somewhere between suspected and known causes of hepatotoxic events would be very helpful.

Dr. Bolton reported that several pharmaceutical companies are screening drugs very early in the drug development process for formation of electrophiles to look at whether they form glutathione conjugates. Dr. Bolton has done the same and found that black cohosh is converted to glutathione conjugates, but the compounds that make these reactive intermediates are not absorbed. More research needs to be conducted to understand potential mechanisms of hepatotoxicity.

Summary and Next Steps

Heather Miller, Ph.D., M.F.S., NCCAM
Adrian Dobs, M.D., M.H.S., Johns Hopkins University
Margaret Chesney, Ph.D., NCCAM

Developing a sound empirical base concerning the safety and efficacy of black cohosh is not as easy as one might wish. Research to demonstrate safety and efficacy of drugs is conducted by the pharmaceutical industry to obtain FDA approval. However, because the regulations that govern drugs do not apply to dietary supplements, a similar motivation to conduct safety and efficacy research does not exist in the botanical industry. Moreover, the economic incentives to conduct research and intellectual property protections may be less clear. The absence of information from industry has left significant gaps in knowledge about these products. Moreover, the lack of standardization of similar products across manufacturers or even across batches produced by the same manufacturer have resulted present hurdles to scientists trying to understand the scientific literature and consumers trying to understand products available in the marketplace. Uncertainties concerning what people have taken (i.e., what was in the bottle) makes it difficult to interpret case reports purporting a link between black cohosh and hepatotoxicity, although the two case reports from Australia had detailed information on the material consumed by the patients.

While the workshop was convened to help understand risks associated with black cohosh, we also need to learn more about any benefits. We hope that ongoing research will contribute to that need. And we hope to learn more about mechanism of action from ongoing and future research.

Meeting participants were asked to address the question that catalyzed the development of this workshop, namely what, if anything, should NCCAM and other Institutes and Centers at NIH supporting clinical studies on black cohosh, do to ensure the protection of subjects? The following summarizes this discussion.

The group was unanimous in its support for characterizing and standardizing botanical products used in research. The group recognized that control of commercially available products is beyond the purview of the NIH, since it is not a regulatory agency. In cases of adverse events associated with black cohosh or other botanical substances, it is critical to obtain as much information about the product taken or, better, a sample of the product for subsequent analysis. Non-standardization of product presents an enormous barrier to understanding the safety and efficacy of botanicals, including black cohosh.

With respect to preparation, possible adulteration, and contamination, investigators should be required to conduct an independent verification of content of material used in research, noting such things as disintegration time, presence of heavy metals, microbial and pesticide analysis, and the like - even in cases where the preparation comes from a reputable source. Verification

should be done for *in vitro* studies as well as studies involving animal and human subjects. The group noted that it would be extremely helpful if publications included more information on the extraction procedures and chemical characterization of products used in studies. Similarly, they noted that study sections reviewing grant applications should be alerted to problems associated with verification of black cohosh and other botanicals. Verification costs should be allowable as part of the research grant budget.

NCCAM's policy on the quality of natural products addresses many of the aforementioned issues. (See http://nccam.nih.gov/research/policies/naturalproducts.htm). NCCAM is continuing to strengthen its policies with respect to quality of botanical products used in research supported by the Center. Applicants will be required to submit documentation supporting verification of substance(s) under study prior to any award being made. NCCAM is also planning to conduct random tests of products used in research projects. Investigators will be required to save and store specimens for such testing. The group noted that the conditions of storage are important to preserve the integrity of study material. This may require storing samples at -20 degrees to slow degradation.

Meeting participants noted that it is critical for NCCAM to communicate these expectations clearly to the field. Recently NCCAM modified its website to make it easier for investigators to find policies and requirements associated with NCCAM-funded and NIH-funded research. We will continue to work on improving communication.

The discussion then turned to Dr. Davis' presentation of data from the murine model of breast cancer. The abstract from this study had raised questions concerning safety of black cohosh because of increased incidence of metastatic lesions in lung among animals fed black cohosh. Participants reported that the results of this study were difficult to interpret, given no difference in overall survival or numbers of tumors when animals fed black cohosh were compared with animals fed the control diet. Moreover, Dr. Davis indicated that work remains to complete the histology component of the research. The study has not yet been published in a peer-reviewed journal. Nevertheless, the workshop participants believed that the study should be replicated and that other research on the effect of black cohosh on different stages of tumor genesis should be pursued using different approaches, as suggested by Dr. Green.

With respect to black cohosh and human breast tissue, there is scant evidence that black cohosh will harm healthy women. Data were presented indicating that black cohosh is not estrogenic. Thus, there appears to be little reason to do mammography on healthy female research subjects participating in studies of black cohosh. However, the use of black cohosh to treat hot flashes associated with treatment for breast and prostate cancer raise additional safety concerns. For women who have been treated for breast cancer, it would seem reasonable to screen for recurrence and metastases, actions that are consistent with standard care. Too little is known about the effect of black cohosh on prostate tissue to say anything more at this time.

The workshop participants expressed interest in ongoing efficacy studies of black cohosh for a range of symptoms and health problem, many of which are associated with menopause. The group noted that there are few long-term clinical studies of this dietary supplement. They also noted that several studies had deficiencies in design that made interpretation of findings difficult. For example, many studies use a cross over design, but it is not clear that all such studies have used a sufficiently long washout period.

Discussions at the workshop noted the number of organ systems with which black cohosh interacts. This raised additional interest in the underlying mechanism(s) of action of this dietary supplement ingredient. For example, the effect on vasomotor symptoms may not be due to any estrogenic properties but rather dopaminergic and/or serotonergic properties. More research is clearly needed to understand the pharmacological mechanisms of action for this botanical.

Hepatotoxicity and black cohosh were topics of extensive discussion. Ultimately, the workshop participants concluded that a balanced approach be taken with respect to this issue. On the one hand, millions of people have taken black cohosh with very few adverse events reported. On the other hand, those cases of hepatotoxicity associated with products that are known to contain black cohosh and believed to be free from other substances of known toxicity raise concern. Thus, the workshop recommended that appropriate safety parameters should be used in clinical studies of black cohosh. Such measures would include monitoring liver function throughout the study period, specifically looking at alkaline phosphatase (AP), aspartate aminotransferase (AST). alanine aminotransferase (ALT), and bilirubin. And depending on what is being studied, investigators should consider screening potential subjects for liver function to exclude individuals with pre-existing liver problems.

At this time, there is no known mechanism with biological plausibility that explains any hepatotoxic activity of black cohosh. Studies using mass spectrometry show that black cohosh contains catechols and phenols, which can be activated to quinone-type intermediates and trapped by glutathione or other sulfhydryls. These phenolic compounds, however, do not appear to be absorbed in animal models, and the conjugates are not detected in blood or urine. However, the possibility cannot be ruled out that such intermediates are generated in vivo. Animal models might provide useful information about what causes idiosyncratic liver damage. It would be helpful to understand the human metabolism of various black cohosh components, to see if these are replicated in specific animal models. And it is likely that we need to look beyond the usual mechanisms to understand how liver damage occurs. Use of genomic microarrays may be useful in exploring such mechanisms.

From an ethical perspective, potential subjects should be told about known risks and benefits from study participation. But the risks and benefits of black cohosh are yet to be determined on several levels. For example, workshop participants were not clear about the relevance of data from the murine model to humans. Metastatic disease was increased in the mice, but it did not alter survival time. As more information is accrued on the efficacy and potential risks of black cohosh, the information included in informed consent can be revisited and revised. The evidence of risk remains equivocal but certainly warrants continued monitoring. The workshop concluded that informed consent should inform potential subjects about known risks and benefits and note conflicting information on rare risks, such as hepatotoxicity, and steps to be taken during the study to monitor for those events. New information should also be shared with the study's Data Safety Monitoring Board (DSMB). And the need to reconsent subjects should be reviewed as new data and publications come forth.

Financial Disclosures: Dr. Dentali serves as the Vice President of Scientific and Technical Affairs for the American Herbal Product Association, which is supported by the membership dues from growers, processors, manufacturers, and marketers of herbal products, including black cohosh. He has also served as a consultant to and employee of Rexall Sundown. Dr. Farnsworth

has served as a consultant to Pharmavite. Dr. Wuttke has received research and travel support from the Bionorica Company.

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Appendix 1

Black Cohosh Workshop

Bethesda Marriott Suites • Salon I and II
6711 Democracy Boulevard, Bethesda, MD

Sponsored by
National Center for Complementary and Alternative Medicine (NCCAM) and
NIH Office of Dietary Supplements
November 22, 2004

Agenda

9:00 AM **Welcome and Charge**
Stephen Straus, NCCAM/ Margaret Chesney, NCCAM
Adrian Dobs, Johns Hopkins University

9:20 AM **Overview of Black Cohosh**
Heather Miller, NCCAM
Qi Ying Liu, NCCAM

10:00 AM **Black Cohosh: What Do We Know About This Botanical?**

Norman Farnsworth, University of Illinois - Preparation and Possible Adulteration and Contamination

Judy Bolton, University of Illinois – Mechanism of Action, Estrogenic Activity, and In Vitro Toxicology Screening Assays

Wolfgang Wuttke, University of Gottingen – Experiments in Rats and Clinical Studies

Edward Sauter, University of Missouri – Effect on Breast

Adrian Dobs, Johns Hopkins University – Effect on Prostate

Larry Walker, University of Mississippi – Discussant

12:30 **Lunch**

1:30 **Black Cohosh, Breast Cancer, and Metastases to Lung: Data from the Mouse Model**
Vicki Davis, Duquesne University

Jeffrey Green, National Cancer Institute – Discussant

2:30 **Black Cohosh and Hepatotoxicity: Case Reports**
Stanley Cohen, U Chicago
Paul Kerlin, Australia

Leonard Seeff, NIDDK - Discussant

4:00 **Summary and Next Steps**
Drs. Chesney, Dobs, and Miller

5:00 **Adjourn**

Appendix 2

Black Cohosh Workshop
Presenter and Participant List

Presenters

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