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A Randomized Phase II Trial of Pioglitazone for Lung Cancer Chemoprevention in High Risk Current and Former Smokers

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Abstract

Lung cancer chemoprevention, especially in high-risk former smokers, has great potential to reduce lung cancer incidence and mortality. Thiazolidinediones prevent lung cancer in preclinical studies, and diabetics receiving thiazolidinediones have lower lung cancer rates which led to our double-blind, randomized, phase II placebo-controlled trial of oral pioglitazone in high risk current or former smokers with sputum cytologic atypia or known endobronchial dysplasia. Bronchoscopy was performed at study entry and after completing of six months of treatment. Biopsies were histologically scored, and primary endpoint analysis tested worst biopsy scores (Max) between groups; Dysplasia index (DI) and average score (Avg) changes were secondary endpoints. Biopsies also received an inflammation score. The trial accrued 92 subjects (47

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pioglitazone, 45 placebo), and 76 completed both bronchoscopies (39 pioglitazone, 37 placebo). Baseline dysplasia was significantly worse for current smokers, and 64% of subjects had mild or greater dysplasia at study entry. Subjects receiving pioglitazone did not exhibit improvement in bronchial dysplasia. Former smokers treated with pioglitazone exhibited a slight improvement in Max, while current smokers exhibited slight worsening. While statistically significant changes in Avg and DI were not observed in the treatment group, former smokers exhibited a slight decrease in both Avg and DI. Negligible Avg and DI changes occurred in current smokers. A trend towards decreased Ki-67 labeling index occurred in former smokers with baseline dysplasia receiving pioglitazone. While pioglitazone did not improve endobronchial histology in this high-risk cohort, specific lesions showed histologic improvement and further study is needed to better characterize responsive dysplasia.

Keywords

Chemoprevention; Lung cancer; Dysplasia; Inflammation; Pioglitazone

Introduction:

Lung cancer is the leading cause of cancer death in the United States and worldwide(1). The majority of US diagnoses occur in former smokers, and effective chemopreventive strategies (beyond smoking cessation) could lead to dramatic improvements in survival(2). Chemoprevention involves the use of agents to reverse or inhibit the carcinogenic process and has been successfully applied to common malignancies other than lung. Chemoprevention may take on additional significance with the implementation of lung cancer screening. Low dose CT scans in high risk populations have been shown to decrease lung cancer death rates(3), and widespread adoption could result in a stage shift and more long term survivors. This group would remain at high risk for a second primary lung cancer and would be ideal for chemopreventive interventions beyond smoking cessation(4). The World Health Organization classification for lung cancer recognizes distinct endobronchial lesions which are precursors of invasive lung cancer(5). For example, the development of squamous cell lung cancer starts with normal epithelium and progresses through hyperplasia, metaplasia, dysplasia (mild, moderate, and severe), and carcinoma *in situ*. To date no intermediate biomarkers have been validated for the interception of lung cancer, in part due to the lack of proven therapy, and histology is currently considered the best marker(6). Our group has shown that the persistence of endobronchial dysplasia on repeat biopsies is associated with an increased risk of developing invasive squamous cell lung cancer(7). By identifying and focusing therapeutic interventions on pre-malignant stages of the disease, reductions in incidence and mortality may become a realizable goal(6).

Products of the arachidonic acid pathway, particularly the prostaglandins (PGs), play a critical role in lung carcinogenesis and chemoprevention. Large epidemiologic studies have shown an association between regular aspirin use and decreased rates of certain cancers. Our group has demonstrated chemoprevention of lung cancer by increasing prostacyclin (prostaglandin I_2, PGI_2) levels in multiple pre-clinical models(8,9). These findings led to a clinical trial showing oral iloprost (a prostacyclin analogue) significantly improved

endobronchial dysplasia in former smokers(10). Additional studies focusing on the chemopreventive mechanism have shown prostacyclin's effects to be independent of the single cell-surface PGI_2 receptor (IP) and may rely on PGI_2's ability to act as a peroxisome proliferator activated receptor gamma (PPARγ) agonist(11). The thiazolidinediones (TZDs) are PPARγ agonists commonly used in the treatment of diabetes and the TZD pioglitazone has been studied in multiple pre-clinical cancer models. Pre-clinical studies of genetically modified PPARγ overexpressors and oral PPARγ agonists have confirmed that PPARγ activation promotes differentiation(12,13), inhibits tumor growth, and prevents progression of pre-invasive lesions in murine models(14). Oral, and more recently inhaled, pioglitazone has been shown in pre-clinical models to prevent both adeno and squamous cell carcinoma as a single agent or in combination with inhaled steroids and metformin(15). Rationale for a role in lung cancer chemoprevention was further supported by a large study focusing on lung, prostate, and colon cancer rates in diabetic Veterans treated with TZDs. Govindarajan and colleagues reported a 33% decrease in lung cancer incidence compared to non-TZD users, suggesting the PPARγ activation may chemoprevent lung cancer. This same group also showed a reduction in head and neck squamous cell cancer by 41–55% with TZD use(16). Additionally, pioglitazone has been studied in oral leukoplakia (a tobacco related pre-malignant lesion,) (17). In this trial twenty-one subjects were treated with once daily pioglitazone for 12 weeks and a partial response was seen in 15 subjects (2 had stable disease and 4 progressed). The combination of pre-clinical and epidemiologic data strongly supported a phase II trial of oral pioglitazone.

Prior randomized phase III lung cancer chemoprevention trials based on epidemiologic studies all proved negative. However, several Phase II studies revealed differences between current and former smokers regarding amount and extent of central airway damage(18–20). Subjects with tobacco smoke exposure, chronic obstructive lung disease, and sputum cytologic atypia have rates of lung cancer greater than 1% yearly and are a high-risk population for prevention studies(21). Based on extensive pre-clinical data and epidemiologic observations we instituted a single-center, double-blind, placebo-controlled phase II trial of pioglitazone in current and former smokers with sputum cytologic or endobronchial atypia using improvement in bronchial dysplasia (*Max)* as the primary endpoint.

Materials and Methods:

Study Design:

The pioglitazone lung cancer chemoprevention study was a phase II, randomized, double-blind, placebo-controlled trial of oral pioglitazone in subjects at increased risk for lung cancer (defined as current or former smokers with ≥ 10 pack year smoking history, at least mild sputum cytologic atypia, airflow limitation [FEV1 % predicted < 0.70], or a history of biopsy proven endobronchial dysplasia). The majority of subjects were recruited from pulmonary medicine clinics. Exclusion criteria included: type I or II diabetes mellitus; severe COPD (GOLD Stage III or IV); prior history of cancer within the past 5 years; history of coronary artery disease or congestive heart failure (LVEF<50%); significant comorbid disease or inability to undergo two bronchoscopies; hypoxemia requiring the use

of supplemental oxygen; and carcinoma *in situ* or invasive cancer on endobronchial biopsy. Sputum was collected and cytology was graded by a single cytopathologist (DTM) using previously published methods(21). Autofluorescence and/or white light bronchoscopy was performed before randomization and after 6 months of treatment, with 6 standard endobronchial sites biopsied (all were carini, identified as RUL, RML, RB6, LUL, LUDB, and LB6), along with all other visually suspicious appearing areas. In addition to bronchoscopy, study subjects had full pulmonary function testing at study entry and conclusion, trans-thoracic echocardiogram prior to study entry to evaluate cardiac function, and high-resolution chest CT at study entry and after 6 months of treatment. The study was conducted according to the Belmont Report ethical guidelines and was approved by the Colorado Multi-Institutional Review Board.

The trial enrolled 92 subjects, and after obtaining written informed consent, participants were randomized to treatment groups using a stratified block randomization with smoking status (current vs. former) as the stratification factor and block sizes of 4. The randomization sequence was generated by the trial biostatistician prior to trial initiation and stored in a password-protected spreadsheet accessible only to the trial biostatistician and study administrator. Subjects were randomized only after confirmation of eligibility and completion of pre-study testing (spirometry, echocardiogram, chest CT, and blood chemistry analyses). Blinding of treatment group for each subject was maintained throughout the trial. Following randomization, subjects were started on either pioglitazone (30 mg) or placebo at dose of 1 tablet QD. Subjects had a monthly clinical evaluation, including EKG and blood chemistry analysis. Following six months of treatment subjects had full pulmonary function testing, a high-resolution chest CT scan, and a second bronchoscopy was performed with repeat biopsies at each of the baseline sites in addition to any new sites suspicious for dysplasia. Adverse events were monitored and reported twice yearly to an independent data safety and monitoring board (DSMB). A final clinical visit occurred one month after completing the trial and subjects are currently undergoing passive follow-up through the Colorado Cancer Registry (i.e. annual phone call). All current smokers were counseled and offered assistance with smoking cessation. This trial was listed and registered on ClinicalTrials.gov (Identifier:). Pioglitazone was purchased by the Denver VA research pharmacy and they produced the identical appearing study medication and placebo tablets.

Biopsy Analysis:

All endobronchial biopsies were formalin fixed, paraffin embedded and stained with hematoxylin and eosin (H&E) for subsequent morphologic evaluation and classification. Biopsies were classified into one of eight WHO defined categories(5) and assigned a score according to the following scale: 1 = normal bronchial epithelium; 2 = reserve cell hyperplasia; 3 = squamous metaplasia without atypia; 4 = mild dysplasia; 5 = moderate dysplasia; 6 = severe dysplasia; 7 = carcinoma *in situ* (CIS); and 8 = invasive carcinoma. All biopsies were graded by the study pathologist (DTM) in a blinded fashion as to treatment group and were read after the completion of each bronchoscopy. Biopsies were also assigned a visual inflammation score. The inflammation scores were generated from microscopic review of H&E stained biopsy slides to characterize percent of cellularity attributed to

inflammatory cells (0 = no significant inflammation, <5%; 1 = mild, 5–25%; 2 = moderate, 25–75%; 3 = severe, >75%).

In addition to WHO histology scoring, epithelial proliferation, measured by Ki-67 immunostaining, was conducted on biopsies from a subset of study participants performed by techniques previously described and implemented in our laboratory(20). In brief, the most dysplastic region of a biopsy was selected, at least 400 cells were graded for Ki-67 positivity, where possible, throughout the entire epithelium and the percentage of positive cells recorded as the Ki-67 proliferative index. The primary antibody used for Ki-67 was Dako clone mib-1 BM28 mouse monoclonal (#B58720) at a dilution of 1:100.

Statistical Design and Analysis:

Endobronchial histology was summarized within each bronchoscopy. The primary endpoint was the change in worst (i.e. maximum, *Max*) histology score after 6 months of treatment. Secondary endpoints included the change in average of all biopsy scores (*Avg*) and in dysplasia index (*DI* - defined as the percentage of biopsies with mild dysplasia (a score of 4) or worse). The pre-specified primary analysis used change in *Max* histology after 6 months calculated using biopsies non-normal at baseline (i.e. those with a baseline histology score > 1). All endpoints were analyzed within four different biopsy site groupings: all biopsies scored (n = 1,169), all biopsies from the six standard endobronchial sites only (n = 1,003), site-matched biopsy pairs from both bronchoscopies (n = 1,030), and site-matched biopsy pairs where the baseline biopsy was non-normal (n = 498).

Of the 92 enrolled subjects, 47 were randomized to pioglitazone and 45 to placebo. There were 44 current smokers enrolled (22 in each treatment arm) and 48 former smokers enrolled (25 in the pioglitazone arm and 23 in the placebo arm). The trial was monitored by an independent DSMB, and no interim analyses of treatment effects on histology were planned or conducted.

All analyses were pre-specified in a written statistical analysis plan (SAP) that followed from the trial protocol. The primary endpoint (and statistical analysis) measured the treatment effect of pioglitazone within former smokers by fitting a regression model $Y = \alpha_0 + \alpha_1 GROUP + \alpha_2 BASELINE$. Y represents the 6-month value of the dependent variable (*Max* histology score), GROUP represents a classification variable for the treatment group (1=pioglitazone, 2=placebo), BASELINE represents the value of the outcome measure at baseline, and α_0, α_1 and α_2 represent the parameter estimates from the general linear model. The test of the difference between groups was the formal test of significance of the α_1 parameter. The primary analysis used the biopsy pairs which were non-normal at baseline. This regression analysis was performed using every combination of histology summary measure (*Max*, *Avg*, and *DI*), every set of biopsy grouping (all, reference sites, matched pairs, and baseline non-normal matched pairs), among all subjects, former smokers and current smokers. In total, 36 regression analyses were performed; all but the primary analysis were considered secondary analyses. Analyses using all subjects (i.e. former and current smokers) included smoking status as a covariate in the regression model. Results from all models are reported as point estimates of the treatment effect, 95% confidence intervals, and 2-sided p-values without adjustment for multiple comparisons.

Results

Study Population:

A total of 6126 subjects were screened in the Denver VAMC pulmonary clinics from 2010 until 2015. Two hundred fifty-nine subjects agreed to provide a screening sputum sample and ultimately 92 subjects were enrolled (Figure 1). The main reasons for not entering the trial included: lack of sputum cytologic atypia; reluctance to undergo multiple bronchoscopies; and travel issues. The study population consisted of 44 current smokers and 48 former smokers, with 47 subjects randomized to pioglitazone and 45 to placebo. The only significant difference in baseline demographic or clinical characteristics between the two study groups was age at enrollment. The placebo group was older than those receiving pioglitazone (62.6 +/− 8.2 vs. 58.6 +/− 9.6 years of age, p=0.036). The groups were well matched for gender, ethnicity, tobacco exposure, time since smoking cessation, sputum cytology, and endobronchial histology (Table 1). Eighty four percent of the study subjects were male due to all recruitment occurring at the Denver VA Medical Center. Baseline bronchoscopy was completed in all 92 subjects, and follow-up bronchoscopies were completed in 76 subjects. Similar dropout rates were observed between the treatment groups (17% pioglitazone vs 18% placebo, p=0.92), with the main reason given as ‘refusing further treatment’ (i.e. not desiring a repeat bronchoscopy). The complete list of reasons for failing to complete the study is included in Figure 1. Five subjects successfully quit smoking during the trial, and two subjects resumed smoking. For analyses these subjects remained classified according to their stratification at randomization.

Histologic Analysis Baseline:

At baseline, there were no significant differences between the pioglitazone and placebo groups in any of the histology-based summary measures (*Max*, *Avg*, and *DI*). Using our entry criteria, 87% (80/92) of the subjects had at least one non-normal biopsy at baseline. Mild dysplasia or worse was observed in 25.1% (157/626) of our baseline biopsies and in 65% (60/92) of subjects. At baseline (and consistent with prior studies(10)), current smokers had more endobronchial dysplasia than former smokers as evidenced by significantly higher *Max* (4.5 vs. 3.5, p=0.005), *Avg* score (2.6 vs. 1.8, p<0.001), and *DI* (34% vs. 16%, p=0.001).

Treatment:

Follow-up bronchoscopy was performed on 39 pioglitazone subjects (19 former smokers, 20 current smokers) and 37 placebo subjects (19 former smokers, 18 current smokers). Overall results combining current and former smokers (depicted in Figure 2) showed no significant difference between treatment groups in the primary endpoint (change in *Max* histology in baseline non-normal pairs: 0.00; 95% CI (−0.79, 0.79); p = 1.00; Supplemental Table 1S). When analyzed according to smoking status, there was also no statistically significant difference between treatment groups (Figure 2 and data contained Table 1S). Former smokers treated with pioglitazone demonstrated a decrease (i.e. improvement) in *Max* histology when compared with former smokers treated with placebo (−0.40, 95% CI (−1.68, 0.89), p=0.53, Table 1S) while current smokers treated with pioglitazone exhibited a mild increase (i.e. worsening) in histology when compared with current smokers treated with

placebo (0.32, 95% CI (−0.69, 1.34), p=0.52, Table 1S). In all 36 regression analyses of treatment effect (Table 1S), there were no statistically significant treatment effects observed. Within former smokers, all 12 analyses resulted in a slight improvement (i.e. decrease) in histology in subjects treated with pioglitazone as compared with placebo, with treatment effects ranging from −0.05 to −0.40. Within current smokers, the treatment effects ranged from −0.14 to 0.32, with 9 of the 12 analyses exhibiting a slight decrease in histology for pioglitazone-treated subjects.

Histologic Response:

Treatment effect was also analyzed using a dichotomous endpoint indicating whether or not a patient responded to treatment. A patient was defined as responding to treatment if the histology summary score (maximum or average) showed an improvement (i.e. decrease) by at least 1 unit. Separate response endpoints were defined using both *Max* and *Avg*. Logistic regression analysis was used to analyze whether response differed by treatment groups. The analysis of response was performed in all 24 combinations of endpoints (*Max, Avg*) and biopsy groupings within all subjects, former smokers, and current smokers. There was no statistically significant treatment effect observed in all analyses.

On a per subject analysis, 65% of subjects (47/72) who completed the trial had at least one site of dysplasia at baseline (23 pioglitazone, 24 placebo). Within this group, 40% (19/47) (of the subjects had their maximum histology regress (defined as improving by at least 1 grade) (48% (1½3) pioglitazone, 33% (8/24) placebo, p=0.31); 43% (20/47) of the subjects had their maximum histology remain the same (30% (7/23) pioglitazone, 54% (13/24) placebo); and 17% (8/47) of the subjects had their maximum histology progress (defined as worsening by at least 1 grade) (22% (5/23) pioglitazone, 13% (3/24) placebo). In former smokers with dysplasia (score ≥ 4) at baseline, there was a trend (−1.33, 95% CI −2.85–0.19, p=0.082) towards pioglitazone improving histology.

Analyzing the data at a biopsy pair level, histologic improvement was seen in 27% (135/502) of the pairs, with improvement as great as 5 units observed. Histology was stable (i.e. did not change) in 48% (241/502) of the pairs, and progressed in 25% (126/502). There were 13 biopsy pairs for which change could not be determined due to an unsatisfactory endobronchial biopsy that could not be scored (7 at baseline and 6 at month 6). Within former smokers treated with pioglitazone, from the 130 scored biopsy pairs, 21% (27/130) exhibited improvement, 59% (77/130) exhibited stability and 20% (26/130) exhibited progression. When further limited to the 41 biopsy pairs which were non-normal at baseline (histologic diagnosis of ≥ 2 on initial biopsy), 66% (27/41) of the pairs improved, 24% (10/41) were stable, and 10% (4/41) worsened. The rates in biopsy pairs with non-normal baseline histology observed among current smokers were 56% (44/78) improved, 23% (18/78) remained stable, and 21% (16/78) worsened. There was no significant difference in the distribution of response to treatment with pioglitazone between former and current smokers (p=0.32).

Inflammation analysis (Figure 3):

All endobronchial biopsies received an inflammation score by the study pathologist, with the scores ranging from 0–3 (corresponding to no inflammation [0], mild [1], moderate [2], and severe [3]). When compared at baseline for the presence or absence of inflammation, former smokers exhibited significantly more inflammation than current smokers (39/48 [81%] vs 26/44 [59%], p=0.02). There were no differences in baseline inflammation between the treatment and placebo groups (Table 1 and Figure 3). A stratified analysis of the primary endpoint (change in *Max* histology) based on the presence of inflammation at baseline showed treatment effects in the same direction in biopsies without inflammation with −0.25 in former smokers (95% CI −2.27–1.78, p=0.72) and −0.43 in current smokers (95% CI −2.00–1.14, p=0.56). In biopsies with inflammation, results were in opposite directions with treatment effects of −0.54 in former smokers (95% CI −1.77–0.70, p=0.38) and 0.84 in current smokers (95% CI −0.55–2.24, p=0.22). For those treated with pioglitazone, 44% of subjects with mild inflammation at baseline improved, improvement rates for those with no inflammation (25%) and moderate inflammation (30%) were less. In the placebo group, 44% of subjects with mild inflammation improved, compared to 18% improvement in those without inflammation and 22% of those with moderate inflammation. The presence of inflammation was not significantly associated with histologic improvement. Of those with any inflammation noted at baseline (n=53), 38% showed an improvement in Max, while only 22% of those without inflammation (n=22) had an improvement in Max (p=0.17).

Ki-67 analysis:

Biopsies from former smokers with baseline dysplasia (score of ≥ 4) had Ki-67 staining completed to determine the proliferative index. A total of 42 pre- and post-treatment biopsy pairs from 19 subjects were evaluated and within this group there were biopsies that exhibited an improved histologic score (n=24), maintained the same score (n=13), and exhibited progression (n=5). A subject-level analysis (n = 19) of the association between baseline Ki-67 staining and change in mean histology score showed that baseline Ki-67 was not predictive of change in histology (p = 0.90). The pioglitazone treatment group exhibited a decrease in the Ki-67 proliferative index, with biopsies from subjects treated with pioglitazone exhibiting an average decrease in Ki-67 of 12%, as compared to placebo subjects who had an average increase of 3% for an overall treatment effect of −15% (p = 0.077).

Adverse Events:

Study subjects were evaluated before the initial bronchoscopy and then had monthly clinic visits to evaluate for treatment related adverse events. Dropout rates were similar in the two treatment groups. The most common adverse events were: hypophosphatemia; hypertension; weight gain and hypocalcemia (Table 2S). There were no statistically significant differences in adverse events between the treatment groups except for hyperglycemia (12 events in placebo subjects compared to 4 events in the treatment group, p=0.028). These side effects, including lower rates of hyperglycemia in subjects on pioglitazone, are consistent with previously published trials of pioglitazone(22). Of note, no adverse cardiovascular events or bone fractures were noted during the trial in the group receiving pioglitazone. One subject

developed hematuria during the trial and was evaluated for bladder cancer (a known risk of cumulative TZD exposure in type 2 diabetics(23)), but a cancer diagnosis was not established. There was a single grade 5 adverse event (death due to alcohol abuse) from a subject treated with pioglitazone. There were no life-threatening adverse events (grade 4) in either group, and 20 severe (grade 3) adverse events; 13 in the placebo group and 7 in the pioglitazone group; 11 of the events were hypertension and 4 events were hypophosphatemia (the remaining five SAEs were each a single occurrence). No significant differences were observed between the two treatment groups for grade 3 adverse events (Table 2S contains the most common adverse events by treatment group).

Discussion

In this randomized, double-blind, placebo-controlled trial of pioglitazone we found that 6 months of treatment did not significantly improve the pre-specified primary histologic endpoint of *Max* histology in former or current smokers. While there were individual lesions that improved (and persisted/progressed), there were no significant differences in the treatment groups of current or former smokers.

Endobronchial histology can be assessed using a variety of measures, including *Max, Avg*, and *DI*, none of which improved during this trial. While prior trials have chosen *Avg* as the primary endpoint, we chose to focus on *Max* as the more advanced lesions may be more likely to reflect invasive cancer risk. The natural history of dysplastic endobronchial lesions is difficult to predict. We published our experience where subjects with multiple bronchoscopic biopsies from the same area were evaluated over time. Subjects with multiple endobronchial dysplasias that persisted or progressed (based on repeat biopsies) had a 7.8 fold higher rate of developing squamous cell lung cancer compared to those with improvement in histologic scores(7). Multiple trials, including the oral iloprost trial, have helped to better define the natural history of endobronchial dysplasia and demonstrate that merely obtaining an endobronchial biopsy does not produce a significant therapeutic effect. Most importantly, this report shows that dysplastic lesions can be targets of chemoprevention trials and, because they typically contain fewer genetic derangements and signaling abnormalities, they may also be amenable to treatment aimed at blocking progression.

Our recruitment model for this trial continues to show that we can successfully identify subjects with endobronchial dysplasia based on smoking history and sputum cytologic atypia. Of the 92 enrolled subjects, 64% (59/92) had at least one dysplastic (mild or worse) biopsy at baseline, and 48% (242/508) of the site-matched biopsy pairs scored at baseline were from histologically non-normal areas. These dysplasia rates compare favorably to our oral iloprost trial where 74% of the study subjects had at least one mildly dysplastic or worse biopsy, and 54% of the matched sites were from histologically non-normal areas(10). Ki-67 was selectively studied in former smokers who had dysplasia at baseline, and pioglitazone exhibited a trend (p=0.077) in decreasing Ki-67 by an average of 12%.

Identifying reliable and validated intermediate endpoint biomarkers for Phase II lung cancer chemoprevention trials has proven difficult. Sustained smoking cessation is the only known

intervention to impact lung cancer death rates(24). In the absence of proven effective agents, intermediate endpoints (endobronchial dysplasia and Ki-67 index) cannot be validated by the Prentice criteria(25), but they are a reasonable approach to assessment for further study in Phase III trials(6). Recently completed Phase II studies have chosen to evaluate the effects of intervention on pre-malignant lesions to further inform larger phase III trials. Phase II studies are now incorporating corollary studies that illustrate the chemopreventive agent has reached the desired target with a biological affect. For example, myoinositol inhibits phosphatidylinositol-3-kinase (PI3K), and some subjects receiving this intervention have gene expression signatures from endobronchial brushings that reflect PI3K inactivation(26). In our study, significantly lower rates of hyperglycemia were noted in the pioglitazone arm thereby confirming a known treatment effect.

For lung cancer chemoprevention to decrease incidence and improve survival, high risk populations must be identifiable. There are many published lung cancer risk models based on age, gender, smoking history, airflow obstruction, family history and radiographic emphysema which can risk stratify current or former smokers(27–29). The presence of COPD (as evidenced by airflow obstruction on spirometry or CT detected emphysema) also significantly increases lung cancer incidence(30,31). Our group previously reported that a cohort of high risk current and ex-smokers with airflow obstruction exhibited an overall rate of incident lung cancer of 1.85 per 100 person-years on longitudinal follow-up(21). Survivors of a previous tobacco associated aerodigestive cancer are an additional high risk population appropriate for phase II or III chemoprevention trials, and prior reports estimate second primary tumor rates in patients with a history of lung cancer at 1–2% per patient per year(4). Second primary lung cancer most commonly develops within 5 years of the initial diagnosis and equally in both genders(32). Advances in lesion characterization and biologic behavior will ultimately assist in identifying the highest risk lesions and should allow for more targeted treatment. Molecular or immunohistochemical analysis may improve the identification of lesions that will progress or which are associated with invasive lung cancer as a manifestation of a field effect(33–35). A recent study by our group comparing persistent to regressive bronchial dysplasia found altered cell-cycle control, inflammatory and adhesion related pathways(36). Of interest, biopsy inflammation was associated with regression, and this supports the results reported by Merrick et al. in their analysis of regressive lesions. For example, an improved understanding of the lesion immune microenvironment may allow for prevention with immune checkpoint inhibitors. Our group has initiated an immunoprevention trial evaluating a checkpoint inhibitor in high risk current and former smokers ().

Previous phase III chemoprevention trials were undertaken with agents without prior positive results in Phase II trials, and some of these agents had not demonstrated strong chemopreventive efficacy in animal models(37,38). Pioglitazone has shown efficacy in pre-clinical models and the results of our trial prove that this agent cannot be used in subjects meeting our entry criteria. Based on clinical experience in treating lung cancer, it is understandable that a single agent may not prevent SCC. A better understanding of dysplasia biology will ultimately allow for the targeted treatment of the highest risk lesions, and an improved understanding of lesion characteristics that predict response to specific agents is needed. This may include, but is not limited to, the following characteristics: dysplasia

grade, persistence of dysplasia on multiple biopsies over time; Ki-67 index; gene expression signatures; and possibly inflammatory characteristics in the lesional microenvironment. Additionally, in this trial we report on a new biopsy parameter, the inflammation score. Success of immune oncology agents in the treatment of NSCLC suggest that the immune microenvironment plays a key role in lesion progression and cancer development. Our group is currently working to characterize the lesional microenvironment to determine if inflammatory characterization (as suggested by the trend toward an improved histologic response in lesions with baseline inflammation) is associated with response.

The current study, similar to many more recent phase II trials, was not powered to provide information about the clinically important endpoint of lung cancer incidence, and no study subjects developed lung cancer during the trial. All biopsy specimens were reviewed by a single pathologist. Dropout rates were similar between treatment groups, although the placebo group did have increased episodes of hyperglycemia. No cardiovascular events occurred in the pioglitazone treatment group during the study.

While smoking cessation will have the greatest impact on lung cancer reduction, effective chemoprevention could have major clinical application in the large population of former smokers. Additionally, the widespread adoption of CT screening in high risk population should increase the rates of stage I disease and lead to a larger group of survivors that remain at high risk for a second lung cancer. Identifying the highest risk lesions and studies of precision chemoprevention are warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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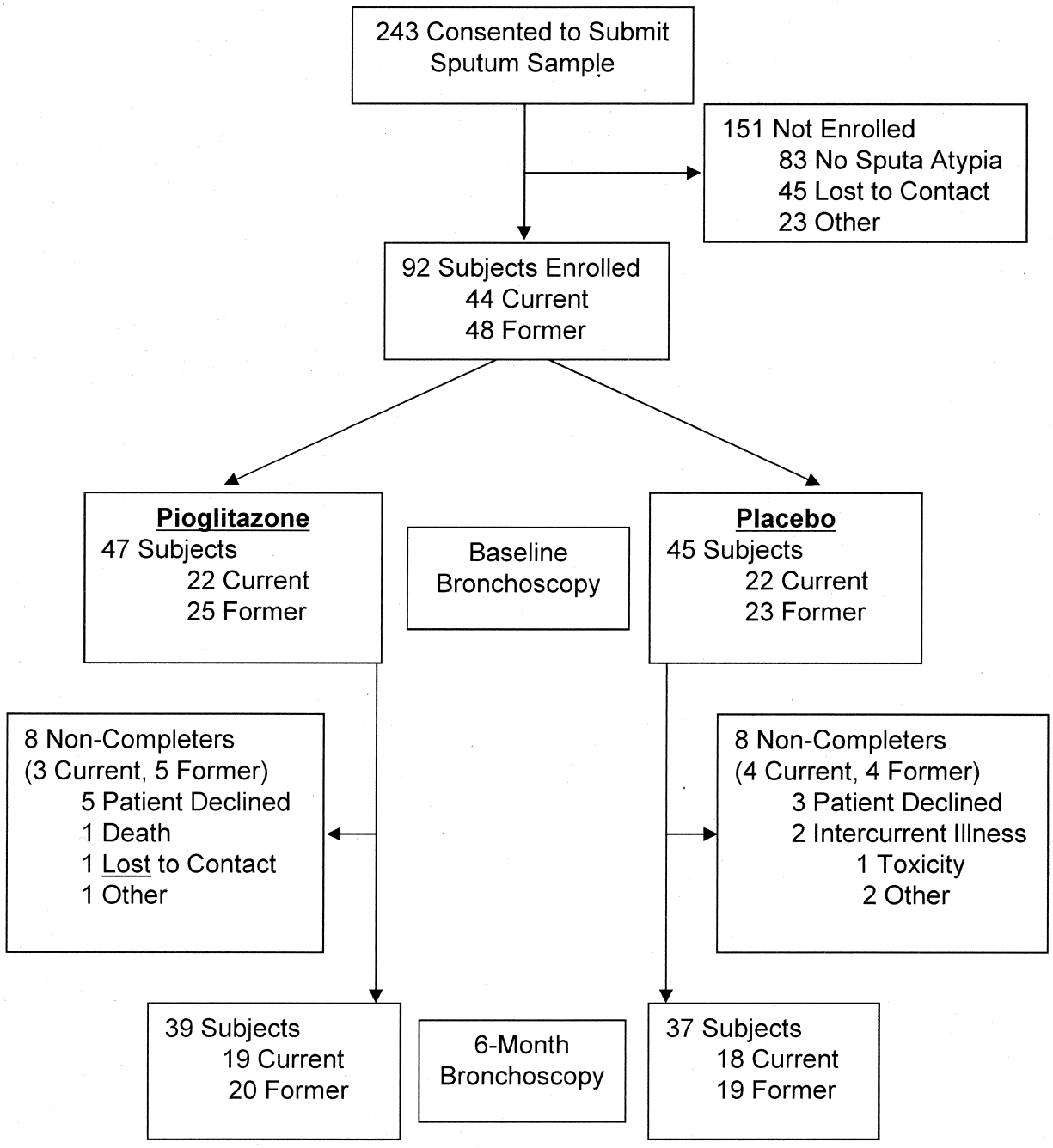

Figure 1:
Trial Flow diagram.

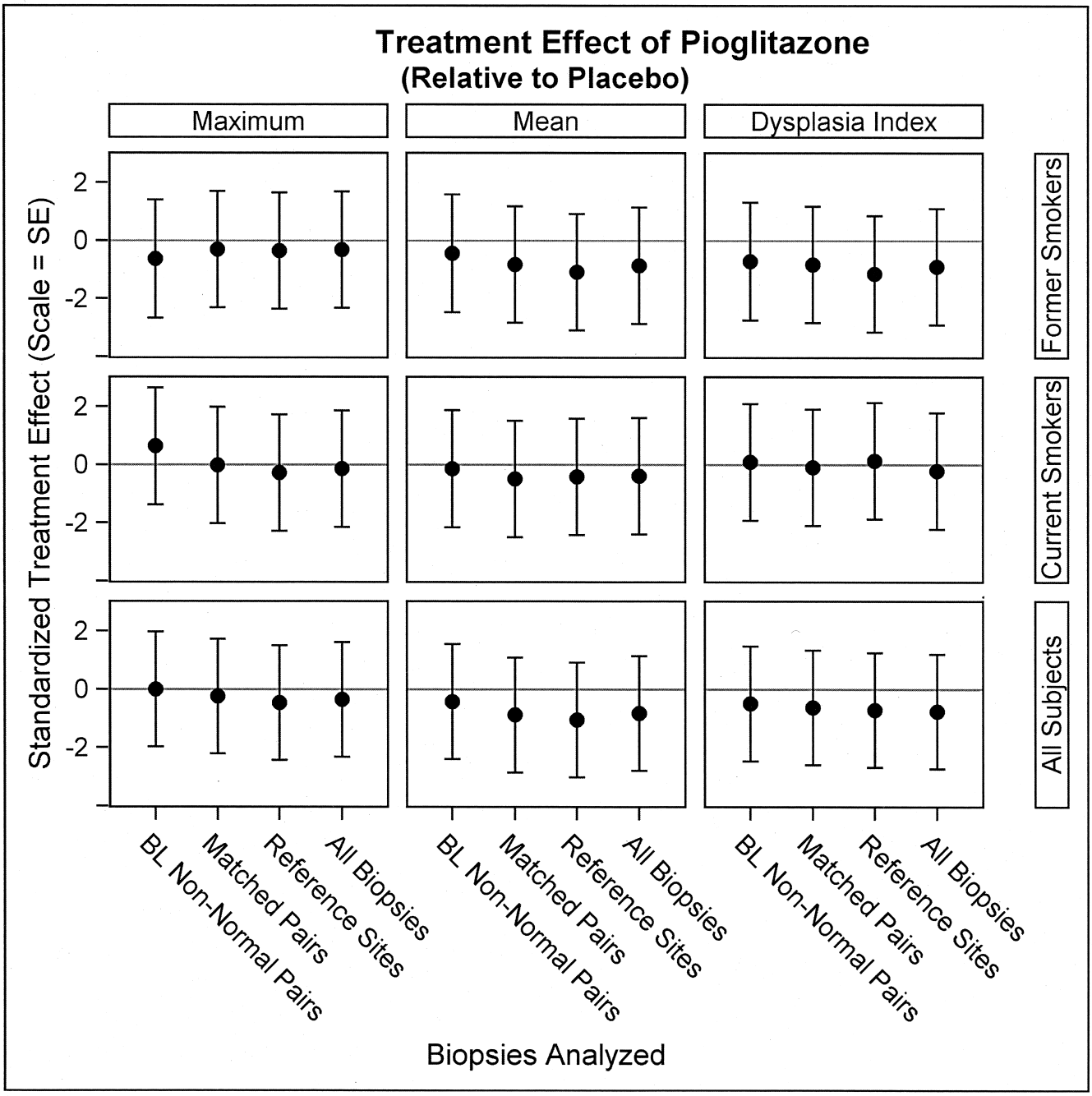

Figure 2: Primary Treatment Effects of Pioglitazone on Endobronchial Histology (Max, Average, Dysplasia Index) in All Subjects, Former Smokers, and Current Smokers

Comparison of Max histology measures on initial and follow-up bronchoscopy of subjects completing the trial (47 pioglitazone subjects, 45 placebo subjects). Biopsies were analyzed in the following groups: baseline (BL) non-normal; matched pairs; reference sites; and all sites. No significant differences are observed in former or current smokers.

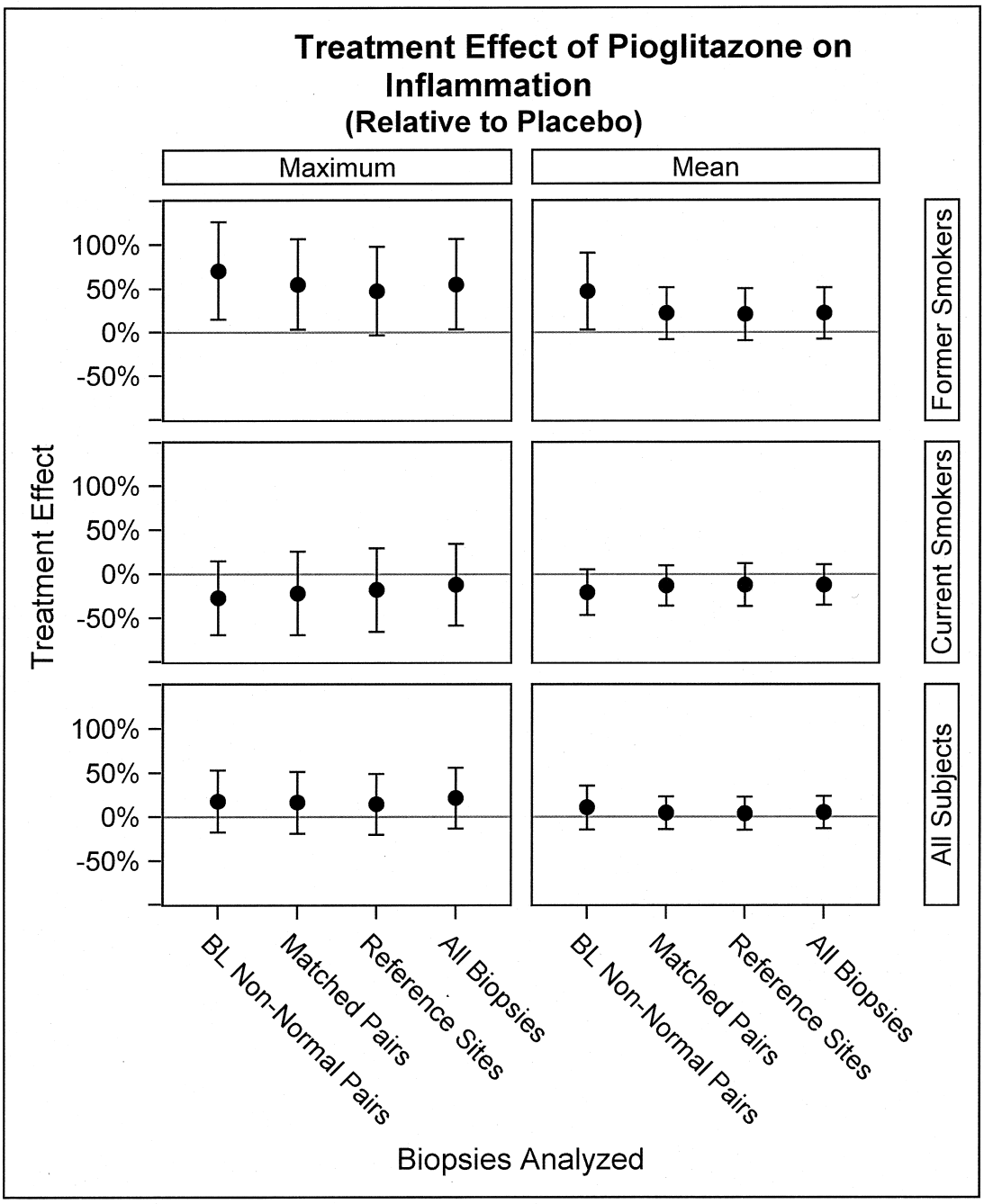

Figure 3: Inflammation results.

All biopsies received an inflammation score (0–3) at baseline and after 6 months of pioglitazone or placebo. Biopsies were analyzed in the following groups: baseline (BL) non-normal; matched pairs; reference sites; and all sites.

Table 1:
Baseline Characteristics of Trial Subjects

Comparison of Baseline Study characteristics (including the presence of inflammation) of subjects on pioglitazone or placebo. The only significant difference observed was older age in the placebo group.

Characteristic		Pioglitazone (N=47)	Placebo (N=45)	Total (N=92)	P-Value
Sex					
Male	N (%)	39 (83.0)	38 (84.4)	77 (83.7)	0.85
Female		8 (17.0)	7 (15.6)	15 (16.3)	
Ethnicity					
Hispanic	N (%)	4 (8.5)	2 (4.4)	6 (6.5)	0.43
Non-Hispanic		43 (91.5)	43 (95.6)	86 (93.5)	
Race					
White	N (%)	44 (93.6)	38 (84.4)	82 (89.1)	0.30
Black		2 (4.3)	6 (13.3)	8 (8.7)	
American Indian		1 (2.1)	1 (2.2)	2 (2.2)	
Age at Enrollment (Years)	Mean (SD)	58.6 (9.6)	62.6 (8.2)	60.5 (9.1)	0.036
Smoking Status					
Current Smoker	N (%)	22 (46.8)	22 (48.9)	44 (47.8)	0.84
Former Smoker		25 (53.2)	23 (51.1)	48 (52.2)	
Age Stopped Smoking	Mean (SD)	49.9 (11.6)	49.4 (13.1)	49.7 (12.2)	0.87
Packs per Day	Mean (SD)	1.2 (0.5)	1.2 (0.4)	1.2 (0.5)	0.59
Smoking Duration (Years)	Mean (SD)	34.9 (11.2)	35.9 (13.3)	35.4 (12.2)	0.72
Smoke Exposure (Pack-Years)	Mean (SD)	41.7 (19.8)	41.0 (19.4)	41.4 (19.5)	0.87
Sputum Classification					
No Sputum Abnormalities	N (%)	4 (8.5)	2 (4.4)	6 (6.5)	0.70
Mild Sputum Atypia		26 (55.3)	25 (55.6)	51 (55.4)	
Moderate Sputum Atypia		10 (21.3)	13 (28.9)	23 (25.0)	
Severe Sputum Atypia		6 (12.8)	4 (8.9)	10 (10.9)	
Unknown		1 (2.1)	1 (2.2)	2 (2.2)	
FEV1 (BL)	Mean (SD)	2.74 (0.82)	2.68 (0.91)	2.71 (0.86)	0.74
FEV1 Predicted (BL)	Mean (SD)	3.45 (0.59)	3.22 (0.66)	3.34 (0.63)	0.086
FEV1 % Predicted (BL)	Mean (SD)	79.8 (20.2)	82.7 (21.1)	81.2 (20.5)	0.50
FVC (BL)	Mean (SD)	3.78 (0.72)	3.73 (0.99)	3.76 (0.86)	0.80
FEV1/FVC Ratio	Mean (SD)	0.71 (0.13)	0.71 (0.12)	0.71 (0.13)	0.88
COPD					
No	N (%)	37 (78.7)	33 (73.3)	70 (76.1)	0.54
Yes		10 (21.3)	12 (26.7)	22 (23.9)	
Had Any Inflammation					0.58
No	N (%)	15 (31.9)	12 (26.7)	27 (29.3)	
Yes		32 (68.1)	33 (73.3)	65 (70.7)	