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# Lycopene in the treatment of prostate cancer*

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*Abstract*: Dietary intake of lycopene is associated with reduced risk of prostate cancer (PCa). We conducted a clinical trial in men with prostate cancer to investigate the biological and clinical effects of lycopene supplementation. Twenty-six men with prostate cancer were randomly assigned to receive a lycopene supplement or no supplement for three weeks before radical prostatectomy. Subjects in the intervention group ($n$ = 15) were instructed to take a tomato oleoresin extract soft gel capsule (Lyc-O-Mato®, LycoRed Company, Beer Sheva, Israel) containing 15 mg lycopene, 1.5 mg phytoene, 1.5 mg phytofluene, and 5 mg tocopherol twice daily with meals. Prostatectomy specimens were evaluated for pathologic stage, Gleason score, volume of cancer, and extent of high-grade prostatic intraepithelial neoplasia (HGPIN). Biomarkers of cell proliferation and apoptosis were assessed by Western blot analysis in benign and cancerous tissue samples obtained from the prostatectomy specimens. Oxidative stress was assessed by measuring the peripheral blood lymphocyte DNA oxidation product 5-hydroxymethyl-deoxyuridine (5-OH-mdU). Plasma levels of lycopene, insulin-like growth factor-1 (IGF-1), insulin-like growth factor binding protein-3 (IGFBP-3), and prostate-specific antigen (PSA) were measured at baseline and after three weeks of study period. After the intervention, more men in the intervention group had smaller (<4 cc) tumors, organ-confined disease without involvement of surgical margins or extra-prostatic tissues, and focal involvement of the prostate with HGPIN compared to the control group. Mean plasma PSA levels were lower in the intervention group compared to the control group. This pilot study suggests that a tomato extract containing lycopene and other tomato carotenoids and phytochemicals may have a potential role in the treatment of prostate cancer. Larger clinical trials are necessary to definitively address potential uses of lycopene or tomato extract in the prevention or treatment of prostate cancer.

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## INTRODUCTION

Prostate cancer is the second leading cause of cancer deaths in males in the United States. It accounts for about 30 % of all cancers that are diagnosed in men. In 2001, the American Cancer Society has predicted 198 100 new cases and 31 500 deaths from prostate cancer in the United States [1]. The incidence of prostate cancer has increased dramatically in the last decade due mainly to the increase in screening using PSA. The prevalence of the precursor lesion, HGPIN, and carcinoma of the prostate increase with aging starting in men in their early thirties [2]. Knowledge of the natural history of development, elucidation of critical genetic and epigenetic pathways, and presence of risk factors for identifying target populations make prostate cancer a good target for prevention [3]. Thus, there is great interest in prostate cancer chemoprevention, which can be defined as the administration of natural and/or synthetic agents that inhibit one or more steps in prostate carcinogenesis [4].

Dietary intake of tomato products and lycopene has been associated with reduced prostate cancer risk [5,6]. Therefore, lycopene has been identified as one of the promising chemopreventive agents. Lycopene and other carotenoids have a number of cancer-preventive biological effects including (a) inhibition of growth and induction of differentiation in prostate cancer cells [7–11], (b) up-regulation of connexin 43 (Cx43) and increased gap junctional intercellular communication [12–16], and (c) prevention of oxidative DNA damage [17–19].

Gap junctional intercellular communication (GJIC) and Cx43 expression levels could be useful intermediate endpoints in prostate cancer chemoprevention clinical trials, because they are decreased in prostate cancer cells [20–22]. Therefore, chemopreventive agents modulating Cx43 expression and/or GJIC would be of great interest [23,24]. Retinoids and carotenoids are potent up-regulators of Cx43 and GJIC [14,25,26]. In particular, lycopene increases GJIC by increasing expression of the gap junctional gene, Cx43 [12–14]. This action correlates strongly with the ability of lycopene and other carotenoids to suppress neoplastic transformation in model cell culture systems [14]. This action of carotenoids has been proposed to have mechanistic significance by enabling the transfer of growth-regulatory signals between normal growth-inhibited cells and preneoplastic cells. Indeed, when neoplastic cells were forced into junctional communication with quiescent normal cells, the neoplastic cells became growth-arrested in direct proportion to their extent of junctional communication [23]. Progressive decreases in the expression of Cx43 with disease severity have been reported in the human prostate [20], and there is evidence in prostatic carcinoma cell lines that some of this loss of junctional communication may result from defects in assembly of Cx43 protein into gap junctions [21]. When functional communication was restored in a human prostatic carcinoma cell line, cells had more normal differentiation, reduced proliferation, and suppressed tumorigenicity [22]. Therefore, Cx43 and GJIC could be used as surrogate endpoint biomarkers (SEBs) or intermediate endpoints in phase II clinical chemoprevention trials for prostate cancer.

It is important to identify men at high risk for prostate cancer so that they can be enrolled in chemoprevention trials. In addition to the presence of HGPIN in the prostate and elevated serum PSA levels, recent studies suggest high-serum IGF-1 and/or low-serum IGFBP-3 as good markers for high prostate cancer risk. Insulin-like growth factors have mitogenic and antiapoptotic effects on normal and transformed prostate epithelial cells [27–29]. IGF-1 is an important mitogen for prostate cells. IGFBPs have opposing actions, in part by binding IGF-1, but also by direct inhibitory effects on target cells [27]. In recent epidemiologic studies, relatively high plasma IGF-1 and low IGFBP-3 levels have been independently associated with greater risk of prostate cancer [30–34]. Two- to fourfold elevated risk has been observed for prostate cancer in men in the top quartile of IGF-1 relative to those in the bottom quartile, and low levels of IGFBP-3 were associated with an approximate doubling of risk [30]. Recent data show that lycopene administration to humans with colon cancer for 1–5 weeks prior to surgery significantly reduces serum IGF-1 levels [35].

## CLINICAL TRIAL OF LYCOPENE IN MEN WITH PROSTATE CANCER

We conducted a pilot study to investigate the biological and clinical effects of lycopene supplementation on the prostate tissues and on serum levels of PSA, IGF-1, and IGFBP-3 in patients with localized prostate cancer [36]. We hypothesized that lycopene supplementation would decrease growth and induce apoptosis in premalignant and malignant prostate cells by up-regulating tissue Cx43 level, decreasing serum IGF-1 level, and decreasing the ratio of bcl-2/bax in patients with localized prostate cancer. In a randomized clinical trial, we enrolled 35 men with clinical stages T1 or T2 localized prostate cancer, who were scheduled to undergo radical prostatectomy. Data were collected from 26 subjects who were assigned to the lycopene arm ($n$ = 15) or the control arm ($n$ = 11) of the study. Nine patients were excluded because they had an incorrect diagnosis (1 patient), dropped out after randomization (2 patients), or had prior hormone therapy (6 patients). A detailed description of the methodology and the results of this trial have been published elsewhere [36].

Subjects were randomly assigned to receive either a mixture of tomato carotenoids (Table 1) with 15 mg lycopene (as a gel capsule containing tomato extract Lyc-O-Mato obtained from LycoRed Company, Beer Sheva, Israel) twice daily with meals, or no supplement for three weeks prior to surgery. Biomarker studies were performed on blood samples collected at baseline and after three weeks of intervention prior to radical prostatectomy. At the time of surgery, entire prostate glands were resected, and specimens were evaluated for pathologic stage, the volume of prostate cancer as well as the extent of HGPIN. Tissue levels of Cx43, bcl-2, and bax were assessed by Western analysis of benign and malignant areas of the tissue samples. Plasma and tissue levels of carotenoids were measured by high-performance liquid chromatography (HPLC). Plasma levels of IGF-1 and IGFBP-3 were measured by enzyme-linked immunosorbent assay (ELISA). Peripheral blood lymphocyte levels of 5-OHmdU was measured by gas chromatography-mass spectrometry.

**Table 1** Contents of Lyc-O-Mato capsules containing natural tomato extract.

| Contents | Percentage (w:w) in 250 mg tomato extract |
|---|---|
| Carotenoids | |
| Lycopene | 5.8–6.2 |
| Phytoene | 0.5–0.7 |
| Phytofluene | 0.5–0.6 |
| Beta-carotene | 0.1–0.2 |
| Total | 6.9–7.7 |
| Other components | |
| Tocopherols | 1.5–2.5 |
| Phospholipids | 14–16 |
| Phytosterols | 0.5–0.7 |
| Tomato oil | 73–76 |

Changes in biological and clinical parameters induced by Lyc-O-Mato supplementation are shown in Table 2. Mean plasma PSA levels decreased by 18 % in the intervention group, while they increased by 14 % in the control group over the study period ($p$ = 0.22). In the intervention group, 11 of 15 patients (73 %) had involvement of surgical margins and/or extra-prostatic tissues with cancer, compared to 2 of 11 patients (18 %) in the control group ($p$ = 0.02). Twelve of 15 patients (80 %) in the lycopene group had tumors that measured 4 cc or less, compared to 5 of 11 (45 %) in the control group ($p$ = 0.22). Multifocal and/or diffuse involvement by HGPIN was observed in 10 of 15 subjects (67 %) in the lycopene group, compared to all 11 subjects (100 %) in the control group ($p$ = 0.05).

**Table 2** Post-treatment clinical and biological endpoints in the Lyc-O-Mato[a] ($n$ = 15) and control ($n$ = 11) groups.

| | Lyc-O-Mato | Control | $p$ |
|---|---|---|---|
| Serum PSA (mean ± SE[b], ng/ml) | 5.6 ± 0.9 | 7.7 ± 1.8 | 0.25[c] |
| Prostate HGPIN[d] ($n$) | | | |
| Focal | 5 | 0 | |
| Multifocal/diffuse | 10 | 11 | 0.05 |
| Prostate tumor volume ($cm^3$) | | | |
| 4< | 12 | 5 | |
| >4 | 3 | 6 | 0.22 |
| Surgical stage ($n$) | | | |
| Confined to prostate | 11 | 2 | |
| Not confined to prostate[e] | 4 | 9 | 0.02 |
| Prostate lycopene level (ng/gm tissue) | 0.53 ± 0.03 ($n$ = 4) | 0.36 ± 0.06 ($n$ = 4) | 0.02 |
| Prostate Cx43 expression (absorbance[f]) | | | |
| In tumor tissue | 0.63 ± 0.19 | 0.25 ± 0.08 | 0.13 |
| In benign tissue | 0.64 ± 0.04 ($n$ = 4) | 0.51 + 0.10 ($n$ = 4) | 0.44 |
| Prostate bax expression | | | |
| In tumor tissue | 1.05 ± 0.29 | 0.68 ± 0.18 | 0.33 |
| In benign tissue | 0.62 ± 0.10 ($n$ = 4) | 0.79 ± 0.11 ($n$ = 4) | 0.28 |
| Prostate bcl-2 expression | | | |
| In tumor tissue | 0.54 ± 0.01 | 0.51 ± 0.06 | 0.59 |
| In benign tissue | 0.63 ± 0.04 ($n$ = 4) | 0.58 ± 0.04 ($n$ = 4) | 0.31 |
| Prostate bax/bcl-2 ratio | | | |
| In tumor tissue | 1.94 | 1.33 | |
| In benign tissue | 0.98 | 1.36 | |

[a]Lyc-O-Mato is a tomato extract. See Table 1 for its composition.
[b]SE denotes standard error.
[c]P value is for comparing the change from pre- to post-intervention PSA in the two groups.
[d]HGPIN denotes high-grade prostatic intraepithelial neoplasia.
[e]Resection margins are positive and/or extra-prostatic invasion is present.
[f]Absorbance corrected for expression of β-actin

Sufficient malignant tissues were available for analysis in four subjects from the lycopene group and in four subjects from the control group. The level of Cx43 protein was 0.63 ± 0.19 optical density (OD) units in the lycopene group compared to the 0.25 ± 0.08 OD units in the control group ($p$ = 0.13). The expression of cell cycle regulatory proteins, bcl-2, and bax, was not significantly different between the two groups, although bax level of the lycopene group (1.05 ± 0.29) was higher than the control group (0.68 ± 0.18).

Tissue samples from benign parts of the gland were available for biomarker analysis in eight subjects in the intervention group and six subjects in the control group. Cx43 level was 0.64 ± 0.12 in the lycopene group compared to 0.51 ± 0.10 in the control group. The expression of bcl-2 was 0.63 ± 0.04 in the intervention group and 0.58 ± 0.04 in the control group, and the expression of bax was 0.62 ± 0.10 in the intervention group and 0.79 ± 0.11 in the control group. None of the differences in the biomarkers of the two groups were statistically significant.

Plasma samples were available from 13 subjects in the intervention group and 10 subjects in the control group. Mean plasma levels of IGF-1 decreased by 233 ± 21 ng/ml to 169 ± 23 ng/ml in the lycopene group ($p = 0.0002$) and from 199 ± 20 ng/ml to 140 ± 16 ng/ml in the control group ($p = 0.0003$). Interestingly, IGFBP-3 levels also decreased in both the intervention and control groups during the study period. Plasma IGFBP-3 levels in the intervention group decreased from 5230 ng/ml to 3924 ng/ml, while in the control group they decreased from 5200 ng/ml to 4070 ng/ml, which were statistically significant ($p = 0.0002$ and $p = 0.0001$, respectively).

In the intervention group, the plasma lycopene level increased in 5 of 11 patients, whereas only 1 of 6 subjects in the control group had an increase (Fisher's exact test, $p = 0.33$). The level of post-intervention plasma lycopene was 23.5 μg/dL in the intervention group and 17.5 μg/dL in the control group ($p = 0.15$). However, there was no significant difference between the two groups with regard to percent change of plasma lycopene level, due to great variability in plasma lycopene levels and small numbers of subjects in each group. Prostatic tissue lycopene levels were 47 % higher in the intervention group (0.53 ± 0.03 ng/gm of prostate tissue) compared to the control group (0.36 ± 0.06), which was a significant difference ($p = 0.02$) despite the small number of samples ($n = 8$).

Peripheral blood lymphocyte levels of 5-OHmdU were similar in both groups before and after intervention. There were no differences between the groups with respect to baseline intake of nutrients assessed by a validated food frequency questionnaire.

## DISCUSSION

The results suggest that 30 mg of lycopene taken daily for three weeks may be sufficient to modulate biological and clinical SEBs of prostate cancer. The microscopic extension of prostate cancer to surgical margins and/or to extra-prostatic tissues appeared to have decreased as a result of lycopene supplementation. This finding has potential clinical implications as the extension of tumor to surgical margins identifies a group of patients with poor prognosis. Patients in the lycopene group had a decrease in the plasma PSA level, which is a clinical parameter of prostate cancer burden. These results suggest that lycopene may have an antitumor effect and perhaps be useful as an adjunct to standard treatments of prostate cancer, such as surgery, radiation therapy, hormones, and chemotherapy. In addition, lycopene supplementation appears to have reduced the diffuse involvement of the prostate gland with HGPIN, which is a precursor of prostate cancer [37], suggesting that lycopene may also have a role in the prevention of prostate cancer.

The mechanism of lycopene's clinical effects remains to be elucidated. Up-regulation of Cx43 expression would be a possible explanation. When the results are analyzed, including all randomized and treated subjects (i.e., 26 subjects plus the 6 subjects that were originally excluded from analysis because of history of hormone therapy), Cx43 expression was significantly higher in the tumors from patients in the lycopene group ($p < 0.05$). However, statistical significance was absent when the analysis was restricted to the 26 eligible and evaluable patients, even though there was an increase in the expression of Cx43 in tumor tissue in patients receiving Lyc-O-Mato. Increased expression of Cx43 and increased junctional communication have previously been shown to occur after treatment of human and murine cells in culture with lycopene [13]. Up-regulated junctional communication has been linked to decreased proliferation in normal and preneoplastic cells [38]. Therefore, our results suggest that lycopene supplementation may decrease the growth of prostate cancer, perhaps by up-regulating Cx43. However, because of small sample size, no definitive conclusions can be reached. Future clinical trials with larger sample sizes and different doses of lycopene are needed to determine the efficacy as well as the appropriate dose and duration of lycopene supplementation in men with prostate cancer or high risk of developing prostate cancer.

Interestingly, the level of pro-apoptotic protein bax increased in tumor tissue, while it decreased in benign tissue in the intervention group compared to the control group (Table 2). On the other hand, the level of anti-apoptotic protein bcl-2 did not change in the tumor tissue and perhaps increased in the

benign tissue in the intervention group compared to the control subjects. These changes were not statistically significant, perhaps because the number of subjects with adequate tissues for analysis was very small.

It should be noted that the lycopene preparation used in this study was a mixture of tomato carotenoids and other tomato phytochemicals (Table 1). Although lycopene was the predominant carotenoid in the capsules, there were significant amounts of phytoene and phytofluene and other bioactive compounds. It is possible that the combination of phytochemicals present in the tomato extract, rather than lycopene alone, was responsible for the observed clinical effects. There are in vitro data suggesting synergistic effects of lycopene with phytoene, phytofluene, and beta-carotene against prostate cancer cells [39].

The differences observed in bioavailability and response to the tomato extract in this study are not easily explained, because the preparation contains the natural tomato oleoresin present in tomato matrix in the Lyc-O-Mato capsules used in this study. Previous studies have shown excellent bioavailability of lycopene from this preparation [40]. However, it is possible that fat and other components of the diet might have influenced the bioavailability of lycopene in our study population. In addition, ingestion of foods containing lycopene (such as tomato, watermelon, pink grapefruit, guava), other carotenoids (beta-carotene, lutein), and micronutrients (tocopherol, zinc, selenium, ascorbic acid) vary widely between individuals and also seasonally. These other dietary components may have important interactions and may have influenced the outcome of the study. The small size of our study does not allow analysis of potential interactions with other nutrients. Our future studies will address potential interactions between different nutrients in modulating genetic and epigenetic pathways in prostate carcinogenesis.

## FUTURE STUDIES

Dose–response to lycopene should be investigated in subjects with localized and advanced prostate cancer. Clinical trials should be conducted in patients with HGPIN or elevated PSA, but without a diagnosis of prostate cancer, as they are at a high risk of developing prostate cancer or of having occult disease. Lycopene could be compared to other promising agents such as vitamin E, selenium, or soy in future clinical trials. We have found significant in vitro [41,42] and clinical activity with soy isoflavones [43] and are currently conducting clinical trials investigating the effects of lycopene alone, or in combination with soy isoflavones, in patients with advanced prostate cancer. Because of the observed in vitro synergistic effects of tomato carotenoids against prostate cancer cells [39], we are planning a clinical trial comparing the effects of pure lycopene with a mixture of tomato carotenoids (Lyc-O-Mato) in patients with localized prostate cancer prior to prostatectomy. Lycopene should also be combined with vitamin E [44] and selenium [45] in future clinical trials. For example, synergistic effects have been observed between lycopene and alpha-tocopherol against prostate cancer cells [46].

## REFERENCES

1. R. T. Greenlee, M. B. Hill-Harmon, T. Murray, M. Thun. *CA Cancer J. Clin.* **51**, 15–36 (2001).
2. W. A. Sakr, G. P. Haas, B. J. Cassin, J. E. Pontes, J. D. Crissman. *J. Urol.* **150**, 379–385 (1993).
3. P. Greenwald, R. Lieberman. In *Prostate Cancer in the Twenty-First Century,* L. Chung, W. Isaacs, J. Simons (Eds.), pp. 499–518, Humana Press, Totowa, NJ (2001).
4. G. J. Kelloff, R. Lieberman, M. K. Brawer, E. D. Crawford, G. Miller. *Prostate Cancer Prostate Dis.* **2**, 27–33 (1999).
5. E. Giovannucci, A. Ascherio, E. B. Rimm, M. J. Stampfer, G. A. Colditz, W. C. Willett. *J. Natl. Cancer Inst.* **87**, 1767–1776 (1995).
6. E. Giovannucci, E. B. Rimm, Y. Liu, M. J. Stampfer, W. C. Willett. *J. Natl. Cancer Inst.* **94**, 391–398 (2002).

7. J. S. Bertram. *Nutrition Rev*. **57**, 182–191 (1999).
8. H. Amir, M. Karas, J. Giat, M. Danilenko, R. Levy, T. Yermiahu, J. Levy, Y. Sharoni. *Nutr. Cancer* **33**, 105–112 (1999).
9. J. Levy, E. Bosin, B. Feldman, Y. Giat, A. Miinster, M. Danilenko, Y. Sharoni. *Nutr. Cancer* **24**, 257–266 (1995).
10. C. K. Park, Y. Ishimi, M. Ohmura, M. Yamaguchi, S. Ikegami. *J. Nutr. Sci. Vitaminol.* **43**, 281–296 (1997).
11. M. Karas, H. Amir, D. Fishman, M. Danilenko, S. Segal, A. Nahum, A. Koifmann, Y. Giat, J. Levy, Y. Sharoni. *Nutr. Cancer* **36**, 101–111 (2000).
12. L.-X. Zhang, R. V. Cooney, J. S. Bertram. *Carcinogenesis* **12**, 2109–2114 (1991).
13. J. S. Bertram, A. Pung, M. Churley, T. J. Kappock, L. R. Wilkins, R. V. Cooney. *Carcinogenesis* **12**, 671–678 (1991).
14. L.-X. Zhang, R. V. Cooney, J. S. Bertram. *Cancer Res.* **52**, 5707–5712 (1992).
15. A. Hotz-Wagenblatt and D. Shalloway. *Crit. Rev. Oncogenesis* **4**, 541–558 (1993).
16. J. S. Bertram and H. Bortkiewicz. *Am. J. Clin. Nutr.* **62**, 1327s–1336s (1995).
17. P. Riso, A. Pinder, A. Santangelo, M. Porrini. *Am. J. Clin. Nutr.* **69**, 712–718 (1999).
18. A. V. Rao, N. Fleshner, S. Agarwal. *Nutr. Cancer* **33***,* 159–164 (1999).
19. L. Chen, M. Stacewicz-Sapuntzakis, C. Duncan, R. Sharifi, L. Ghosh, R. van Breemen, D. Ashton, P. E. Bowen. *J. Natl. Cancer Inst.* **93**, 1872–1879 (2001).
20. H. Tsai, J. Werber, M. O. Davia, M. Edelman, K. E. Tanaka, A. Melman, G. J. Christ, J. Geliebter. *Biochem. Biophys. Res. Commun.* **22***,* 64–69 (1996).
21. P. P. Mehta, B. L. Lokeshwar, P. C. Schiller, M. V. Bendix, R. C. Ostenson, G. A. Howard, B. A. Roos. *Mol. Carcinog.* **15***,* 18–32 (1996).
22. P. Mehta, C. Perez-Stable, M. Nadji, M. Mian, K. Asotra, B. A. Roos. *Gene.* **24***,* 91–110 (1999).
23. P. P. Mehta, J. S. Bertram, W. R. Loewenstein. *Cell* **44**, 187–196 (1986).
24. M. Z. Hossain, L. R. Wilkens, P. P. Mehta, W. Loewenstein, J. S. Bertram. *Carcinogenesis* **10**, 1743–1748 (1989).
25. M. Rogers, J. M. Berestecky, M. Z. Hossain, H. M. Guo, R. Kadle, B. J. Nicholson, J. S. Bertram. *Mol. Carcinog*. **3**, 335–343 (1990).
26. G. S. Goldberg and J. S. Bertram. *In Vivo* **8**, 745–754 (1994).
27. R. Rajah, B. Valentinis, P. J. Cohen. *Biol. Chem.* **272**, 12181–12188 (1997).
28. P. Cohen, D. M. Peehl, R. G. Rosenfeld. *Hormone Metab. Res.* **26***,* 81–84 (1994).
29. P. Cohen, D. M. Peehl, G. Lamson, R. G. J. Rosenfeld. *Clin. Endocrinol. Metab.* **73***,* 401–407 (1991).
30. J. M. Chan, M. J. Stampfer, E. Giovanucci, P. H. Gann, J. Ma, P. Wilkinson, C. H. Hennekens, M. Pollak. *Science* **279**, 563–566 (1998).
31. E. Giovannucci. *Horm. Res.* **51***,* 34–41 (1999).
32. C. S. Mantzoros, A. Tzonou, L. B. Signorello, M. Stampfer, D. Trichopoulos, H. O. Adami. *Br. J. Cancer* **76**, 1115–1118 (1997).
33. A. Wolk, C. S. Mantzoros, S. O. Andersson, R. Bergstrom, L. B. Signorello, P. Lagiou, H. O. Adami, D. J. Trichopoulos. *Natl. Cancer Inst.* **90**, 911–915 (1998).
34. M. Pollak, W. Beamer, J. C. Zhang. *Cancer Met. Rev.* **17**, 383–390 (1998–99).
35. Y. Sharoni and Y. Levy. Personal communication and unpublished observations.
36. O. Kucuk, F. Sarkar, W. Sakr, Z. Djuric, F. Khachik, M. Pollak, F. Khachik, Y.-W. Li, M. Banerjee, D. Grignon, J. S. Bertram, J. D. Crissman, E. J. Pontes, D. P. Wood Jr. *Cancer Epidemiol. Biomarkers Prev.* **10**, 861–868 (2001).
37. W. A. Sakr. *Eur. Urol.* **35**, 474–478 (1999).
38. M. Z. Hossain and J. S. Bertram. *Cell Growth Diff.* **5**, 1253–1261 (1994).
39. Y. Sharoni and Y. Levy. Personal communication and unpublished observations.
40. A. V. Rao and S. Agarwal. *Nutr. Cancer* **31**, 199–203 (1998).

41. J. N. Davis, B. Singh, M. Bhuiyan, F. H. Sarkar. *Nutr. Cancer* **32**, 123–131 (1998).
42. J. N. Davis, O. Kucuk, F. H. Sarkar. *Nutr. Cancer* **35**, 167–174 (1999).
43. M. Hussain, F. H. Sarkar, Z. Djuric, M. N. Pollak, M. Banerjee, D. Doerge, J. Fontana, S. Chinni, J. Davis, J. Forman, D. P. Wood, O. Kucuk. *J. Nutrition* **132**, 575S–576S (2002).
44. O. P. Heinonen, D. Albanes, J. Virtamo, P. R. Taylor, J. K. Huttunen, A. M. Hartman, J. Haapakoski, N. Malila, M. Rautalahti, S. Ripatti, H. Maenpaa, L. Teerenhovi, L. Koss, M. Virolainen, B. K. Edwards. *J. Natl. Cancer Inst.* **90**, 440–446 (1998).
45. L. C. Clark, B. Dalkin, A. Krongrad, G. F. Combs Jr., B. W. Turnbull, E. H. Slate, R. Witherington, J. H. Herlong, E. Janosko, D. Carpenter, C. Borosso, S. Falk, J. Rounder. *Br. J. Urol.* **81**, 730–734 (1998).
46. M. Pastori, H. Pfander, D. Boscoboinik, A. Azzi. *Biochem. Biophys. Res. Commun.* **250**, 582–585 (1998).