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Cancer Prevention Research

Research Article

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A Randomized, Placebo-Controlled, Preoperative Trial of Allopurinol in Subjects with Colorectal Adenoma

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Abstract

Inflammation and oxidative stress play a crucial role in the development of colorectal cancer (CRC) and interference with these mechanisms represents a strategy in CRC chemoprevention. Allopurinol, a safe molecular scavenger largely used as antigout agent, has been shown to increase survival of patients with advanced CRC and to reduce CRC incidence in long-term gout users in epidemiologic studies. We conducted a randomized, double-blind, placebo-controlled preoperative trial in subjects with colorectal adenomatous polyps to assess the activity of allopurinol on biomarkers of colorectal carcinogenesis.

After complete colonoscopy and biopsy of the index polyp, 73 subjects with colorectal adenomas were assigned to either placebo or one of two doses of allopurinol (100 mg or 300 mg) and treated for four weeks before polyp removal. Change of Ki-67 labeling index in adenomatous tissue was the primary endpoint. Secondary endpoints were the immunohistochemical (IHC) expression of NF-κB, β-catenin, topoisomerase-II-α, and terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) in adenomatous polyps and normal adjacent colonic tissue.

Compared with placebo, Ki-67 levels were not significantly modulated by allopurinol, whereas β-catenin and NF-κB expression levels decreased significantly in adenomatous tissue, with a mean change from baseline of −10.6%, 95% confidence interval (CI), −20.5 to −0.7, and −8.1%, 95% CI, −22.7 to 6.5, respectively. NF-κB also decreased significantly in normal adjacent tissue (−16.4%; 95% CI, −29.0 to −3.8). No dose–response relationship was noted, except for NF-κB expression in normal tissue.

Allopurinol can inhibit biomarkers of oxidative activation in colon adenomatous polyps and normal adjacent tissue. Further studies should define its potential chemopreventive activity. *Cancer Prev Res; 6(2); 74–81. ©2012 AACR.*

Introduction

Colorectal cancer (CRC) is a major neoplasm worldwide and both its prevalence and mortality are increasing (1). Although CRC clinical symptoms develop late, its precursor lesion adenoma can easily be detected (2, 3). Because the development of an adenoma into CRC may take an average of 10 to 15 years (2, 4), chemoprevention may be a practical approach to reduce CRC incidence (4, 5).

Chronic inflammation associated with microbial infection directly contributes to the etiology of approximately 20% of all epithelial cancers (6). The chronic inflammatory microenvironment in colon cancer tissue is characterized by immune dysregulation and elevated levels of reactive oxygen species (ROS), including superoxide, hydrogen peroxide, and singlet oxygen (7, 8).

Allopurinol, a structural analog of hypoxanthine, has been used in the treatment of gout for many of years and has a well-defined safety profile (9, 10). This drug is also commonly used in the treatment of inflammatory bowel disease because of its anti-inflammatory properties (10). Its activity leads to the reduction of uric acid through the inhibition of xanthine oxidase, an enzyme producing ROS (i.e., superoxide anions) by catalyzing the hydroxylation of many purine substrates, including hypoxanthine, which is converted to xanthine and then to uric acid (10, 11). Although little is known about the role of xanthine oxidase in carcinogenesis, an increase of this enzyme has been

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found in different types of human cancers (12) and mouse skin tumors (13). Elevation of xanthine oxidase activity during carcinogenesis, including the promotion phase, was also reported (14). Several natural products in edible plants, including genistein and green tea, are known to act as xanthine oxidase inhibitors (15) and have been reported to display cancer preventive activity in preclinical models (16–19). Allopurinol is able to prevent early alcohol-induced liver injury in rats, most likely by inhibiting the oxidant-dependent activation of the NF-κB pathway (20), and to decrease ROS production in animal models, thus preventing the activation of mitogen-activated protein kinase [MAPK; p38, extracellular signal–regulated kinase (ERK) 1 and ERK 2]-–NF-Kβ pathways (21).

A population-based case–control study conducted in Israel (22) showed that the use of allopurinol for at least 5 years was associated with diminished risk of CRC [OR, 0.33; 95% confidence interval (CI), 0.16–0.71] after adjustment for other known risk factors. Allopurinol has also been shown to increase survival of patients with advanced CRC (23, 24). So far, however, no clinical trial has investigated the effect of allopurinol on CRC risk modulation.

Window-of-opportunity, preoperative studies may provide insight into the preventive potential of drugs by modulating intraepithelial neoplasia and adjacent normal mucosa, thus targeting the field cancerization effect in colon carcinogenesis (25–28). The present proof-of-principle trial evaluated a 4-week administration of allopurinol in subjects with colorectal adenomas more than 1 cm in diameter. While no surrogate endpoint biomarkers have been clinically validated for colon carcinogenesis, we selected the nuclear Ki-67 proliferation antigen-labeling index (LI) as the primary endpoint variable given its clinical validation in different neoplasms (29, 30). Moreover, the immunohistochemical (IHC) expression of NF-κB and β-catenin were selected as secondary endpoints for their role in oxidative activation (31, 32), whereas topoisomerase-II-α and terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) were selected as markers of proliferation and apoptosis for their putative role in colon carcinogenesis progression (33, 34).

Materials and Methods

Eligibility criteria and study design

Subjects of ages 18 years or older with at least 1 histologically confirmed adenoma with diameter 1 cm or more were eligible for trial entry. Exclusion criteria were: prior CRC, flat or hyperplastic adenomas, grade >1 alterations of hepatic and renal function, anticoagulant therapy with dicumarol, acute gout, and use of allopurinol within the last 6 months. Because nonsteroidal anti-inflammatory drugs and calcium supplementation may interfere with proliferation and apoptosis, information about concomitant medications was carefully collected.

The study was a randomized, double-blind, placebo-controlled, multicenter clinical trial. The primary endpoint was the change of Ki-67 LI in adenomatous colon tissue. Secondary endpoints included IHC expression of NF-κB, β-catenin, topoisomerase-II-α, and TUNEL, and circulating ultrasensitive C-reactive protein (CRP) and insulin-like growth factors (IGF; IGF-1, IGFBP-3). The study was approved by the hospital Institutional Review Boards (IRB; EUDRACT code: 2006-001084-27) and all subjects signed an informed consent.

Treatment plan and study procedures

The study was conducted in 3 centers: E.O. Ospedali Galliera [Genoa (coordinating center), Italy], European Institute of Oncology (EIO; Milan, Italy), and National Institute for Cancer Research (Genoa, Italy). Randomization list was centralized at the coordinating unit and stratified by center. Treatment assignment was blinded and a sealed list indicating the content of each set of bottles was kept at the coordinating center and was available for consultation only for safety reasons.

After complete colonoscopy and biopsy of the index polyp and adjacent normal-appearing rectal mucosa, subjects with histologically confirmed adenomas were assigned, within a week from the baseline biopsy, to either placebo or 1 of 2 clinical doses of allopurinol (100 mg or 300 mg) and treated for 4 weeks until the day before endoscopic polypectomy. Polyps were tattooed whenever their multiplicity or anatomic site (e.g., sigmoid) could make identification for polypectomy difficult at second colonoscopy. The experimental treatment duration was tailored to the current standard clinical practice as polyps more than 1 cm in diameter are removed for safety reasons during a second colonoscopy after blood coagulation tests are available. Moreover, an interval of 4 weeks between polyp detection and polypectomy is within the waiting time range in many national health system centers.

Given the presumed antiproliferative effect of polyethylene glycol, colonic cleansing before colonoscopy was obtained using sodium phosphate, using a divided-dose regimen, the first the evening before the procedure and the second 10 to 12 hours later, as previously described (35).

The drug and placebo were purchased through the coordinating hospital Pharmacy, encapsulated to ensure blinding, packaged and labeled for the study. Samples of normal colonic tissue were also collected at baseline and end-of-study colonoscopy. Fasting blood samples were taken between 8 and 10 am at baseline and on the day of polypectomy for hematology, biochemistry, and serum biomarkers. Treatment adherence was assessed by pill count. Toxicity was evaluated using the National Cancer Institute-Common Terminology Criteria for Adverse Events version 3.0 (NCI-CTCAE; ref. 36).

Analytic methods

Immunohistochemistry. Immunohistochemistry was carried out by staining formalin-fixed, paraffin-embedded 3-μm tissue sections of colon biopsies with the following antibodies: Ki-67 mouse monoclonal clone 30–9 (Ventana); topoisomerase-II-α, mouse monoclonal clone Ki-S1 (Dako); β-catenin, mouse monoclonal clone 14 (Cell Marque); and NF-κB, mouse monoclonal clone F-6 (Santa

Cruz). Sections were stained using the automatic immunostainer Benchmark XT (Ventana); the sections were deparaffined and antigen-retrieval was conducted with citrate buffer high pH for 30 minutes and incubated with primary antibody tested by optimal dilution according to the data sheet for topoisomerase-II-α 1:500 and NF-κB 1:50 and treated for an automated IHC staining for β-catenin and Ki-67. Monoclonal antibodies (mAb) were then incubated for 40 minutes (Ki-67 and β-catenin), or 1 hour (topoisomerase-II-α and NF-κB), at 37°C followed by addition of the polymeric detection system (Ventana). An appropriate positive tissue control was used for each staining run; the negative control consisted of the entire IHC procedure on adjacent sections in the absence of the primary antibody. The sections were counter-stained automatically with Gill's-modified hematoxylin and then cover-slipped. The sections were evaluated by Leica DMLA light microscope with ×40 magnification and using the image analysis Leica QWin software to count the percentage of positive nuclear areas for Ki-67, topoisomerase-II-α, and NF-κB over the total nuclear areas in 10 section fields. The immunostained sections were examined with an optical microscope (×10 magnification) to find the location with greater immunoreactivity. Cells with unequivocal granular staining were considered positive. Leica-QWin software calculated automatically the percentage of positive cells as the result of the average of 10 consecutive fields evaluated at a ×40 magnification. The electronic system provided for each field considered the ratio between the positive area and the total nuclear area of the histologic component selected, which was fully adjustable.

Ki-67 processing was done according to recent guidelines to avoid differences due to preanalytic or analytic methods (37). TUNEL was evaluated on 3-μm thick sections using the ApopTag In Situ Apoptosis Detection Kits (Chemicon International) for apoptosis count of DNA fragmentation, according to the manufacturer's instructions. The entire length of the crypt was analyzed whenever possible and all assessments were made only on dysplastic tissue. However, the crypt base of the normal mucosa was excluded from Ki-67 measurement as values are physiologically the highest and are not representative of the average normal mucosa (38).

Study power and statistical analysis

The primary endpoint was the pre- versus posttreatment difference in Ki-67 LI between placebo and each allopurinol arm at the study endpoint (polyp removal). We initially calculated a total of 25 subjects per arm with $\alpha = 0.05$ and $1 - \beta = 0.85$, one-sided test, and 10% dropout rate, to show a 40% reduction in Ki-67 LI with any allopurinol versus placebo, assuming a SD of Ki-67 difference equal to 40%. Using an ANOVA linear model, we considered orthogonal

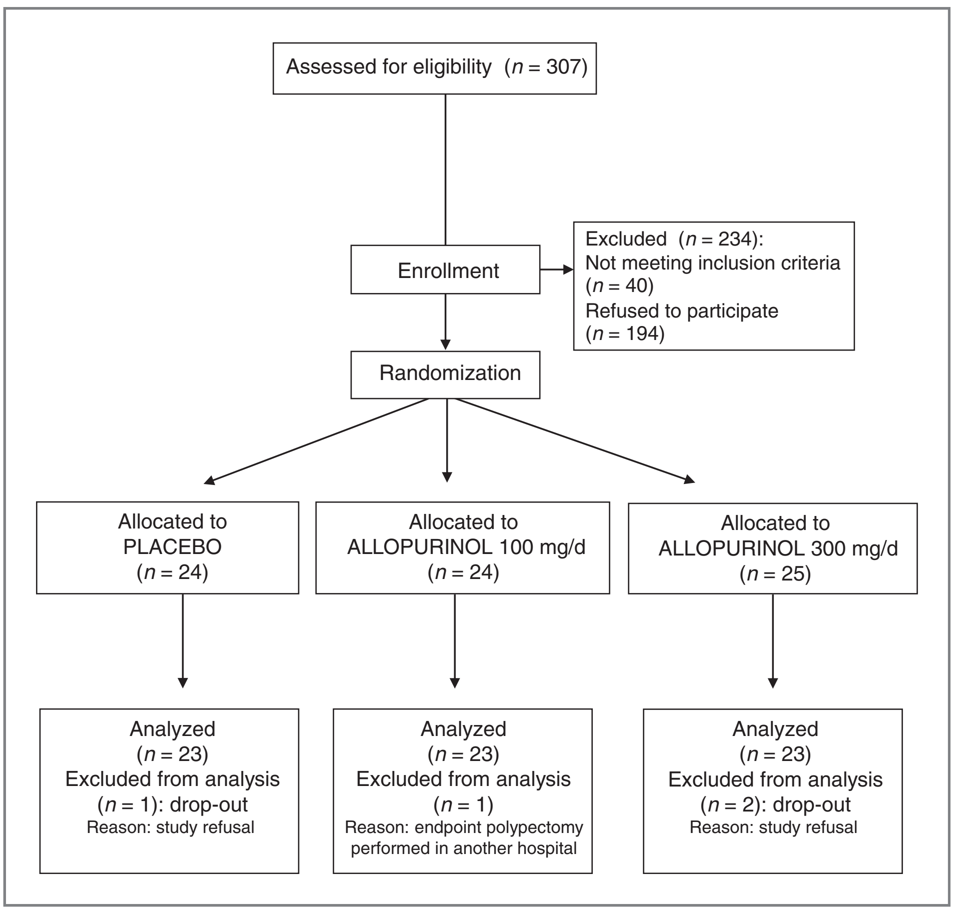

Figure 1. Participant flow diagram.

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Table 1. Baseline subjects characteristics by allocated arm

	Placebo ($n = 24$)	Allopurinol 100 mg/d ($n = 24$)	Allopurinol 300 mg/d ($n = 25$)	P*
Age, y (mean ± SD)	61 ± 8	62 ± 8	61 ± 8	0.9
Sex (male/female)	13/11	14/10	12/13	0.8
Body mass index, kg/m^2 (mean ± SD)	25.9 ± 3.4	24.3 ± 2.9	25.1 ± 2.8	0.4
Smoking habits (yes/no/former)	7/9/8	6/8/10	5/10/10	0.9
Alcohol consumption (yes/no)	16/8	15/9	18/7	0.8
Family history for colon cancer, *n* (%)	6 (25)	10 (42)	7 (28)	0.5
Family history for other cancers, *n* (%)	16 (67)	14 (58)	11 (44)	0.2
Treatment adherence, % median (min–max)	100 (90–100)	100 (81–100)	100 (93–100)	0.8
Polyp max diameter, mm, median (min–max)	15 (10–25)	18 (10–40)	15 (10–30)	0.5

*χ^2 test for categorical variables, ANOVA *F* test for continuous variables.

linear contrasts to test the differences between placebo and each allopurinol arm. The power of contrasts were 82% to detect a 50% difference between allopurinol 300 mg and placebo and 60% to detect a 30% difference between allopurinol 100 mg and placebo. Level of significance of contrasts was adjusted for multiplicity using the Bonferroni correction. As no significant difference emerged between the 2 doses, we pooled allopurinol arms to increase statistical power.

Univariate associations between arms at baseline were assessed using χ^2 for categorical variables and ANOVA *F* test or the Kruskal–Wallis *U* test for continuous variables. A nonparametric test for trend across ordered groups was used when appropriate. Linear regression modeling was used to test the post–pretreatment difference in biomarker levels at the endpoint among arms (response variable), adjusting for baseline values and age, sex, body mass index, smoking habits, alcohol consumption, family

Table 2. Biomarkers expression levels in adenomatous tissue by allocated arm

	Placebo ($N = 24$)	Allopurinol, 100 mg/d ($N = 24$)	Allopurinol, 300 mg/d ($N = 25$)	Allopurinol vs. placebo[a]	P
Ki-67 LI, %					
Baseline (median, IQR)	44, 20–66	45, 30–73	44, 24–71		0.7
Surgery (median, IQR)	66, 40–78	65, 38–80	62, 40–74		
Δ (Mean, 95% CI)	+18.7 (8.2–29.3)	+11.4 (1.6–21.3)	+14.4 (5.1–23.7)	−3.2 (−13.4 to 6.9)	0.5*
Topoisomerase-II-α, %					
Baseline (median, IQR)	18, 7–28	20, 10–36	17, 12–23		0.9
Surgery (median, IQR)	25, 19–32	19, 14–38	26, 13–38		
Δ (Mean, 95% CI)	+4.5 (−3.5 to 12.5)	+1.7 (−5.2 to 8.6)	+4.5 (−3.4 to 12.3)	+1.8 (−4.9 to 8.4)	0.6*
β-Catenin, %					
Baseline (median, IQR)	2, 0–40	1, 0–40	1, 0–7		0.8
Surgery (median, IQR)	16, 30–74	8, 2–60	6, 3–15		
Δ (Mean, 95% CI)	+12.1 (2.7–21.5)	+3.9 (−4.8 to 12.6)	+4.5 (0.1–8.9)	−10.6 (−20.5 to −0.7)	0.03*
NF-κB, %					
Baseline (median, IQR)	51, 8–85	80, 10–90	80, 30–90		0.5
Surgery (median, IQR)	75, 6–90	60, 35–90	70, 30–90		
Δ (Mean, 95% CI)	+9.7 (−2.0 to 21.3)	−0.4 (−10.8 to 10.0)	−1.4 (−16.5 to 13.7)	−8.1 (−22.7 to 6.5)	0.3*
TUNEL-positive cells, %					
Baseline (median, IQR)	0, 0–2	1, 0–2	0, 0–1		0.6
Surgery (median, IQR)	2, 0–8	2, 1–9	2, 0–7		
Δ (Mean, 95% CI)	+3.2 (1.2–5.2)	+5.9 (−0.3 to 12.1)	+4.0 (−0.1 to 8.0)	+0.8 (−3.2 to 4.7)	0.7*

Abbreviations: IQR, interquartile range; Δ, change, defined as: (polypectomy − baseline) tissue expression levels.
**P* for treatment, allopurinol vs. placebo, from the ANCOVA model.
[a]ANCOVA model considering allopurinol arms (100 mg + 300 mg) vs. placebo arm, adjusted for baseline biomarkers levels, age, sex, body mass index, smoking habits, alcohol consumption, family history for cancer, and treatment adherence.

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history for CRC, and treatment adherence. The treatment effect on the change of each biomarker's level was considered as a dummy variable in the linear model. Interactions between treatment and covariates were tested, but no significant effect modification was noted. No multiple testing correction was used in the analyses of secondary endpoints. Normality of distributions was achieved and models assumptions were assessed using residual plots. Two-tailed *P* value of 0.05 was used to define nominal statistical significance, and analyses were conducted using STATA software (version 11; StataCorp.).

Results

Subject characteristics

From May 13, 2006 through May 31, 2010, 73 subjects were included, 24 in the placebo arm, 24 on allopurinol 100 mg, and 25 on allopurinol 300 mg. The participant flow diagram is illustrated in Fig. 1. The baseline subjects' characteristics are summarized in Table 1. No significant differences among arms were found. The mean ± SD treatment duration was 28 ± 5 days. All polyps could be identified and removed. The median polyp diameters were comparable in the 3 arms and treatment adherence was very high (overall mean = 98%). No subject was on therapy with anti-inflammatory drugs or calcium supplementation.

Tissue biomarkers

The biomarker expression levels in adenomatous tissue at baseline biopsy and endpoint removal and their changes by allocated arm are shown in Table 2 and Fig. 2. There was a trend to an increase in all biomarkers in the polypectomy specimens compared with the baseline biopsy, which was statistically significant for Ki-67 in all 3 arms. Treatment with allopurinol did not significantly affect Ki-67 LI changes, nor did modulate changes in topoisomerase-II-α and TUNEL (Table 2). At variance, allopurinol blunted the increase in β-catenin and NF-κB levels in the polypectomy specimen observed in the placebo arm, even though the effect was statistically significant only for β-catenin.

The biomarker expression levels in normal tissue at baseline biopsy and endpoint removal and their changes by allocated arm are shown in Table 3 and Fig. 2. Compared with placebo, allopurinol treatment significantly decreased NF-κB levels, whereas no significant change was observed on the remaining biomarkers.

The effect of allopurinol on β-catenin in adenomatous tissue and NF-κB in normal tissue is illustrated, for all the 3 study arms, in Fig. 3. Although allopurinol blunted the increase of β-catenin expression in adenomatous tissue between baseline biopsy and the polypectomy specimen (Fig. 3A), the effect on NF-κB consisted of a decreased expression from baseline biopsy to polyp removal, with a dose–response relationship (*P* trend = 0.013; Fig. 3B).

Safety

Treatment was well tolerated, and no grade 2 or higher adverse events were registered (data not shown).

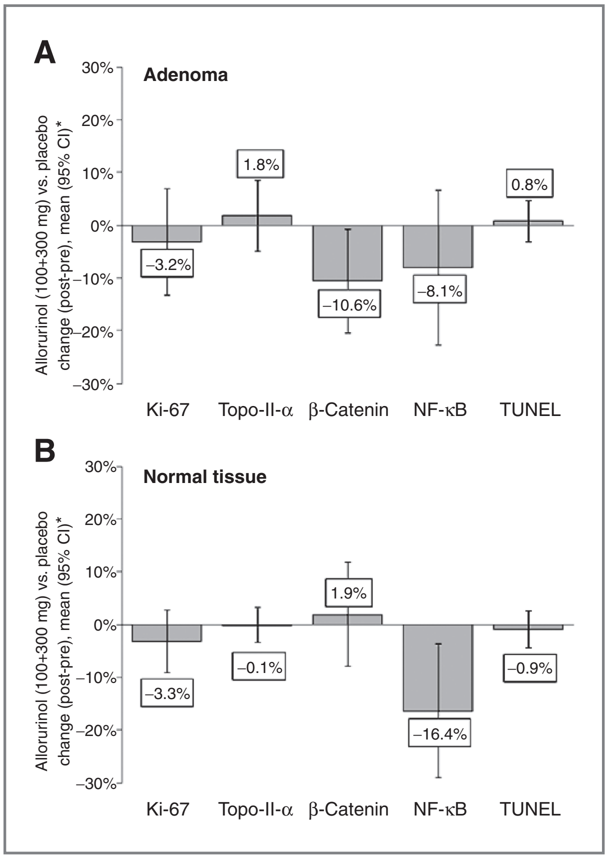

Figure 2. Treatment effect on the change(post–pre values) of biomarkers in adenoma (A) and normal tissue (B). *ANCOVA model considering allopurinol arms (100 mg + 300 mg) versus placebo arm, adjusted for baseline biomarkers levels, age, sex, body mass index, smoking habits, alcohol consumption, family history for cancer, and treatment adherence.

Discussion

Because inflammation and colon carcinogenesis are strongly associated (6–8), anti-inflammatory drugs and/or natural compounds with anti-inflammatory properties have been assessed as preventive agents in several randomized controlled trials in subjects at increased risk for CRC. Although aspirin and COX-2 inhibitors showed high efficacy on the reduction of adenoma recurrence and advanced adenoma incidence, gastrointestinal and cardiovascular toxicity were significant and have limited their broad use (5).

We tested the preventive potential of an old drug, such as allopurinol, with a well-defined good safety profile, in a proof-of-principle, window-of-opportunity trial on tissue biomarkers of colorectal carcinogenesis. Nox1-derived ROS stimulate the expression of NF-κB-linked antiapoptotic proteins (39), thus exerting a cancer-promoting effect both by inducing mitogenic stimuli and by increasing resistance to programmed cell death of transformed cells (40). Allopurinol has recently been shown to be associated with a lower risk of CRC in a population-based case–control study (22) and to increase survival of patients with advanced CRC

Table 3. Biomarkers expression levels in normal tissue by allocated arm

	Placebo ($N = 24$)	Allopurinol, 100 mg/d ($N = 24$)	Allopurinol, 300 mg/d ($N = 25$)	Allopurinol vs. placebo[a]	*P*
Ki-67 LI, %					
Baseline (median, IQR)	7, 3–10	6, 1–12	4, 2–13		0.9
Surgery (median, IQR)	8, 3–19	6, 1–16	10, 3–18		
Δ (Mean, 95% CI)	+4.2 (0.1–8.4)	−0.7 (−7.3 to 5.9)	+2.9 (−1.5 to 7.3)	−3.3 (−9.2 to 2.7)	0.3*
Topoisomerase-II-α, %					
Baseline (median, IQR)	3, 1–7	2, 1–3	2, 1–4		0.5
Surgery (median, IQR)	3, 1–6	3, 1–5	4, 2–5		
Δ (Mean, 95% CI)	−0.5 (−3.2 to 3.1)	+0.5 (−3.0 to 4.0)	+0.3 (−1.6 to 2.3)	−0.1 (−3.4 to 3.3)	0.9*
β-Catenin, %					
Baseline (median, IQR)	0, 0–0	0, 0–2	0, 0–1		0.8
Surgery (median, IQR)	0, 0–20	0, 0–20	1, 0–10		
Δ (Mean, 95% CI)	−4.0 (−14.8 to 6.8)	−9.8 (−21.1 to 1.6)	−0.9 (−9.7 to 7.8)	1.9 (−7.9 to 11.7)	0.7*
NF-κB, %					
Baseline (median, IQR)	20, 0–50	50, 0–70	40, 0–70		0.4
Surgery (median, IQR)	30, 0–60	15, 0–50	15, 0–40		
Δ (Mean, 95% CI)	+5.3 (−5.5 to 16.2)	−12.4 (−24.1 to 0.7)	−14.6 (−26.6 to −2.5)	−16.4 (−29.0 to −3.8)	0.01*
TUNEL-positive cells, %					
Baseline (median, IQR)	0, 0–0	0, 0–1	0, 0–0		0.5
Surgery (median, IQR)	1, 0–2	0, 0–2	0, 0–1		
Δ (Mean, 95% CI)	+2.1 (−1.0 to 5.1)	+2.9 (−0.6 to 6.4)	+0.2 (−0.1 to 0.5)	−0.9 (−4.4 to 2.6)	0.6*

Abbreviations: IQR, interquartile range; Δ, change, defined as: (polypectomy − baseline) tissue expression levels.
*P for treatment, allopurinol vs. placebo, from the ANCOVA model.
[a]ANCOVA model considering allopurinol arms (100 mg + 300 mg) vs. placebo arm, adjusted for baseline biomarkers levels, age, sex, body mass index, smoking habits, alcohol consumption, family history for cancer, and treatment adherence.

(23, 24). Our hypothesis was that inhibiting xanthine oxidase and reducing the intracellular ROS production by allopurinol might inhibit activation of the MAPK–NF-κB signaling pathways, and therefore decrease cell proliferation as measured by Ki-67 and increase apoptosis as measured by TUNEL.

In our study, allopurinol did not significantly influence Ki-67 LI changes, nor did it significantly modulate changes in topoisomerase-II-α and TUNEL in either adenomatous or normal colonic tissue. A possible interpretation of our results is that allopurinol, acting as a molecular scavenger, may not interfere with downstream mechanisms related to cellular proliferation/progression and apoptosis.

A clear limitation to our study is the lack of important variables that might have provided more insight about the impact of allopurinol on the carcinogenesis state of flat mucosa and adenomas, including, for example, markers of oxidative activation and metabolism, such as the glutathione-S transferase system. In our study, the assessment of oxidative stress was limited to a single marker and does not reflect the complexity of the metabolic, buffering, and reactive metabolites associated with intracellular oxidative stress. The same can be suggested also about the MAPK–NF-κB signaling pathway, in which a contemporary assessment of this system would consist of identifying and quantifying phosphorylated metabolites of MAPK and its downstream intermediates.

Despite these limitations, we found, compared with placebo, a statistically significant blunted increase of β-catenin in patients treated with allopurinol, irrespective of the dose, and a statistically significant dose-dependent reduction of NF-κB levels in normal tissue. A trend to a reduction of NF-κB, even if not statistically significant, was shown also in adenoma tissue. While an increase in β-catenin levels and subsequent β-catenin/Tcf-lymphoid enhancer factor complex formation are important events in the early stage of colonic carcinogenesis (41–44), the transcription factor NF-κB has also been linked to multiple aspects of oncogenesis, including the control of apoptosis, cell cycle, differentiation, angiogenesis, and cell migration (45). Therefore, our results reinforce the notion that inhibition of β-catenin and NF-κB by allopurinol may function as stand-alone cancer-preventive compound.

An interesting finding is the trend to a higher biomarker expression in the polypectomy specimen compared with the baseline biopsy, which was mostly evident for Ki-67 in the placebo arm. While this is a common observation in presurgical studies of patients with breast cancer (46–48), which has mainly been attributed to the stronger inhibitory effect of formalin fixation on antigen expression in smaller specimen volumes such as the baseline

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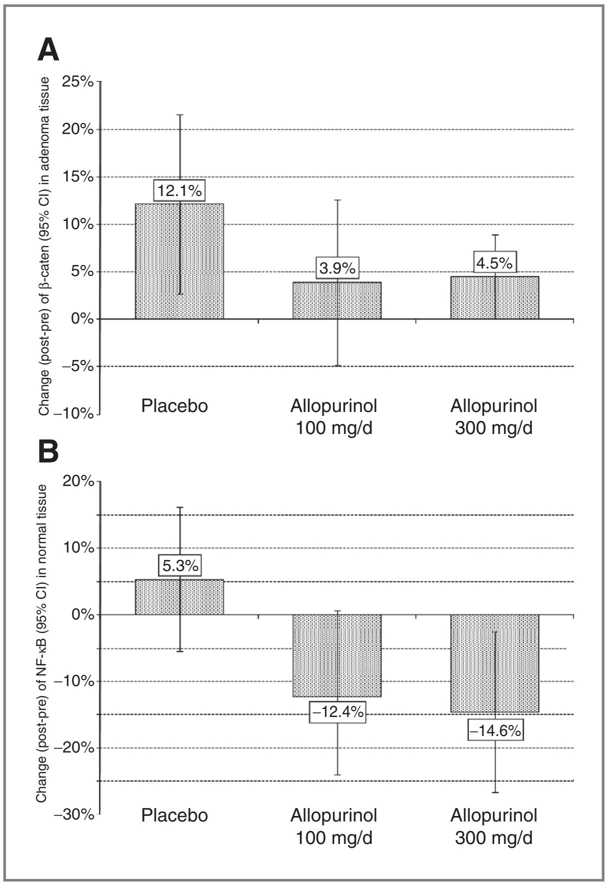

Figure 3. Mean (95% CI) change (post–pretreatment difference) of β-catenin in adenomatous tissue (A) and NF-κB in normal tissue (B).

biopsy (37), additional methodologic factors should be considered, including the difficulty in the IHC assessment and orientation of small biopsy specimens. A proinflammatory effect of the biopsy acting as a proliferative stimulation or a real proliferative increase 4 weeks apart cannot either be excluded. In the case of β-catenin, indices of less than 10% and for TUNEL, 1% or less, suggest that β-catenin expression or DNA fragmentation are rare events in normal mucosa but also, surprisingly, in adenomatous tissue. This then raises the concern that the adenomatous tissue sample may have been metaplastic rather than dysplastic, and we might have missed the cellular turnover that one might expect to see in a dysplastic portion of an adenoma. Or, perhaps low labeling indices are indeed the case in adenomas as well as in flat mucosa. Finally, a potential limitation of the results is the lack of correction for multiple testing as regards the secondary endpoints.

In conclusion, in this trial, the first to our knowledge ever conducted to assess the activity of allopurinol in reducing CRC risk biomarkers in humans, we were unable to find any modulation of proliferation/apoptosis either in adenomatous or in normal colonic tissue. However, we found an activity of allopurinol in oxidative activation biomarkers of CRC. That inflammatory mediators were modulated is interesting and suggests there could be a role for allopurinol in combination with agents having differing mechanisms of action, including aspirin, COX-2 inhibitors and natural antioxidants, such as curcumin and anthocyanins. These findings, combined with the excellent drug safety profile and the very low cost, should encourage further investigations on allopurinol as a potential chemopreventive agent for adenoma recurrence and CRC.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: M. Puntoni, D. Branchi, A. Argusti, E. Meroni, B. Gatteschi, R. Benelli, A. DeCensi
Development of methodology: M. Puntoni, D. Branchi, A. Argusti, S. Zanardi, C. Crosta, E. Meroni, P. Michetti, R. Bandelloni, S. Boccardo, B. Gatteschi, R. Benelli, A. DeCensi
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): D. Branchi, C. Crosta, E. Meroni, F. Munizzi, P. Michetti, G. Coccia, G. De Roberto, L. Turbino, E. Minetti, M. Mori, A. DeCensi
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M. Puntoni, M. Mori, S. Salvi, B. Gatteschi, R. Benelli, A. DeCensi
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Cancer Prevention Research

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