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This is the author's manuscript

Original Citation:

Availability:

This version is available http://hdl.handle.net/2318/1556209 since 2016-02-18T15:51:25Z

Published version:

DOI:10.1002/pros.22999

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(Article begins on next page)

05 June 2024

A randomized double-blind placebo controlled phase I–II study on clinical and molecular effects of dietary supplements in men with precancerous prostatic lesions. Chemoprevention or "chemopromotion"?

Paolo Gontero[1*], Giancarlo Marra[1], Francesco Soria[1], Marco Oderda[1], Andrea Zitella[1], Francesca Baratta[2], Giovanna Chiorino[3], Ilaria Gregnanin[3], Lorenzo Daniele[4], Luigi Cattel[2], Bruno Frea[1] and Paola Brusa[2]
1Department of Surgical Sciences, Città della Salute e della Scienza, University of Turin, Turin, Italy
2Department of Scienza e Tecnologia del Farmaco, University of Turin, Turin, Italy
3Cancer Genomics Laboratory, Fondazione Edo ed Elvo Tempia, Biella, Italy
4Department of Pathology, Città della Salute e della Scienza, University of Turin, Turin, Italy

Abstract

BACKGROUND

Antioxidants effectiveness in prostate cancer (PCa) chemoprevention has been severely questioned, especially after the recent results of the Selenium and Vitamin E Cancer Prevention Trial. We present the results of a double-blind randomized controlled trial (dbRCT) on the pharmacokinetic, clinical, and molecular activity of dietary supplements containing lycopene, selenium, and green tea catechins (GTCs) in men with multifocal high grade prostatic intraepithelial neoplasia (mHGPIN) and/or atypical small acinar proliferation (ASAP).

METHODS

From 2009 to 2014, we conducted a dbRCT including 60 patients with primary mHGPIN and/or ASAP receiving daily lycopene 35 mg, selenium 55 µg, and GTCs 600 mg, or placebo for 6 months. Pharmacokinetic analysis were performed with UV–Visible spectrophotometric assay under standard (SC) and accelerated (AC) conditions. Upon plasma lycopene concentrations falling within the expected range (1.2–90 mcg/l) and no side-effects of grade >1, study proceeded to phase II (n = 50). After unblinding of results, eight men (4 per arm, 2 without and 2 with PCa, respectively) were randomly selected and totRNA extracted from "non-pathological" tissues. MicroRNA profiling was performed with the Agilent platform. Raw data processing used R-statistical language and linear models for microarray analysis.

RESULTS

Samples were stable except for lycopene, showing significant degradation (SC = 56%, AC = 59%) and consequently stabilized under vacuum in a dark packaging. Mean plasmatic lycopene concentration was 1,45 ± 0,4 µM. At 6 months, 53 men underwent re-biopsy and 13 (24.5%) were diagnosed with PCa (supplementation n = 10, placebo n = 3 [$P = 0.053$]). At a mean 37 months follow-up, 3 additional PCa were found in the placebo group. No significant variations in PSA, IPSS, and PR25 questionnaires were observed. Stronger modulation of miRNAs was present on re-biopsy in the supplementation group compared to the placebo, including: (i) overexpression of miRNAs present in PCa versus non-cancer tissue; (ii) underexpression of miRNAs suppressing PCa proliferation; (iii) detection of 35 miRNAs in PCa patients versus disease-free men, including androgen-regulated miR-125b-5p and PTEN-targeting miR-92a-3p (both upregulated).

CONCLUSION

Administration of high doses of lycopene, GTCs, and selenium in men harboring HGPIN and/or ASAP was associated with a higher incidence of PCa at re-biopsy and expression of microRNAs implicated in PCa progression at molecular analysis.

INTRODUCTION

Prostate cancer (PCa) has always been viewed as an ideal target for chemoprevention due to its long natural history and its high incidence 1. The influence of diet, race and environmental factors on PCa development is well documented by epidemiological studies [1-3]. Over the last decade, several potential chemopreventive agents have been employed for PCa, including selenium, lycopene, and green tea catechins (GTCs), due to their antioxidant and anti-proliferative properties [4-6]. In particular, selenium was shown to decrease both PSA and androgen-receptor transcripts, while GTCs inhibited angiogenesis and 5-a-reductase activity, with a possible protective action in patients with HGPIN [4, 6, 7]. These properties, together with a low toxicity, seemed ideal to employ these natural compounds as PCa chemopreventive agents, if confirmed in human subjects [4, 6, 7].

On this basis, in 2009 we started a double-blind Phase I–II randomized controlled trial (RCT) to assess the chemopreventive ability of a combination of lycopene, selenium and GTCs at the highest non-toxic concentrations in men with multifocal high grade prostatic intraepithelial neoplasia (HGPIN) and/or atypical small acinar proliferation (ASAP). Such patients, believed at high risk of developing PCa, seemed the ideal target for chemoprevention, considering that the likelihood of finding a cancer on re-biopsy in men with multifocal HGPIN and ASAP has been estimated at 39–55% and 34–60%, respectively [8]. The disposition toward vitamin E and selenium in PCa chemoprevention has dramatically changed with the recent disclosure of results from a large subset analysis of Selenium and Vitamin E Cancer Prevention Trial (SELECT) [9], showing that these compounds failed to prevent, but rather promoted PCa development. In 2014, in parallel with these new developments, we unblinded the results of our study, which are presented and discussed thereafter.

MATERIALS AND METHODS

Study Design, Galenic Formulation, and Study Endpoints

After institutional board approval (protocol number CEI/489), between November 2009 and April 2014 we prospectively enrolled patients aged between 45 and 75 years, with histological diagnosis of multifocal HGPIN (≥2 positive cores) or ASAP on 12-core primary transperineal prostate biopsy. Exclusion criteria included previous or ongoing intake of dietary supplements potentially interfering with PCa natural course and/or 5-α-reductase inhibitors, and the presence of manifest neoplastic or any other severe clinical condition. Eligible subjects were then randomized in double blind into two groups. One group received a dietary supplementation containing selenium–methionin, lycopene, and green tea extracts for a total of 55 μg of selenium, 35 mg of lycopene, and 600 mg of polyphenols per day (Supplementation Group). Supplementation compounds choice and respective dosages were established using the maximum non-toxic dose of the dietary substances according to the highest level of evidence for PCa chemoprevention at the time the study was initiated [10]. A pregelatinized maize starch excipient (quantum sufficit) was added in the capsule composition to avoid dietary intolerance. The other group received a placebo containing the excipient only (Placebo Group). For both groups, preparations were given as hard capsules, 3 per day per 6 months. All components were purchased from Farmalabor s.r.l. (Canosa di Puglia, Italy).

The study design is summarized in Figure 1. We began Phase I by enrolling ten patients, randomized to take supplementation or placebo for 1 month. All underwent clinical visits at enrolment and after 1 month to collect data on PSA, IPSS score, quality of life (QoL) (through the EORTC-QLQ-PR25 questionnaire), adverse events (according to the Common Terminology Criteria for Adverse Events Version 4 (CTCAEv4), and patient compliance (through oral questions and the number of empty drug packages returned). Plasma samples were analyzed for pharmacokinetic assessment at enrolment and at each follow-up visit.

Having verified the absence of CTCAEv4 toxicity grade >1, 50 additional patients were included in Phase II for a total of 60 men [30 + 30] randomized in the two groups. All parameters collected at the study entrance were re-assessed at the 6 months follow-up visit, when all the patients underwent a 14- to 20-core re-biopsy. All patients were further followed up until November 2014 with PSA testing, and DRE every 6 months.

Primary endpoint of Phase I was the evaluation of chemical stability, tolerability, and blood concentrations of lycopene, whereas the assessment of disease (PCa and/or HGPIN/ASAP incidence) at re-biopsy in the two groups constituted the main endpoint of Phase II. Variations of PSA, IPSS, QoL, and compliance to treatment during follow-up and between the two groups were secondary endpoints, together with the evaluation of microRNA (miRNA) expressions in a subgroup of patients.

Stability and Pharmacokinetic Assessment

Different temperatures (T) and relative humidity (RH) levels were applied according to EMA (European Medicines Agency) guidelines for medicinal products to evaluate the substances and the pharmaceutical form for chemical stability in standard (SC) (T = 25 ± 2°C, RH = 60 ± 5%) and in accelerated conditions (AC) (T = 40 ± 2 °C, RH = 60 ± 5%)[11]. Tests were performed every 30 days for a total of 36 months for SC and 3 months for AC. Analysis was performed with UV–Visible spectrophotometric assay.

Pharmacokinetic evaluation of lycopene plasmatic levels was then performed to ensure appropriate bioavailability, due to its high instability. To quantify lycopene in plasma samples a standard procedure was developed starting from the method used by Talwar et al.[12]. Lycopene plasma levels were assessed through an HPLC method using a UV detector (from Shimdzu, SPD-10A VP UV–Vis detector, LC-10AD VP liquid chromatograph). All chemicals were of analytical grade (from Sigma–Aldrich, Milan, Italy). Experiments were performed in the absence of direct sunlight at ambient temperature. Analytical chromatographic conditions were: a Purospher® STAR RP-18e [5 μm] LiChroCART® 250-4.6 column; a mobile phase of acetonitrile and tetrahydrofuran [86:14, v/v] with a flow-rate of 1.1 ml/min; an injection volume of 200 μl; absorption was measured at 472 nm wavelength (max lycopene wavelength absorption). The elution time of lycopene in plasma samples was approximately 15 min. Stock solution of lycopene (0,1 mg/ml) was prepared in a mixture of dichloromethane—hexane 25:75 (v/v). and then stored at −20 °C. The solution was stable for at least 1 month. Calibration curves (0.5–5 μg/ml of lycopene) were built using either solutions of standards or spiked plasma standards. Analysis of plasma samples was then performed. To prevent lycopene degradation the galenic preparation was stored in a dark polypropylene package under nitrogen atmosphere. This package was placed in a polypropylene bag under vacuum.

Molecular Assessment Using microRNA Expression Profiling

Comparison of miRNA levels between first and second biopsy was carried out in eight men (four placebo and four active treatment, all of them being selected randomly, two amongst those having no evidence of disease and two amongst those diagnosed with PCa at second biopsy within each arm, respectively). Tissue samples (16 in total) were extracted from areas of the paraffin block corresponding to normal tissue adjacent to "pathological" areas when present (HGPIN/ASAP at first biopsy and HGPIN/ASAP or PCa at second biopsy). After macro dissection totRNA was extracted using miRNeasy FFPE kit (Qiagen) and miRNA expression profiling was then carried out using the one-color labeling method (Agilent Technologies). Labeling, hybridization and washing were performed following manufacturer protocols. Samples were hybridized to human miRNA microarrays (Version 19, Agilent Technologies). Slides were scanned with an Agilent C dual-laser microarray scanner and images analyzed with Feature Extraction software. Raw data from molecular analysis were processed using R and the limma (linear models for microarray analysis) package for normalization and class comparison, while TMev software was used for hierarchical clustering analysis on differentially expressed miRNAs.

Statistical Analysis

Statistical analysis of clinical outcomes was performed using T, χ^2, and Fisher exact test, when appropriate. For molecular analyses, second minus first biopsy miR intensities were singled out as variables to be tested in: (i) one-class comparison, to detect miRNAs differentially expressed on re-biopsy for all eight patients and for the placebo or the supplementation group respectively; (ii) two-class paired comparison, to identify miRNAs differently changed on re-biopsy in the supplementation vs the placebo group or in patients who had PCa diagnosis vs those who did not. Moderated t-statistics was used, with a significance level of $P < 0.05$.

RESULTS

Phase I: Stability and Pharmacokinetic Assessment

No alterations up to 3 years were found both under SC and AC for green tea extract and selenomethionine. All tested samples were considerably stable over time: the mean variation detected during the stability tests, both in SC and AC, was lower than 10%. On the contrary, the lycopene extract was found particularly sensitive to temperature, light and atmospheric oxygen showing significant degradation under SC and AC after only one month. The degradation in SC was 56%, in AC was 59%. When the unpackaged powder was directly exposed to light and oxygen, the degradation was 52% after only 4 days.

To guarantee the stability of lycopene, the extract was then stored under vacuum in a dark packaging: the extract was stable up to 36 months, the tested samples showing absorbance variations in SC lower than 10%. The developed HPLC method allowed to highlight the presence of lycopene in the plasma: mean plasmatic lycopene concentration was 1.45 ± 0.4 μM.

Phase I–II: Clinical Assessment

Mean patients age was 63.3 (SD 7) with no significant differences being found between the two arms. Three subjects discontinued treatment: two had indication for re-biopsy at 3 months because of high PSA velocity (placebo n = 1, supplementation n = 1), while one in the supplementation group complained of abdominal pain (CTCAEv3 grade 2). All three patients were considered in the intention to treat analysis. Treatment was well tolerated (no recorded toxicities) in all the remaining cases.

Clinical and pathological features are shown in Table I. The two groups were balanced as for the finding of ASAP and HGPIN or their combination at baseline. Mean PSA and DRE, with no substantial differences across groups at baseline,

did not significantly change in the 6 months period in both arms. Fifty-three patients out of sixty underwent re-biopsy after a mean of 7 months from previous biopsy; 6 refused the re-biopsy, while 1 patient died for unrelated causes. At re-biopsy, PCa was found in 13 cases: 10 in the supplementation group and 4 in the placebo group (P = 0.053). Gleason score (GS) did not differ significantly between the two groups; no GS ≥8 was present. At a mean follow-up of 37 months, three more neoplasms were found after an additional re-biopsy due to persistent PCa suspicion, all in the placebo group; GS was 6 in 2 patients and 9 in 1. Out of the total of 16 PCa patients, 12 underwent active treatment (radical prostatectomy n = 11, external beam radiation therapy n = 1) and 4 were placed on active surveillance (AS). At last follow-up all treated men were alive and disease free. One AS patient died for causes other than PCa. A significant reduction of both HGPIN and ASAP findings compared with baseline was found at re-biopsy, with no difference between the two arms (Table I). IPSS and PR25 did not show any significant variation during the study and between groups (Table II).

Phase II: Molecular Assessment Using microRNA Expression Profiling

Post- versus pre-treatment analysis on samples from all eight patients, irrespective of treatment, gave a list of 48 modulated miRNAs whose level was mainly altered in the supplementation group. This observation was confirmed when the four patients in the placebo group were analyzed separately, yielding only 5 slightly modulated miRNAs. On the other hand, 39 miRNAs with a significant and strong modulation were found when the supplementation group was considered separately. The expression differences of these 39 miRNAs in all 8 patients are shown in Figure 2A. Among those with increased level after supplementation, we found a consistent group of microRNAs already identified as overexpressed in cancer versus non cancer stroma (namely, let-7f-5p, miR-100-5p, miR-130a-3p, miR-23a-3p; [19]) and/or in PCa versus normal prostate tissue (namely, miR-26b-5p, let-7i-5p, let-7d-5p, miR-16-5p, miR-199a-5p, miR-214-3p, miR-15a-5p, miR-29b-3p, miR-30e-5p, and miR-34a-5p; [20, 21]). Upregulation of miR-15a-5p, already demonstrated in the progression of PCa from HGPIN to metastasis, was also present [13]. Among the miRNAs downregulated by supplementation, the most modulated microRNA was miR-494, with a known oncosuppressive role in PCa [14, 21]. Two-class paired analyses identified 35 microRNAs differently modulated in the patients with a PCa diagnosis at re-biopsy compared to those without cancer (Fig. 2B). Notably, several miR-let-7 family members, miR-193b-3p, miR-92a-3p, miR-10b-5p, miR-103a-3p, miR-100-5p, miR-16-5p, and miR-125b-5p were upregulated in PCa cases. In Figure 3, intersections of modulated miRNAs in all the comparisons carried out are shown, with circles proportional to the number of miRNAs and names indicated for miRNAs resulting from more than one analysis.

DISCUSSION

According to our results, a dietary supplementation at the highest non-toxic doses of selenium, lycopene and GTCs would exert a negative rather than positive impact on HGPIN/ASAP patients in terms of risk of PCa development. In our study, PCa incidence was three times higher in patients taking supplementation compared to those who did not.

In the past, a PCa protective role for the investigated supplements was suggested by several studies [15, 16], most of which affected by strong limitations: the amount of the dietary intakes were usually badly reported, blood concentrations of supplements were rarely assessed, and cut-offs defining low-, medium-, and high-intake were often inaccurate. Furthermore, self-administered questionnaires were the most common method to assess the dietary intake, but were highly heterogeneous and often unreliable. Finally, significant confounding factors related to different lifestyle habits in those taking or not the investigated substance are also likely to be present 6.

In line with our findings, recent RCTs questioned the PCa protective ability of selenium [22, 23]: the SELECT trial, the largest cancer prevention study ever performed, and the SWOG S9917 failed to show any PCa preventive effect of selenium methionine in disease-free men with or without HGPIN, respectively [17, 24]. Similarly, a Canadian RCT investigating the rate of progression from HGPIN to PCa did not discover any protective property for selenium given in combination with soy and vitamin E [25].

Furthermore, recent subset analyses of SELECT have warned about the use of selenium [9], showing that selenium supplementation did not benefit men with low baseline selenium status and increased the risk of high-grade PCa among men with high baseline selenium status.

GTCs have been associated to a 90% PCa risk reduction in a small RCT on patients with HGPIN [4]; these results were confirmed after 2 years of follow-up as statistically significant, despite a high patient dropout rate [22]. However, the protective role of catechins was recently questioned by a trial which showed similar PCa progression rates between patients taking 400 mg GTCs a day and placebo (ClinicalTrials.gov Identifier NCT00596011). A recent meta-analysis found no significant association between tea consumption and reduced PCa risk, overall (OR = 0.86, 95%CI 0.69–1.04) and in stratified analysis according to population and tea type [16].

As for lycopenes, serum concentrations are unrelated to PCa incidence according to Health Professionals Follow-up Study and Prostate, Lung, Colorectal, and Ovarian Cancer Screening Study [23, 26]. The PCa Prevention Trial including 9.559 participants found no correlations between dietary intake and PCa [27]. The PCa protective ability of lycopenes has been

suggested only in one small RCT on HGPIN patients: Mohanty et al. [5] found three times lower PCa incidence in the lycopene group compared to placebo. Nevertheless, these results were not statistically significant, selection of HGPIN patients was performed with sextant biopsies or TURP, and rates of re-biopsies were different in the two groups. According to other studies, the concentrations of lycopene in prostatic tissue are lower in men progressing from HGPIN to PCa compared to those who do not [28]. An issue of studies investigating lycopene chemopreventive ability is the poor correlation of plasmatic level dosage following their intake. We ascertained the sensitivity of lycopene to environmental conditions (temperature, light, atmospheric oxygen) and devised a specific packaging to guarantee the stability of both the extract and the galenic preparation before entry into the bloodstream. Plasma lycopene concentration of treated subjects (1.45 ± 0.4 μM) in our study was consistent with the one reported for products containing lycopene stabilized by the association with other antioxidants [29]. The confirmation of appropriate blood lycopene concentration, along with the use of the highest non-toxic doses also of selenium and GTCs, strengthens our results, supporting the absence of preventive properties for the supplementation.

Our clinical findings are supported by molecular analyses comparing miRNA levels before and after treatment in tissue samples adjacent to pre- or neoplastic lesions. The dietary supplementation was associated with expression patterns not clearly in keeping with a chemopreventive effect. In the supplementation group, we identified upregulation of miRNAs with a putative tumor suppressive role in PCa, such as the miR-23a∼27a∼24-2 cluster which might control the Wnt pathway [30] known to activate transcription of proliferation genes; however, most of them exhibit context-dependent functions [20, 31] and may also exert oncogenic roles. In the same group, we found overexpression of known oncomiRs such as miR-199a, miR-92a, and miR-30e, as well as of miR-16 [32]. The latter was peculiarly overexpressed in cases with PCa at re-biopsy. In addition miR-494, previously found downregulated in cancerous versus normal prostate tissues [17] and targeting CXCR4 16 and PTEN 33 to suppress the proliferation, invasion, and migration of PCa, resulted underexpressed in the supplementation arm. Taken together, molecular findings reveal overexpression of several oncogenes and underexpression of oncosuppressors related miRNAs in prostatic tissue following a 6 months oral intake of our dietary supplementation. This could explain the negative or as null as placebo effect on PCa prevention. Martinez et al. [34] found that antioxidant administration may increase the risk of prostate carcinogenesis in animal models with selected genotypes and subsequently investigated the SELECT biomarker case-control cohort [30]; according to the authors, genetic background may modulate the effects of selenium supplementation increasing PCa risk.

With regards to our secondary endpoints, no significant differences before and after treatment were found in PSA, IPSS, and QoL, in contrast with groups reporting a beneficial effect on urinary tract symptoms and/or a reduction of PSA levels for each of the three investigated supplementation components [4, 5, 7, 28, 18].

Our study has some limitations. Amongst them are the relatively small number of patients and the simultaneous use of three compounds which does not allow precise evaluation of each substance, and the absence of assessment for family history of PCa. Molecular analysis was not performed in all patients due to cost-related issues. Nonetheless, this study has the strength of being a well-designed double-blinded RCT: innovative at the time of its conceiving, now it confirms the warnings coming from SELECT and more recent studies, at least for selenium [9, 30]. About our target population, it is worth reminding that we do not have clear data on the natural history of HGPIN/ASAP yet, nor on their real risk in terms of PCa development [8]. This may account as a serious limitation of a study having PCa incidence as an endpoint. That said, we must remember that nowadays 39–51% of new PCa patients use dietary supplements, hoping to have some beneficial effects [33, 35]. Once again, a warning must be issued about the uncontrolled use of supplements containing selenium, and possibly lycopenes and GTCs. According to our results, a dietary supplementation of high doses of selenium, lycopene and GTCs cannot be recommended in patients with a history of HGPIN, ASAP, or PCa.

CONCLUSION

Our clinical and molecular analyses suggest that dietary supplementation with high doses of selenium, lycopene, and green tea catechins in HGPIN/ASAP patients carries a higher risk of PCa at re-biopsy compared with placebo. Whilst the "oncogenic" potential of these products still remains to be demonstrated, no PCa protective ability was shown. All these things considered, the use of these supplements should be avoided in this category of patients.

ACKNOWLEDGMENT

The study was supported by an unrestricted grant from the Regional Government of Piedmont, Italy, from the Bank of Piedmont, Turin, Italy and from MIUR University of Turin "Fondi Ricerca Locale (ex 60%)".

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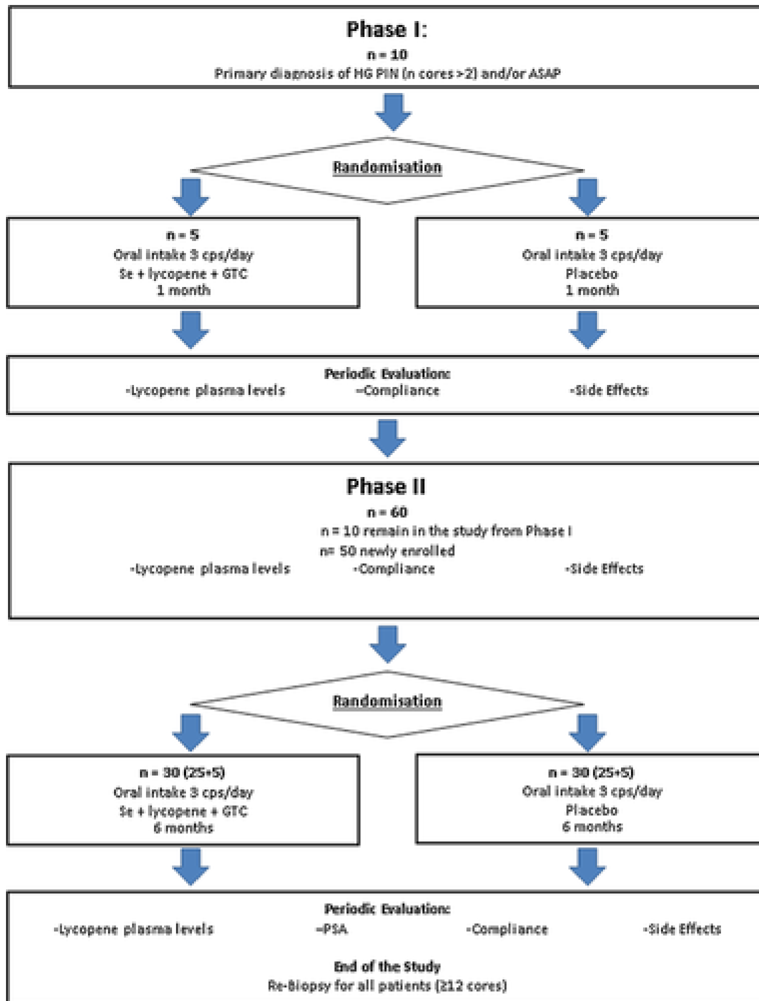

Figure 1

Study Flowchart. HG PIN, High Grade Prostatic Intraepithelial Neoplasia; ASAP, Atyipical Small Acinar Proliferation; Se, Selenium; GTC, Green Tea Catechins.

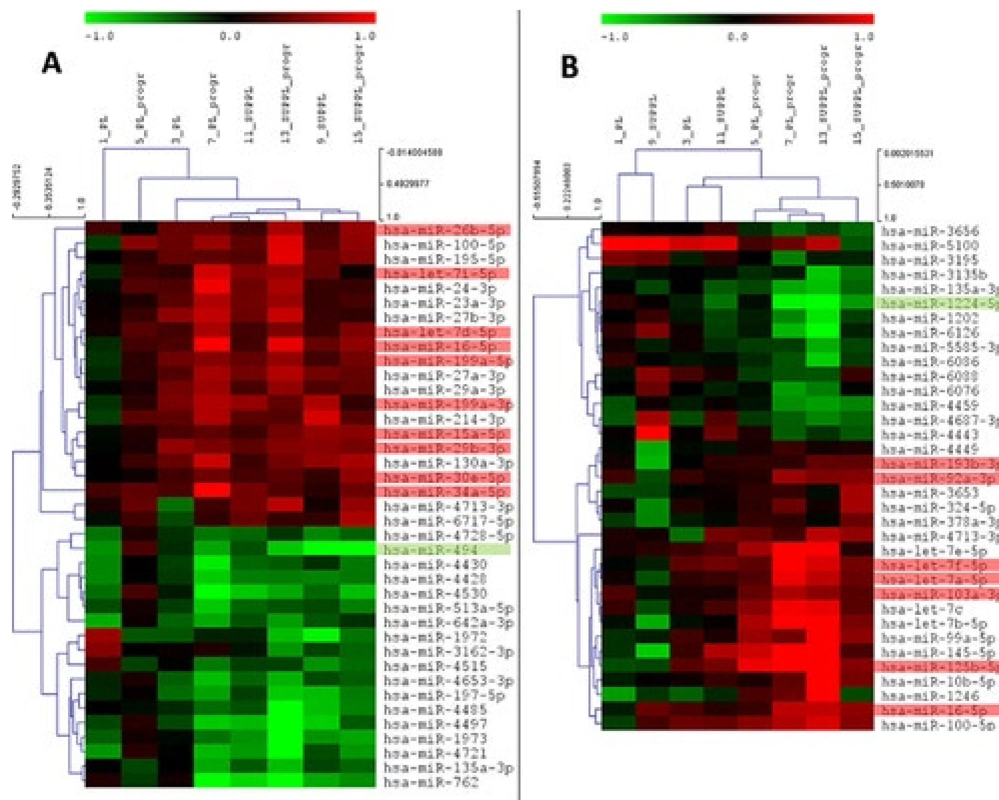

Figure 2

(A) Hierarchical clustering of 39 miRNAs differentially expressed on re-biopsy among men in the supplementation group, using Pearson correlation as similarity metrics and average as linkage method. Columns represent the miRNA profiles of the four men of the placebo group (on the left) or in the supplementation one (on the right). Each square represents the difference of miRNA log intensities in second vs first biopsy tissue samples, with a color scale from green (downregulation, where −1 means a twofold expression decrease on re-biopsy) to red (upregulation, where +1 means a twofold expression increase on re-biopsy). The list of miRNAs was obtained by one class comparison on the supplementation group, using the limma package (moderate t-test P-value cut-off: 0.01 and fold-change cut-off: 1.25). Highlighted in red (green) the names of miRNAs already found overexpressed (underexpressed) in prostate cancers versus normal prostate tissue in another study carried out by our group and/or in 13-17.

(B) Hierarchical clustering of 35 miRNAs differently modulated in the four patients who progressed respect to those who did not, obtained by two-class paired analysis with limma (moderate t-test P-value cut-off: 0.05 and fold-change cut-off: 1.25). Pearson correlation was used as similarity metrics and average as linkage method. Each square represents the difference of miRNA log intensities in second versus first biopsy tissue samples. Highlighted in red (green) the names of miRNAs already found overexpressed (underexpressed) in prostate cancers versus normal prostate tissue in another study carried out by our group and/or in ref. 14, 15, 17, 18.

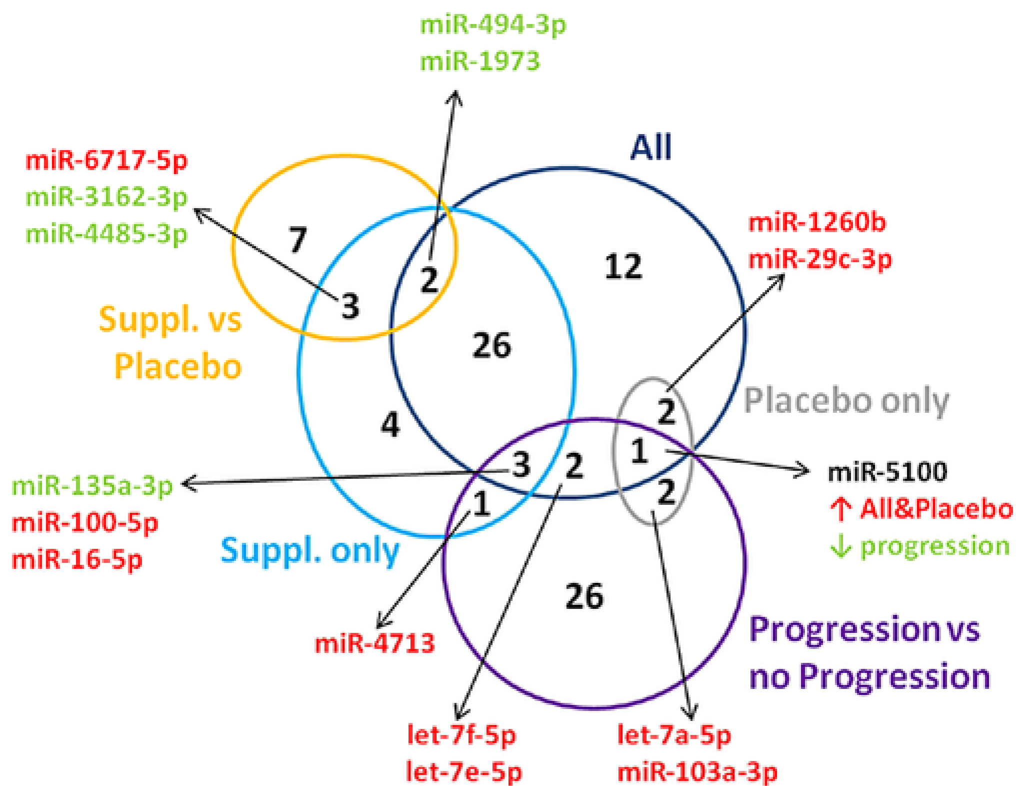

Figure 3
Venn diagram of the lists of differentially expressed miRNAs found in the class comparison analyses carried out. miRNAs in green characters are down-regulated on re-biopsy in one-class comparison or less strongly modulated in two-class paired comparison. miRNAs in red are up-regulated or more strongly modulated.

Table I. Baseline and Six Months Characteristics and Outcome Measures

	Baseline				6 Months			
Variable	**All patients n = 60**	**Placebo n = 30**	**Treatment n = 30**	***P***	**All patients n = 53**	**Placebo n = 26**	**Treatment n = 27**	***P***
Age mean (SD)	63.3 (7.0)	62.6 (8.2)	64.1 (5.7)	0.41				
PSA mean (SD)	6.3 (3.2)	6.5 (3.7)	6.2 (2.6)	0.74	6.2 (4.6)	6.5 (4.3)	6.0 (4.9)	0.74
DRE positive n (%)	21 (34.8)	8 (26.7)	13 (43.3)	0.28	20 (37.7)	6 (23.1)	13 (48.1)	0.09
Biopsy cores taken								
Mean (SD)	14.9 (3.3)	15.1 (4.4)	14.8 (1.8)	0.73	15.5 (2.8)	15.0 (2.0)	15.7 (3.3)	0.64
ASAP n (%tot)								
Overall	32 (53.3)	17	15	0.62	9 (17.0)	3	6	0.47
Monofocal	24	11	13		8	3	5	
Multifocal	8	6	2		1	0	1	
HGPIN n (%tot)	18 (30.0)	9	9	1.00	4 (7.5)	2	2	1.00
HGPIN + ASAP n (%tot)	10 (16.7)	4	6	0.73	2 (3.8)	1	1	1.00
PCa Overall n (%tot)					13 (24.5)	3 (5.7)	10 (18.9)	
Gleason 6 n					6	1	5	0.05
Gleason 7 n					7	2	5	
Gleason ≥8 n					0	0	0	
Negative n					40	23	17	

- All participants were Caucasians.
- PSA, prostate specific antigen; DRE, digital rectal examination; HGPIN, high grade prostatic intraepithelial neoplasia; ASAP, atypical small acinar proliferation; PCa, prostate cancer.

Table II. IPSS and Quality of Life Variations According to the PR25 Questionnaire Overall and in the Two Groups During the Study Period

	All n = 53	Placebo n = 26	Treatment n = 27	*P*
IPSS variation	+1.0	−0.4	+2.4	0.22
PR25 variation				
Urinary symptoms (SD)	+1.1 [5.4]	+2.1 [5.4]	0 [5.2]	0.14
Bowel symptoms (SD)	0 [0]	0 [0]	0 [0]	1.0
Sexual activity (SD)	−2.5 [21.7]	+4.2 [23.9]	−8.5 [11.8]	0.27
Sexual functioning (SD)	+4.2 [3.0]	−6.3 [3.4]	+3.1 [3.2]	0.38

- PR25, EORTC-QLQ-PR25 questionnaire.