A randomized double-blind placebo-controlled phase IIB trial of curcumin in oral leukoplakia

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Abstract

Oral leukoplakia is a potentially malignant lesion of the oral cavity, for which no effective treatment is available. We investigated the effectiveness of curcumin, a potent inhibitor of NF-κB/COX-2, molecules perturbed in oral carcinogenesis, to treat leukoplakia. Subjects with oral leukoplakia (n=223) were randomized (1:1 ratio) to receive orally, either 3.6g/day of curcumin (n=111) or placebo (n=112), for six months. The primary endpoint was clinical response obtained by bi-dimensional measurement of leukoplakia size at recruitment and 6 months. Histological response, combined clinical and histological response, durability and effect of long-term therapy for an additional six months in partial responders, safety and compliance were the secondary endpoints. Clinical response was observed in 75 (67.5%) subjects [95% Confidence Interval (CI): 58.4-75.6] in the curcumin and 62 (55.3%) (95%CI: 46.1–64.2) in placebo arm (p=0.03). This response was durable, with 16 out of the 18 (88.9%) (95% CI: 67.2–96.9) subjects with complete response in curcumin and 7/8 subjects (87.5%) in placebo arm, demonstrating no relapse after six months follow up. Difference in histological response between curcumin and placebo was not significant (HR: 0.88, 95%CI: 0.45–1.71, p=0.71). Combined clinical and histological response assessment indicated a significantly better response with curcumin (HR: 0.50,95%CI: 0.27–0.92, p=0.02). Continued therapy, in subjects with partial response at six months did not yield additional benefit. The treatment did not raise any safety concerns. Treatment of oral leukoplakia with curcumin (3.6g for six months), thus was well tolerated and demonstrated significant and durable clinical response for six months.

Introduction

Oral leukoplakia is the most common and well-defined potentially malignant lesion (1, 2) of the oral cavity. The reported annual malignant transformation rate is 1% with a total lifetime risk of 42% (2-4). It is a predominantly white lesion of oral mucosa that cannot be compared to other lesions without malignant transformation potential such as candidiasis, lichen planus, frictional keratosis, leukodema, and lupus erythematosis (5). Several novel technologies have been introduced in the recent past that can detect oral malignant lesions at the pre-neoplastic stage (6, 7). However, there exists no effective strategies to treat oral leukoplakia.

Surgical excision of leukoplakia has significant morbidity with a relapse rate of 26 to 35% (8, 9). Although chemoprevention by retinoids has a clinical response rate of up to 67% (10, 11), high toxicities and relapse following cessation of therapy has prevented its clinical use (12). All subsequent chemoprevention trials, with various agents so far, have been ineffective (13).

Concordance of several pre-clinical and observational clinical studies of curcumin, a polyphenol extracted from the plant *Curcumin longa*, have established anti-proliferative, pro-apoptotic and pro-differentiation properties of the drug, in diverse epithelial malignancies including that of the oral cavity (14-17). A prior phase I clinical trial has demonstrated that curcumin is well tolerated at a dosage as high as 8 g/day for 3 months (18). In this study, we investigated the efficacy and safety of curcumin in treatment of oral leukoplakia.

Materials and Methods

Study design and participants

Curcumin Chemoprevention in Oral Potentially Malignant Lesion (OPML) trial (CCP-OPML-06) was a multi-center, double blind, randomized, placebo-controlled trial to test whether curcumin can, effectively and safely, treat oral leukoplakia. This trial was funded by the Department of Biotechnology (DBT), Government of India. The study was carried out in accordance with the International Conference on Harmonization recommendation on Good Clinical Practice. The study protocol was approved by Institutional Research Review Boards and registered with Drugs Controller General of India (F.No.4-129/06-DC) and the clinical trial registry of Indian Council of Medical Research (CTRI/091/000113). The safety and efficacy data was reviewed by an Independent Data and Safety Monitoring Board (IDSMB). The clinical trial schema, eligibility criteria (19) and patient disposition are given in **Figure 1**. The principal inclusion criteria were the presence of clinical and histologically confirmed oral leukoplakia of size more than 15 mm^2 in area and with any linear dimension more than 1 cm, no previous biopsy or treatment for head and neck cancer and no chemopreventive treatment prior to 3 months of accrual, Zubrod performance of 0 to 2, normal hematological and biochemical parameters and ability to participate in the trial and sign informed consent. The main exclusion criteria was the presence of oral submucous fibrosis.

The trial was led by a steering committee of lead principal investigator, principal investigators of three participating clinical sites and central laboratory. This steering committee directed all aspects of the study including design, data gathering, analyses and manuscript preparation. A clinical research organization (Manipal-Acunova, India)

had the responsibilities for medical monitoring, data management and statistical analysis.

Recruitment randomization and masking

The subjects with clinical evidence of oral leukoplakia, as per the WHO consensus criteria (5), were recruited from the outpatient clinics of head and neck oncology services of three academic medical centers in India. These were Regional Cancer Center, Trivandrum, Amrita Institute of Medical Science, Kochi and Chennai Dental Research Foundation, Chennai. The recruitment period was from June 2007 to December 2011. Subjects stratified by study sites were randomly assigned in a 1:1 ratio to receive curcumin or placebo. The randomization was done centrally at the CCP-OPML study center located at Manipal-Acunova, Bangalore (with no external input) by telephone or fax to each of the three recruitment centers located in Trivandrum, Kochi and Chennai. The study was conducted in a double-blind fashion without revealing the treatment assignments to patients or investigators except the study statistician and pharmacists.

Procedures

The investigational product was curcumin, reconstituted with turmeric oil and dispensed in capsules (BCM95®–Biocurcumax™). Pre-clinical pharmacokinetic studies have demonstrated that its bioavailability was, approximately, six times more than curcumin alone (20). Curcumin was dispensed in calendar packs of three 600 mg capsules in a twice daily regimen consumed orally after food (3.6 g/day) for six months. The placebo capsules containing cellulose was identical in physical characteristics and dispensed

similar to the curcumin. The curcumin and placebo were procured from Arjuna Natural Extracts, India.

After determining the eligibility and baseline clinical evaluation, all the subjects underwent incision biopsy of the lesion using a 5mm punch biopsy. The subjects who meet the clinical and histological diagnosis of leukoplakia and other inclusion/exclusion criteria were randomized to receive either curcumin or placebo, in a twice daily regimen, for six months. At the end of 6 months of intervention, the lesion was measured to determine the primary endpoint of clinical response and biopsy was performed for histologic response. Those subjects demonstrating clinical Partial Response (PR) were continued on their respective treatment (either curcumin or placebo) for 6 additional months to determine the effect of long-term treatment. The remaining subjects with Complete Response (CR) and Stable Disease (SD) were observed for six months, without intervention, to determine durability of response. Clinical and histopathological assessments of the subjects were repeated at the end of twelve months. The patient disposition and clinical follow up results are given in **Supplementary Fig S1**.

Physical examination, laboratory tests including complete blood count, serum biochemistry and urine analysis were performed at baseline, 6 and 12 months after randomization. In addition, patients were reviewed once a month to evaluate concomitant medication, lesion(s) size measurement, compliance and adverse events. The risk habits were defined by the presence of tobacco (smoking/chewing) and/or alcohol habits. Smoking habits was defined by usage of either cigarettes or `beedi' a local form of cigar, wherein tobacco is rolled inside tobacco leaves, some patients used both. The risk habits were evaluated and the subjects were counseled on risk-habit

cessation prior to enrollment and during the monthly reviews. The counseling was carried out by a medically trained study coordinator.

The lesions were measured using disposable paper rulers during monthly follow up visits. The area of a lesion was obtained by multiplying the longest diameter by the greatest perpendicular diameter. In case of multiple lesions, the largest lesion was considered as the index lesion. Total area was obtained from the sum of areas of all the lesions. On appearance of new lesions or clinical progression, the lesion was re-biopsied. Any subject with histological progressive disease (hPD) was terminated from the study and appropriate treatment was offered. The histopathology diagnosis and grading of dysplasia were determined by the pathologist at each of the clinical sites using the WHO oral dysplasia criteria for inclusion in the study (21). Prior to initiation of the trial, three pathologists involved in the study reviewed and agreed upon the diagnostic criteria. The panel of three pathologists reviewed the slides independently at the central laboratory and reported consensus of observations to determine histological response.

Outcomes

Primary Efficacy Endpoint: The primary efficacy endpoint of this study was the clinical response based on the comparison of the lesion size at baseline and at six months. A standard treatment response criteria (22) was used: CR- disappearance of the lesion, PR- 50% or greater decrease in the sum of products of diameters of all measured lesions, SD- neither sufficient shrinkage to qualify for PR or sufficient increase to qualify for PD and PD- any increase of more than 25% in the sum of products of diameters of all measured lesions.

Secondary endpoints: The secondary endpoints of this study included the following-

(1) Histological response: The histological response criteria at follow up in comparison to baseline result of the index lesion is defined as follows: histological Complete Response (hCR)- complete reversal of dysplasia/hyperplasia to normal epithelium, histological Partial Response (hPR) - regression of the degree of dysplasia, histological Stable Disease (hSD)- no change in the degree of dysplasia and histological Progressive Disease (hPD)- any increase in severity grade.

(2) Combined Response: A previously described oral leukoplakia staging system that integrates both clinical and histological criteria was utilized to determine the combined response criteria (23). The definition of combined response criteria is as follows - Complete response: Clinical CR and Histologic CR, Partial response: Clinical PR and Histologic PR or SD, Stable Disease: Clinical SD and Histologic PR or SD, Progressive disease: Clinical PD and/or Histologic PD.

(3) Durability of Response: Additional six month clinical follow up was done for those subjects who showed clinical CR or SD at six month evaluation to determine the durability of response. The response evaluation at six months (Clinical, histological and combined response) was correlated with that at 12 months.

(4) Effect of continued intervention: The patients with clinical PR were continued on the intervention (either curcumin or placebo) for additional six months to determine *effect of long-term intervention*. The responses were evaluated with the same response criteria as carried out at the 6 month evaluation.

(5) Compliance: The compliance to medication was reviewed on a monthly basis. Compliance is calculated based on the formula given below:

$$\text{Compliance (\%)} = \left[1 - \frac{\text{Expected no. of capsules to be consumed} - \text{Actual no. of capsules consumed}}{\text{Expected no. of capsules to be consumed}}\right] * 100$$

The subject was deemed to be compliant if the average monthly pill count is ≥ 80% (i.e. ≤ 20% capsules remained at end of each month). The unblinded pharmacist counted the pills at each monthly review and the results were recorded on the Drug Compliance Form. The average compliance of each treatment group was calculated separately.

(6) Safety Evaluation: Adverse events (AE) were classified according to criteria from Medical Dictionary for Regulatory Activities (MedDRA), version 8.1 (21). The total number of AE(s), based on their frequency, causality, as well as severity, were compared between treatment and control arms. AE(s) were described as unrelated, unlikely, possibly related, probably related and definitely related to the study medication.

Statistical analysis

The trial was designed with a statistical power of 90%. Therefore, a sample size of 223 subjects was required to detect 25% more clinical response in the treatment arm when compared to the placebo arm. The power calculation assumed a dropout rate of 30%. All the efficacy analyses were performed on the intent-to-treat (ITT) population, with primary efficacy end points determined for all patients by follow up clinical examination at six months. For those subjects whose six months endpoint measurements were not available, the ITT analysis utilized the most recent measurements to determine the clinical response outcome. Additionally, the analysis of the clinical outcomes was also

conducted after excluding the patients whose six months endpoint measurements were not available. For both the approaches, the proportion of patients with CR or PR were grouped together as responders and SD or PD were grouped together as non-responders. Separate descriptive analyses were carried out with histological response and combined clinical and histological response as endpoints. The response rates were presented as percentage and 95% confidence intervals (CI). Comparison between responses in curcumin and placebo groups was carried out using Chi-square and Fisher Exact tests. Statistical significance was defined as p-value <0.05. Additionally, the clinical response to curcumin therapy versus placebo at six months was compared individually in sub-groups stratified by demographic and clinical factors using binomial logistic regression analysis. The results were presented as odds ratios and p-values.

For safety evaluation, the proportion of subjects with adverse events (AE) were classified by MedDRA® SOCs and Preferred Terms and summarized by treatment arm as treatment-emergent adverse events (24). An AE was considered to be treatment-emergent, if the onset of the AE was subsequent to the administration of the study medication. The safety population consisted of all those who have received at least one dose of study medication. AEs were also summarized by severity as mild, moderate, severe, life threatening and death related and by relationship to study medication as unrelated, unlikely, possibly related, probably related and definitely related to study medication.

Role of funding source

The funding agency had no role in study design, collection of data, data analysis, and interpretation of the results or writing the report. All authors have access to the entire

raw data. The authors confirm the completeness and veracity of the data and analysis. The corresponding authors had the final responsibility on the decision to submit for publication.

Results

Of the 280 subjects screened, 223 subjects met all the inclusion and exclusion criteria and were enrolled in the study and formed the intent-to-treat population. Disposition of these subjects are outlined in COSORT Flow Chart in **Figure 1**. One hundred and eleven patients were randomized into the curcumin arm and 112 patients in the placebo arm. The demographic, risk profile and clinical characteristics were comparable in the two groups (**Table 1**). Ninety four subjects in each arm had endpoint measurement completed at 6 months. The reasons for withdrawal from the trial are given in supplementary data (**Supplementary Fig S1**). Eleven patients in curcumin arm and 14 patients in placebo arm had at least one post-baseline evaluation, which was used for the ITT analysis for clinical response. Therefore, 105 subjects in the treatment arm and 108 subjects in the placebo arm were available at the end of six months for evaluation of primary endpoints. Sixty-eight and 67 evaluable patients were available for follow up till twelve months in the treatment and placebo arm respectively.

Drug compliance

Compliance was evaluated at six months of intervention in 105 patients from the curcumin arm and 108 patients from the placebo arm. The drug compliance at 6 months was 83.8% (n=88) (95% CI: 75.6 – 89.6) in curcumin arm and 83.3% (n=90) (95% CI:

77.1 – 90.6) in placebo arm. The drug compliance in PR subjects at 12 months was 79% in curcumin arm and 84% in placebo arm.

Clinical response at 6 months

In the ITT population of 105 subjects in curcumin arm and 108 patients in placebo arm, a clinical response (CR+PR) was observed in 75 subjects (67.5%, 95% CI: 58.4 - 75.6) in curcumin arm and in 62 subjects (55.3%, 95% CI: 46.1 – 64.2) in placebo arm. Thirty (27.0%, 95% CI: 19.6 – 36.0) subjects in curcumin arm and 46 (41.1%, 95% CI: 32.4 – 50.3) subjects in placebo arm were non-responders (SD+PD). Statistically significant difference in clinical response rate was observed between the curcumin and the placebo arms (p=0.03) (**Figure 2, 3A & B and Supplementary Table ST1**). When the analysis was repeated with the subjects who had completed 6 month endpoint evaluation (94 subjects in each arm), the results were consistent with the ITT analysis (**Supplementary Table ST2**). Clinical response (CR+PR) rate was 74.5% in curcumin arm versus 57.4% in placebo arm. The difference in clinical response rate observed between the curcumin and the placebo arms was statistically significant (p=0.02).

Histological Response at 6 months

Histological response (hCR+hPR) was observed in 25 (22.5%, 95% CI: 15.7 – 31.3) subjects in the curcumin arm and in 23 (20.5%, 95% CI: 14.1 - 28.9) subjects in the placebo arm. No response (hSD+hPD) was noted in 60 (54.1%, 95% CI: 44.8 – 63.0) and 63 (56.3%, 95% CI: 47.0 – 65.1) subjects in the curcumin and placebo arms, respectively. Data of 26 subjects in both the curcumin and placebo arms was not available. The difference in histological response rates between curcumin arm and the

placebo arm was not statistically significant (p=0.71). (**Figure 2 and Supplementary Table ST1**)

Combined clinical and histological response at 6 months

Combined response (CR+PR) was noticed in 65 (58.6%, 95% CI: 49.3 – 67.3) subjects in the curcumin arm and 50 (44.6%, 95% CI: 35.8 – 53.9) subjects in the placebo arm. No response (SD+PD) was noticed in 28 (25.2%, 95% CI: 18.1 – 34.1) and 43 (38.4%, 95% CI: 29.9 – 47.6) subjects in the curcumin and placebo arm, respectively. Data of 18 and 19 subjects in the curcumin and placebo arms, respectively, was not available. The difference in combined response rates between the curcumin and placebo arms was statistically significant (p=0.03). (**Figure 2 and Supplementary Table ST1**)

Correlation of clinical and histology response

Of the 67 subjects in the curcumin arm with clinical response, only 23 (34.3%) showed histological response; whereas 14 out of 16 clinical non-responders were histological non-responders. The result is summarized in **Supplementary Table ST3**. The Kappa correlation coefficient was 0.13 (p=0.06) for the curcumin group and 0.27 (p=0.001) for placebo group (Data not provided).

Sub-group analyses based on clinical and pathologic characteristics

Univariate and multivariate logistic regression analyses were conducted comparing risk of non-response (SD/PD) in curcumin arm to the placebo arm. The univariate analysis showed a significantly lower odds of SD/PD in curcumin arm compared to placebo arm

(OR: 0.54, 95% CI – 0.31- 0.95, p: 0.03). Similarly, significantly lower odds of SD/PD in curcumin arm was noticed after adjusting individually for significant factors such as presence of dysplasia, lesion type (homogenous vs. non-homogenous), smoking tobacco use, smokeless tobacco use, alcohol use and habit cessation through trial period. On multivariate analysis, adjusting for the key factors of habit cessation, presence of dysplasia and lesion type (homogenous vs. non-homogenous), curcumin showed a significantly lower odds of SD/PD compared to placebo (OR: 0.40, 95% CI – 0.21- 0.76, p: 0.005)

The clinical response to curcumin therapy versus placebo at six months was also evaluated individually in all the sub-groups stratified by demographic, clinical and pathologic variables such as age, gender, sub-site, morphology of lesion (homogeneous and non-homogeneous leukoplakia, erythroplakia; **Supplementary Fig S2**), histology of lesion, type of habits, abstinence or continuation of habits. The ratio of responders to non-responders in each arm and the risk odds ratio based on logistic regression analysis are presented separately for each of these variables in **Figure 4**. A significant reduction in risk of SD/PD with curcumin versus placebo was noted with homogenous leukoplakia lesions (p=0.03), non-dysplastic lesions (p=0.003), smokeless tobacco use (p=0.03), alcohol use (p=0.04), either tobacco/alcohol use (p=0.01) and in subjects who continued to use tobacco/alcohol through the trial period (p=0.009).

Durability of response

To evaluate durability of response, subjects with CR and SD at 6 months were followed up for an additional six months after completion of the intervention. The results are summarized in **Table 2**. Among 22 subjects with CR at 6 months in the curcumin arm, 4

were lost to follow up. Of the 18 subjects available at follow up, 16 (88.9%) continue to have CR at 12 months (95% CI: 67.2 – 96.9). In the placebo group, out of 12 subjects with CR, 4 subjects were lost to follow up, while 7 (87.5%, 95% CI: 52.9 – 97.8) remain to have CR at 12 months follow up, p=0.99. Among the subjects with SD at 6 month evaluation, durable response (CR/PR/SD) at 12 months was noted in 7 (70%, 95% CI: 39.7 – 89.2) and 14 (82.4%, 95% CI: 59.0 – 93.8) in the curcumin and placebo arms, respectively (p=0.64). Thirteen in the curcumin arm and 15 in the placebo arm, who were lost to follow up, were not included in the analysis. Similarly, when histological and combined response criteria were evaluated, no statistically significant difference in durability of response was noted between the two study arms (data not shown).

Effect of longer-term treatment of Curcumin

103 subjects with clinical PR at six months were allowed to continue on curcumin (n=53) or placebo (n=50) for additional six months to determine any additional benefit on longer term curcumin therapy (**Supplementary Table ST4**). At the end of twelve months, CR or PR was seen in 29 (54.7%, 95% CI 41.5 – 67.4) subjects in curcumin arm and 30 (60.0%, 95% CI 46.2 – 72.4) subjects in placebo arm. Stable Disease (SD) or Progressive Disease (PD) was seen in 7 (13.2%, 95% CI 6.6 – 24.8) subjects in curcumin arm and 10 (20.0%, 95% CI 11.2 – 33.0) subjects in placebo arm. No statistically significant difference was observed between the two groups, suggesting that treatment longer than six month may not have additional benefit.

Safety results

IDSMB that monitored the study did not identify any safety concerns. The safety study population consisted of 223 subjects (111 in the curcumin arm and 112 in the placebo arm). Daily dose of 3.6g of curcumin was well tolerated by the study population during the study period. A total of 61 (27.4%) subjects experienced at least one adverse event during the study period, 26 (23.4%) in curcumin arm and 35 (31.3%) in placebo arm. There no statistically significant difference in major AEs between in the curcumin and placebo arms. (**Supplementary Table ST5**).

Moderate/severe AEs were recorded in four patients in the curcumin and included anaemia, skin/subcutaneous tissue disorders and hypertension. The placebo group recorded 18 patients with moderate/severe AEs, which included epistaxis and pleural effusion , anaemia, hypertension, increase in levels of creatinine/lactate dehydrogenase and infections (leptospirosis, urinary and respiratory tract infections). Four subjects from curcumin arm and 1 from placebo arm withdrew from the study due to AEs/SAEs. One subject in each arm experienced severe AE (n=1), while all other AEs reported in study were mild or moderate. Three subjects with AEs were assessed to be possibly related to treatment in the curcumin arm. Three subjects with AEs were assessed to be possibly related and one to be probably related to treatment in the placebo arm. No death was reported among the study population during the study period. The details of AEs are provided in **Supplementary Table ST5**.

Discussion

Since the first description of chemoprevention of oral leukoplakia about twenty-five years ago (11), there have been several attempts to develop a safe and clinically effective chemopreventive agent. Clinical use of chemoprevention strategies for oral leukoplakia

has been hampered by the high rate of relapse and occurrence of adverse events with the interventions (12). This was highlighted in a recent meta-analysis and in the Cochrane review, which are summarized in **Supplementary Table ST6** (25-27). In the present study, we report curcumin to be a clinically effective and safe agent to treat oral leukoplakia, suggesting its chemopreventive potential in oral cancer.

Though in chemoprevention trials incidence of cancer may be used as the primary endpoint, all, but two, of the 26 reported trials have employed clinical response as a surrogate primary endpoint, as was adopted in the present study. This is primarily due to the need for longer follow-up period and larger sample size if cancer incidence is considered as the endpoint. The reported clinical response of previous oral leukoplakia chemoprevention trials ranges from 4 to 67 percent (25). Vitamin A or retinoid has been the most investigated chemopreventive agent for oral leukoplakia, with a reported response rate of 45 to 67% (26-28). In the present study, we observed 67.5 percent clinical response rate (complete or partial) and 20.7 percent stable disease after six months of intervention. This response rate is better than most of the previously reported oral leukoplakia chemoprevention trials (**Supplementary table 6**). It is to be noted that the validity of clinical response, as a surrogate endpoint for cancer development in oral leukoplakia, has not been established.

Histologic response as an endpoint is used infrequently in oral chemoprevention trials and has yielded inconsistent results (25-27). Subjective nature of oral dysplasia diagnostic criteria and high inter-observer variability has been attributed as the primary reason. In the present study we did not observe significant improvement in histologic response despite improvement in clinical response. We have attempted to overcome the frequently reported inter-observer variability encountered in oral dysplasia grading by

using well-defined oral dysplasia diagnostic criteria and obtaining a consensus report of three pathologists for histologic response evaluation. The possibility of technical artifacts during biopsy cannot be ruled out in the study. It has been reported that incisional biopsy under diagnosed 73.3% of the patients, in comparison to pathologic examination of excised oral leukoplakia lesions (29). There is a possibility that the biopsy may have geographically missed the worst histological region. In addition, after chemoprevention intervention, the persistent lesion may represent histologically higher-grade lesion that may be sampled during the follow up biopsy. These inherent methodological challenges for using histological outcomes as endpoint in oral chemoprevention studies need to be considered while interpreting the results.

One of the consistent findings of all previous chemoprevention trials in oral leukoplakia to date, has been relapse of lesions after cessation of therapy. It ranged from 50 percent with topical Bleomycin (30), 54 to 64 percent with vitamin A and beta carotene (31), and 56 percent with retinoid (11). In the present study, we observed clinical and histologic relapse only in 7.7% and 7.3% respectively in the curcumin arm after six months of follow up.

An interesting observation, in the current study, was the relatively high response and the low relapse rates in the placebo arm compared to previous reports. It has been shown that tobacco cessation alone can result in clinical response in 43.2 to 58.3 percent of subjects with leukoplakia (32, 33). This study employed a structured habit cessation counseling program. This was carried out by medically trained study coordinators prior to enrollment of subjects to the study and during each monthly review. This resulted in high rates of habit cessation (28% in the curcumin and 29% in the placebo), which might have contributed to the better response and lower relapse rate in both arms. This

argument is further supported by the sub-group analyses that showed the response rate in the placebo arm to be relatively higher (67.9%) in subjects who quit tobacco/alcohol, compared to the subjects who continued using tobacco/alcohol (51.5%). Whereas, in the curcumin arm, the response rates were similar between subjects who quit (74.1%) and who did not quit (73.2%) risk habits. Furthermore, logistic regression analysis showed a lack of difference in risk between the curcumin arm versus placebo among subjects who quit habits (p=0.61).

Sub-group analyses of the data showed that curcumin has a significantly better chemopreventive potential than placebo in smokeless tobacco users, alcohol users and in subjects who continued to use tobacco or alcohol during the trial. These results seem to support the previous studies that have hypothesized that curcumin may prevent alcohol and tobacco-induced carcinogenesis by regulating pathways involving NF-kB COX-2 and AKT/MTOR (34). However, it must be noted that these results were based on our post-hoc sub-group analyses. Therefore, additional studies may be needed to further confirm the efficacy of curcumin in tobacco and alcohol users.

Chemoprevention trials have used varying durations of intervention ranging from 14 days to 12 months. In the current study, we did not observe improvement in response rate following continued therapy after six months in subjects with partial response. This implies an absence of additional benefit in treating subjects with curcumin for periods more than six months. Further studies are required to understand the mechanism of resistance in order to optimize the curcumin therapy.

One of the major hurdles in chemoprevention clinical trials has been the toxicity associated with medical interventions (12). Since only one third of all oral leukoplakia

undergo malignant transformation, it is essential that the interventions should be less toxic. In this study, daily dose of 3.6g of curcumin is found to be well tolerated. Though compliance to 3.6g dispensed in six capsules per day was satisfactory in a study setting, one concern may be its feasibility in clinical practice. It is to be noted that the 3.6g dose was used in this study based on the previously established tolerance dose (15) and not the efficacy dose. Thus, if the compliance in clinical practice proves to be an issue it could be of value to determine efficacy of a lower dose of curcumin.

Low durable response rate and high toxicity were encountered in most of the oral leukoplakia chemoprevention intervention trials during the past three decades. Findings from this study suggest that curcumin may serve as an effective treatment for oral leukoplakia, with good tolerance and durable clinical response. It is to be noted that there was no statistically significant difference in the durability of response between the placebo and curcumin arms. Further long-term studies are required to determine whether the clinical response in oral leukoplakia can be translated to a decrease in oral cancer development. In addition, molecular biomarkers, rather than a reliance on dysplasia, and molecular marker based-targeted interventions are required for risk stratification, response evaluation and to improve effectiveness of chemoprevention (35, 36).

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Figure Legends

Figure 1: CONSORT Flow Diagram showing inclusion and exclusion criteria, and patient disposition

Figure 2: Clinical, histopathological and combined response rates for curcumin and placebo arms at six months Difference in response rates (CR/PR) between the Curcumin group and the placebo group was evaluated using Chi-square test. * represents statistically significant p-values

Figure 3: Clinical response to curcumin therapy (A) 2cm x 05cm homogenous leukoplakia at baseline. (B) Complete clinical response to 3.6gm curcumin after 6 months of treatment

Figure 4: Comparison of the response between curcumin arm and placebo arms based on clinical/demographic variables Odds ratio of no response (SD/PD) at 6 months and 95% confidence intervals calculated based on binary logistic regression models comparing curcumin to the placebo arm. * Includes use of smoking tobacco, smokeless tobacco and alcohol. **Subjects without any risk factors# were not considered for the 'Change in risk habits during trial' analysis

Table 1: Baseline demographic and clinical characteristics of 223 subjects enrolled in the Curcumin Chemoprevention Trial

Variable	Categories	Curcumin (N=111*)	Placebo (N=112*)
Age- year	Median (Range)	54 (30 – 74)	55 (26 – 74)
Gender- N (%)	Male	79 (71.2)	82 (73.2)
	Female	32 (28.8)	30 (26.8)
Any habits – N (%)	Yes	104 (93.6)	100 (89.3)
	No	7 (6.4)	12 (10.7)
Smoker- N (%)@	Yes	61 (54.9)	51 (45.5)
	No	50 (45.1)	61 (54.5)
Smoking Status N (%)$	Current	35 (31.5)	26 (23.2)
	Former	20 (18)	17 (15.2)
Alcohol – N (%)**	Yes	54 (48.6)	39 (34.8)
	No	57 (51.4)	73 (65.2)
Alcohol Status N (%)$	Daily	11 (9.9)	5 (4.5)
	Non-daily	27 (24.3)	24 (21.4)
Chewing Areca nut/tobacco N (%)	Yes	81 (72.9)	77 (68.7)
	No	30 (27.1)	35 (31.3)
Chewing Status N (%)	Current	50 (45.0)	45 (40.2)

	Former	31 (27.9)	32 (28
Site of lesion N (%)	Tongue and floor of mouth	22 (19.8)	18 (16.1)
	Buccal Mucosa	75 (67.6)	88 (78.6)
	Both (Buccal mucosa and Tongue and floor of Mouth)	14 (12.6)	6 (5.3)
Size of the lesion (Baseline)	Median (Range) in mm2	596 (50 - 15000)	500 (40 - 30000)
Morphologic type- N [#]	Homogenous leukoplakia	116	146
	Non-homogenous leukoplakia	61	50
	Erythroplakia	10	8
Baseline Histology N (%)[$]	Non-dysplastic	54 (49.5%)	52 (46.4%)
	Dysplastic	55 (50.4%)	56 (50%)

[@]In the curcumin arm 23% used cigarettes only, 10% used beedi/cigars only, and 67% used both cigarettes and beedi/cigars. In the placebo arm 37% used cigarettes only, 16% used beedi/cigars, and 47% used both cigarettes and beedi/cigars.

*Considered as denominator for calculating percentages

#Many patients had more than one lesions, hence the data is given number of lesions and not in percentage

$ Patients for whom risk factor status and histology grading was not available were not included

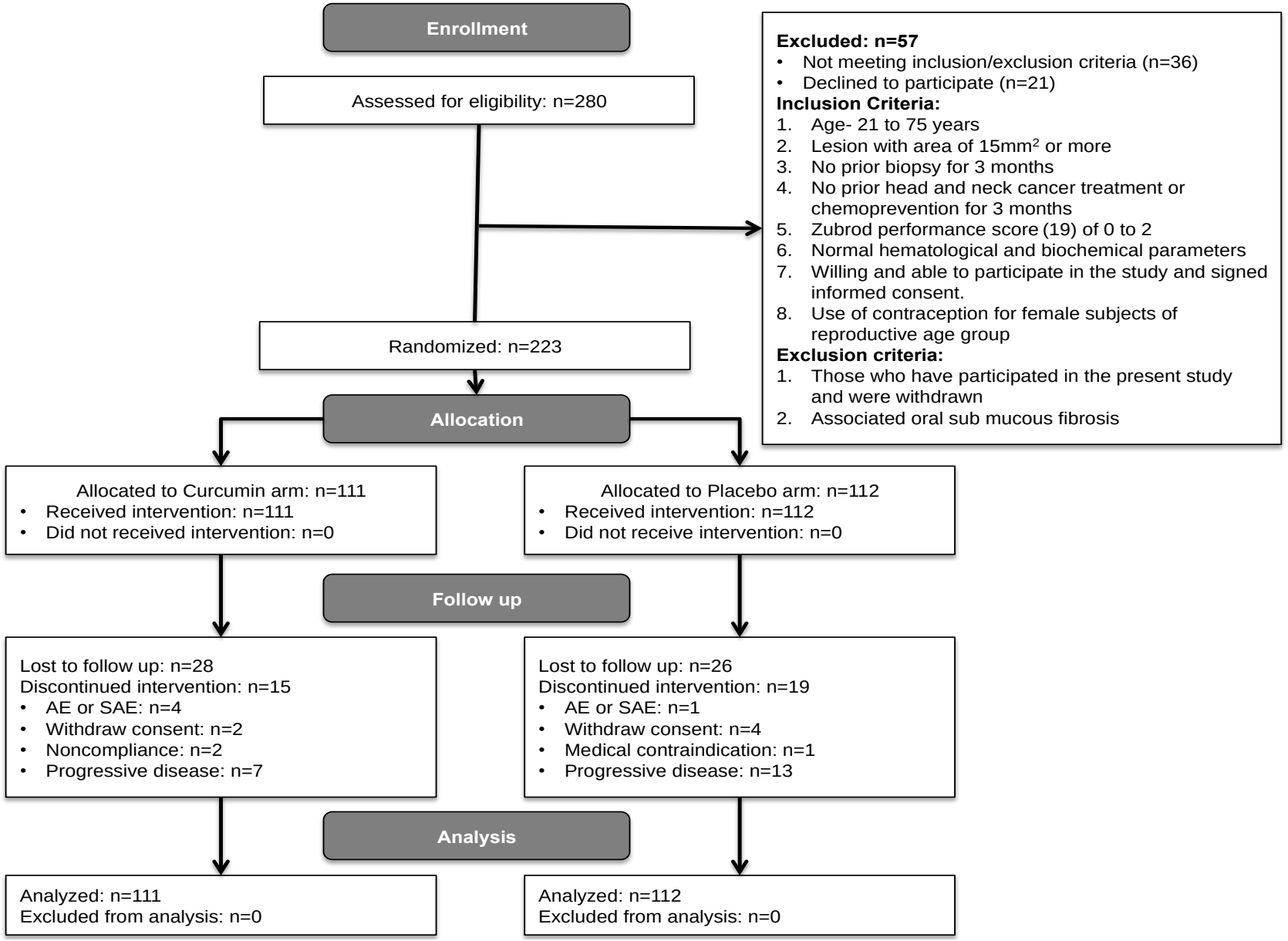
**p-value statistically significant. p-value: 0.04

Table 2: Analysis of durability of clinical response at 12 months in patients who had complete response or stable disease at 6 months (intent-to-treat population)

Clinical Response at 12 months compared to 6 months evaluation	**Curcumin N (%)**	**Placebo N (%)**
Patients with Clinical *CR at 6 months		
Durable response (*CR) at 12 months	16 (88.9)	7 (87.5)
Disease relapse (*PD at 12 months)	2 (11.1)	1 (12.5)
Missing	4	4
Patients with Clinical *SD at 6 months		
Durable response (*CR/*PR/*SD at 12 months)	7 (70.0)	14 (82.4)
Disease relapse (*PD at 12 months)	3 (30.0)	3 (17.6)
Missing	13	15

*CR: Complete Response, *SD: Stable Disease, *PR: Partial Response

Figure 1



*AE: Adverse Events, *SAE: Serious Adverse Events

Figure 2

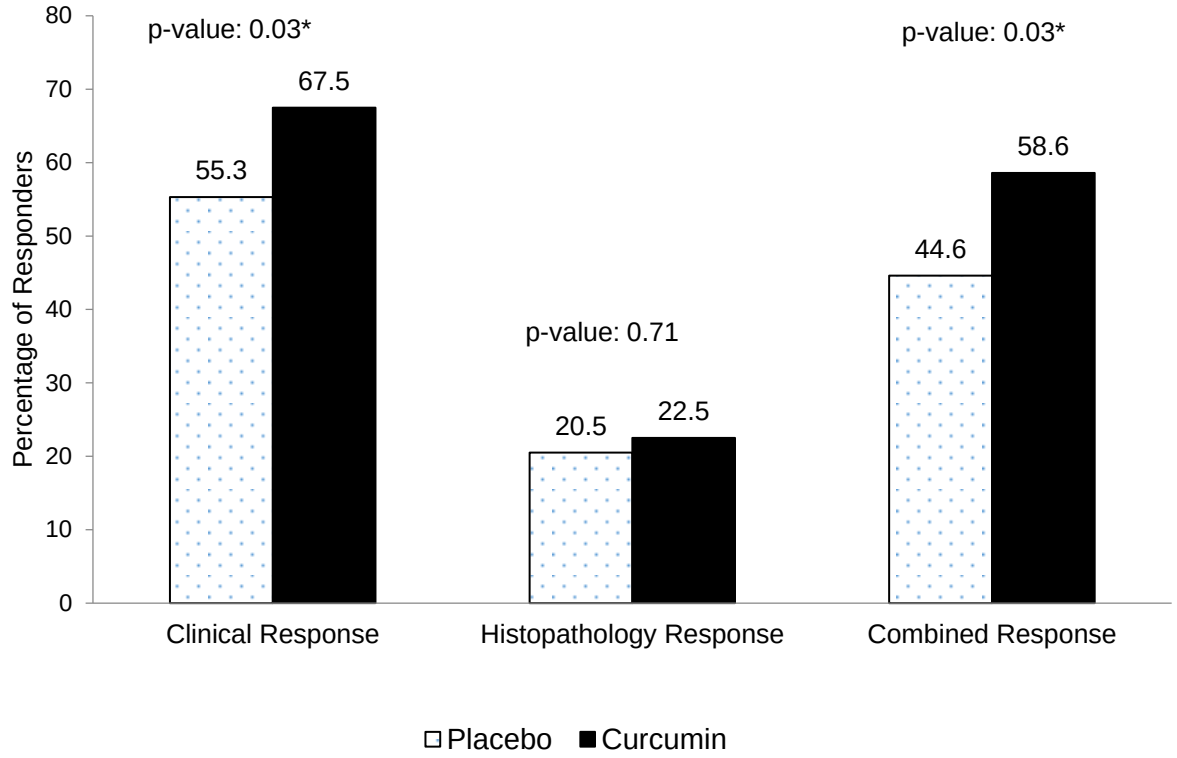


Figure 3

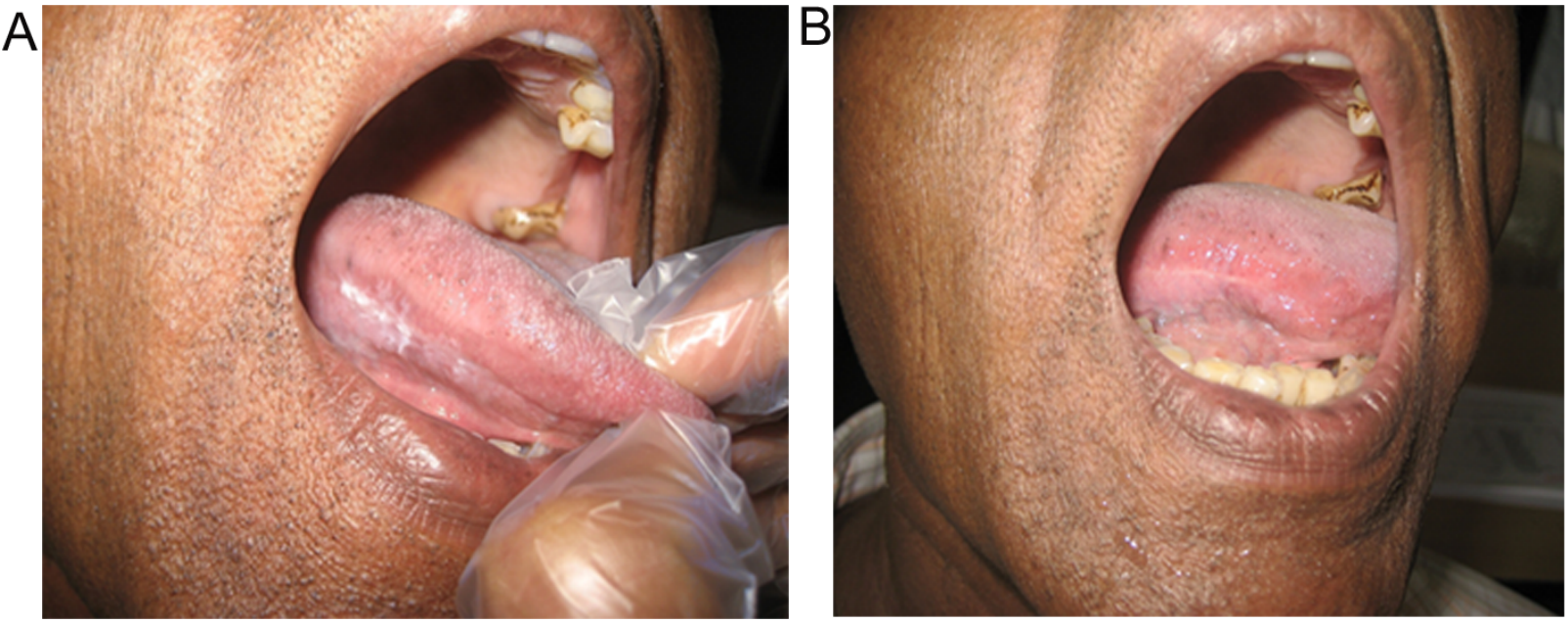
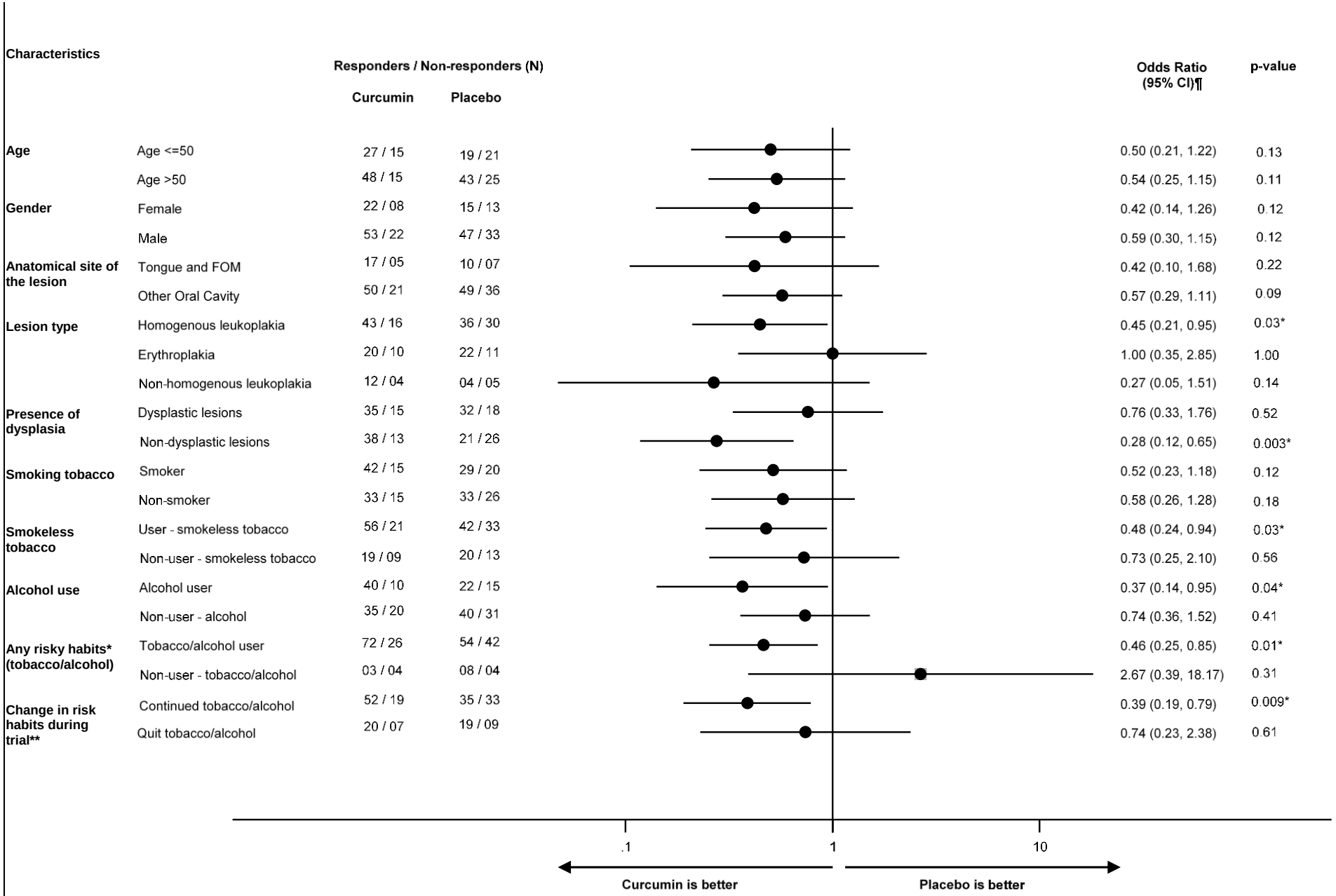


Figure 4



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Cancer Prevention Research

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