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Food Extracts for Chemoprevention: Quo Vadis?

*Perspective on Thomasset et al., p. 625*

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The development of chemoprevention agents is hard and unglamorous work. By 1980 there was sufficient epidemiologic evidence to suggest that certain diets and non-smoking lifestyles were protective against the development of many epithelial cancers, including breast, prostate and colon cancer (1). In the early 1980s, other investigators and I considered the feasibility of performing diet intervention trials, even fantasizing about randomizing the citizens of City X against those of City Y. Procedural and design issues and unfavorable grant reviews conspired against this possibility. Subsequently, dietary studies have been conducted in defined cohorts (2, 3), but broad society-oriented studies have been limited to marketing, such as the 5-a-day program (4). Several groups have labored to develop food extracts or concentrates for chemoprevention, although doing so has been extremely difficult. For example, our group and colleagues at the University of Pennsylvania have struggled to bring a Bowman-Birk inhibitor concentrate (BBIC), which is a chymotrypsin inhibitor-rich concentrate of soybeans, to the clinic (this work, including its trials and tribulations, is reviewed in ref. 5). A major early obstacle to this type of natural-agent development is that once a medical benefit is claimed, the FDA requirements become the same for a concentrate as for a proprietary drug. After 17 years of development, including pilot, phase I and phase IIa trials (6, 7), we are nearing completion of a large randomized trial of BBIC in patients with oral leukoplakia.

Notwithstanding these formidable obstacles and barriers, important work in this area is progressing with, for example, the “first in humans” cancer chemoprevention trial of oral mirtocyan, an extract from the food bilberries, reported by Thomasset et al. in this issue of the journal (8). Achieving the “Perfect Trial” is nigh onto impossible, and the dissection of this study in this perspective is not meant to reflect aspersions on what it has achieved but rather to serve as a frame of reference, and perhaps guidance, for investigators brave enough to enter the field, which very much needs committed soldiers if this chemoprevention strategy is going to continue to develop and mature in a meaningful way. The major issues addressed in the following paragraphs are posed in a question-and-answer format and are summarized in Table 1.

What evidence supports the use of the bilberry extract mirtocyan for chemoprevention or for the cancer patients targeted in this pilot trial?

- Epidemiologic evidence: none presented.
- Experimental evidence: results in animal models, including skin, esophageal and several colon carcinogenesis models, indicate efficacy of mirtocyan at reasonable doses in reducing malignant tumors; the extract was well-tolerated with no obvious toxicities. The preclinical evidence from gastrointestinal studies by this and other groups was particularly strong.
- Clinical evidence: There is no apparent clinical evidence of safety concerns over mirtocyan extracts (at reasonable doses) based on the history of bilberries as a foodstuff (www.nlm.nih.gov/medlineplus/druginfo/natural/patient-bilberry.html#Safety) and on trials testing effects on visual acuity (www.mirtoselect.com).

How well characterized is the candidate extract, mirtocyan, and its putative active components (anthocyanins) and metabolites?

The analysis techniques of high performance liquid chromatography-visible spectroscopy and tandem mass spectrometry and characterization of the chemical contents of the extract were elegant and well-described. Although the characterization methods were outlined, content reproducibility from batch to batch was not addressed.

How relevant are the mirtocyan doses tested (1.4, 2.8, 5.6 g), and is the tested duration of administration (7 days) adequate?

As explained by Thomasset et al. in their Discussion section, the lowest tested dose of mirtocyan (1.4 g/day) equates to ~370 g of fresh bilberries per day. This calculation translates to a consumption of about 13 ounces (4/5 lb) of bilberries per day. Furthermore, 1.4 g of mirtocyan per day represents 5 times the level of anthocyanins contained in the normal daily Western diet, and the highest tested dose (5.6 g/day) represents almost 20 times the normal dietary level of anthocyanins and a daily consumption of about 52 ounces (3 1/4 lbs) of fresh bilberries, which, of course, explains why an extract needs to be used rather than the food itself! Therefore, these doses are super-pharmacologic and immediately raise the specters of relevance and long-term safety, especially in view of the past history of excess lung cancers associated with β-carotene in trials to prevent lung cancer (9) and a probably increased number of prostate cancers in the vitamin E arm of the Selenium and Vitamin E Cancer Prevention Trial (SELECT; ref. 10). Mirtocyan was given for a very short time of 7 days, which, in view of the negative (and/or uninformative) pharmacodynamic tissue outcomes (discussed below), may have been insufficient to achieve tissue saturation and equilibrium.

Overall and on balance, however, the evidence and safety data from available preclinical and animal-dosing studies supported conducting a “first in humans” trial of the mirtocyan...
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Table 1. Selected key issues in assessing a “first in humans” trial of an extract (concentrate) for cancer chemoprevention

<table>
<thead>
<tr>
<th>1. Single compound, multiple compounds, extract, or food</th>
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<tr>
<td>2. Prior evidence of benefit: epidemiologic, experimental, clinical</td>
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<td>3. Prior evidence of toxicity: experimental, clinical</td>
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<td>4. Pharmacology</td>
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<tr>
<td>(a) Extract components: Characterization and reproducibility from batch to batch</td>
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<td>(b) Pharmacokinetics: Dosing and duration issues and similarity between mice and humans</td>
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<td>5. Pharmacodynamics</td>
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<td>(a) General markers not necessarily linked to drug mechanism or activity but selected based on carcinogenic progression, either cellular (e.g., proliferation, apoptosis) or molecular (e.g., key signaling pathway alterations)</td>
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<tr>
<td>(b) Specific markers selected based on mechanistic activity of the agent</td>
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<td>(c) Effect on relevant marker in tissue of interest?</td>
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<tr>
<td>6. Trial Design</td>
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<tr>
<td>(a) Adequate number of cases.</td>
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<tr>
<td>(b) Comparability of pre- and post-intervention results.</td>
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</table>

extract. Furthermore, anthocyanins, the active components of mirtocyan, are polyphenols, as are curcumin and components of green tea that are showing promise for cancer chemoprevention.

How strong were the design and implementation of the trial?
The design of the trial, which enrolled patients scheduled for surgery to remove a primary colon cancer or its metastases in the liver, had the following strengths and weaknesses:

- This was a prospective trial of patients randomized with respect to dose level.
- Retrospective consent (after diagnosis of cancer) was obtained to use diagnostic tissue specimens, and so no presurgical biopsy samples of normal colorectal tissue were obtained, although postsurgical biopsies of normal colorectal tissue were obtained from the resection specimens.
- It is unclear where normal tissue was obtained from the liver resection specimens.
- Measuring endpoints in two different tissues introduces an unnecessary level of complexity since different organ sites are likely to affect the metabolism of any agent, especially one as complex as an extract, quite differently.
- As mentioned above, it seems unlikely that a maximal effect on the tissue markers of interest would have occurred within the short, 7-day trial.
- Biopsies were obtained after the tissue resection. Although the authors state that pathologic examination showed no evidence of necrosis, assessment for absence of cadaverine, a sensitive maker of necrosis, would have been more reassuring.
- The sample size was small-22 participants spread over 3 doses-and no placebo or other control group was included, two crucial considerations for a biomarker-assessment trial. This problem supersedes all others since detection of an effect may or may not have been possible, especially with the relatively large coefficient of variations and standard deviations of the biomarker measurements. More robust statistical design considerations would have been important.

How informative were the trial’s assessments of Ki-67 as a proliferation marker, cleaved caspase-3 as an apoptosis marker in the tissue (by immunohistochemistry), and plasma insulin-like growth factor 1 (IGF-1) and IGF binding protein-3 (IGFBP-3)?

Ki-67 is a time-honored, rather crude proliferation measure of questionable value (11); recent data suggest that other measurements are better. Cleaved caspase-3 is a reasonable measure for a broad assessment of apoptosis in tissue specimens. Although the rationale for IGF-1 and IGFBP-3, favorite current markers of many investigators, vis-à-vis anthocyanins (the active components of mirtocyan) is indirect, they are implicated as targets of other polyphenols such as silibinin and curcumin, which are structurally similar to anthocyanins. Therefore, markers that are more closely related to the direct mechanism of action of anthocyanins should have been included, as has occurred in early drug-development clinical trials of other agents. Examples of clinically tested directly related pairs of markers/agents include tissue polyamines/difluoromethylornithine (12), tissue prostaglandin E$_2$/nonsteroidal anti-inflammatory drugs (NSAIDs; ref. 13, 14) and urinary prostaglandin E metabolite/NSAIDs (synthetic agents; ref. 15) and phospho-AKT/myoinositol (a natural agent; ref. 16).

Is the interpretation of the results reasonable, and what can one conclude?
The pharmacodynamic results, although largely negative, are of interest. The limited evidence concerning any differences in plasma IGF-1 or IGFBP-3 was a provocative trend of reduced circulating IGF-1 in all patients/doses combined (versus pre-intervention levels); the different doses of mirtocyan produced no evidence of apoptosis in colon tumor tissue. Proliferation as measured by Ki-67 was decreased by 7% in tumor tissue assessed post-mirtocyan versus that assessed prior to mirtocyan, and the decrease was evident only at the lowest dose of mirtocyan. Mirtocyan also failed to affect markers of oxidative DNA damage. The absence of a placebo control group, however, hinders interpretation as many measurement biases that are not easily detected can be hard wired into procedures (17). If the results of this study lead to further study, lower doses of mirtocyan for a longer duration should be used. With regard to this dose recommendation, many circumstances in biology have suggested that more is not always better.

In addition to the many caveats already discussed, another caveat arises from the most striking finding of Thomasset et al., which is the discordance between plasma and tissue of the anthocyanin metabolites in mice (reported earlier by the same group) compared with that in humans (8). Although, as anticipated, the plasma concentrations were similar between mice and humans, the concentrations in human tissue were from 1/10 to 1/20 those in mice. This unexpected pharmacokinetic and pharmacodynamic result is important and suggests that either the duration of dosing was too short to have achieved tissue saturation or, unfortunately, that there are differences between mice and humans in processing anthocyanins in colon (and perhaps liver) tissues. If the latter explanation is correct, then bilberries are unlikely to be effective chemoprevention in humans, at least for colorectal carcinogenesis.
Overall, the early translational results of Thomasset et al. with this extract are not encouraging. Follow-on studies in humans would need to be better designed. Also, a study of the tissue distribution of anthocyanins in humans would seem to be the order of the day before proceeding to additional studies in specific organ sites. A recent commentary by Stoner is well worth consulting for anyone interested in learning more about the development of foodstuffs for preventing cancer (18).

What goes into a strong rationale and design for a food-extract trial?
The following rationale and design elements are important to the prospects for a successful outcome of “first in humans” carcinogenesis trials of an extract:

1. Strong epidemiologic and/or preclinical experimental data.
2. Chemical components of the extract should be well-characterized and reproducible from batch to batch.
3. The extract should be given in a reasonable dose and for an adequate duration.
4. Tissue of normal and/or tumor should be obtained and processed via identical procedures both before and after the extract intervention.
5. The choice of markers of pharmacodynamic effect should be based on characterized mechanistic effect(s) of the extract and/or its component(s).
6. The numbers of samples should be adequate, a placebo and/or control group should be included, and the statistical assessment should be sufficiently robust to allow a meaningful interpretation.

Developing agents for the chemoprevention of human cancer is a long and tedious process, although results for single agents including hormonal agents in breast and prostate cancer prevention (19, 20) and NSAIDs in colorectal cancer prevention (21) indicate that efficacy is possible; the toxicity of single agents at active doses, however, has precluded their widespread adoption to date. The recent achievement of a low-dose combination of the polyamine synthesis inhibitor difluoromethylornithine and the non-selective NSAID sulindac in markedly reducing colorectal adenomas (12) suggests that re-exploring the role of complex combinations of natural chemopreventives, as found in bilberries (and many other fruits and vegetables), is a reasonable thing to do. Intervention trials with dietary food prescriptions, whether wholesale changes in diet or a single or multiple fruits and/or vegetables (“little chemical factories”), is once again well worth pursuing. A task not for the “faint of heart” though, and investigators who choose it may find themselves feeling a bit like the imperiled St. Peter as he fled from Rome and asked, “Quo vadis?”

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

References
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