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Authors

Linden, Kenneth G
Leachman, Sancy A
Zager, Jonathan S
et al.

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A Randomized, Double-Blind, Placebo-Controlled Phase II Clinical Trial of Lovastatin for Various Endpoints of Melanoma Pathobiology

Kenneth G. Linden[1,2], **Sancy A. Leachman**[3], **Jonathan S. Zager**[4], **James G. Jakowatz**[1], **Jaye L. Viner**[5], **Christine E. McLaren**[1,6], **Ronald J. Barr**[2], **Philip M. Carpenter**[1,7], **Wen-Pin Chen**[1], **Craig A. Elmets**[8], **Joseph A. Tangrea**[9], **Sung-Jig Lim**[10], **Alistair J. Cochran**[11], and **Frank L. Meyskens Jr**[1]

[1]Chao Family Comprehensive Cancer Center, University of California, Irvine, California, USA

[2]Department of Dermatology , University of California, Irvine, California, USA

[3]Huntsman Cancer Institute, University of Utah, Salt Lake City, Utah, USA

[4]Moffitt Cancer Center, Tampa, Florida, USA

[5]Takeda Cambridge – USA, Cambridge, Massachusetts, USA

[6]Department of Epidemiology, University of California, Irvine, USA

[7]Department of Pathology, University of California, Irvine, USA

[8]University of Alabama at Birmingham, Birmingham, Alabama, USA

[9]Division of Cancer Prevention, National Cancer Institute, Rockville, Maryland, USA

[10]Kyung Hee University, Seoul, Republic of Korea

[11]University of California, Los Angeles, California, USA

Abstract

Based on large cardiovascular clinical trials of lipid lowering agents that showed a considerable decrease in incidence of primary melanomas in the active agent arm, we have carried out a randomized, double-blind clinical trial examining the impact of lovastatin on various biomarkers of melanoma pathogenesis. Subjects with at least two clinically atypical nevi, were randomized to receive oral lovastatin or placebo for a six month period. Clinical, histopathologic, and molecular biomarkers were evaluated for change in the two groups. 80 subjects were randomized, evaluable, and included in the analyses. Lovastatin showed no benefit in comparison to placebo in the primary endpoint of decreasing the level of histopathologic atypia, nor in any of the secondary endpoints of decreasing clinical atypia, impact on nevus number, nor in showing significant changes in any of the molecular biomarkers. There were no significant differences in adverse

Corresponding author: Kenneth G. Linden, Department of Dermatology & The Chao Family Comprehensive Cancer Center, University of California Irvine Medical Center, 101 The City Drive, Orange, CA 92868 (714) 456-3719, (714) 456-8524 (fax), kglinden@uci.edu.

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event profiles for lovastatin compared to placebo. The lovastatin arm did show a significant and considerable decrease in total serum cholesterol and serum LDL levels compared to placebo, an expected result. This finding bolsters confidence in subject compliance. Given the results of this trial, it is concluded that if lovastatin were to lower the incidence of melanoma, it would appear not to be doing so by reversing atypia of precursor atypical nevi over the six month time frame studied. Further research into the pathogenesis of melanoma and in other potential chemopreventive agents is needed.

Keywords

melanoma; chemoprevention; statins; lovastatin; atypical nevi

INTRODUCTION

Although recent advances in treatment of Stage IV melanoma are exciting and encouraging, the long-term prognosis for melanoma once it has disseminated remains dismal. Given this, it is imperative that research be carried out not only on therapeutics for melanoma, but also on other aspects, including prevention and detection. Chemoprevention of melanoma is a little explored area (1) that deserves more attention, especially given the failures in therapeutics for advanced melanoma.

Two large cardiovascular clinical trials demonstrated a significant reduction in the incidence of melanoma in the lipid-lowering agent arm vs. the control arm (2, 3). However clinical trial evidence is not uniformly in support of an effect of lipid-lowering agents on melanoma incidence with a recently published prospective analysis of association between use of statins and melanoma risk in the Women's Health Initiative showing no difference in risk between those using statins and those not (4). Also, two meta-analyses showing no decreased risk with statin use(5, 6). However, an epidemiologic study in the Netherlands found that though statins did not seem to influence the incidence of melanoma, their use was associated with a reduced Breslow thickness in melanomas upon diagnosis, possibly suggesting a beneficial effect of statins on melanoma progression (7). Also, a recent large case-control study of 300,000 subjects conducted in the Netherlands showed an overall reduction in cancer incidence among statin users, but not in melanoma incidence (8). In addition to clinical trial data, various in vitro, animal model, and theoretical work has lent support to the concept of statins as potential chemoprevention or chemotherapy agents (9-11). There are several theoretical mechanisms where by statins could influence melanoma development. They might function through their action on HMG Co-A reductase to change the geranylation for farnesylation patterns of key cell cycle regulatory proteins such as those in the RAS pathway (9, 11). Also statins may be acting by immunomodulation through possible effects on steroids known to be generated in the skin, including melanoma cells (12, 13).

Since atypical (dysplastic) nevi are precursors to melanoma and can be considered precancerous lesions (14, 15), reduction of atypia clinically and histopathologically by a chemopreventive agent should lead to a reduction in risk of these lesions developing

melanoma and would provide strong evidence of a chemopreventive effect. To address this, we conducted a randomized, placebo-controlled phase II trial testing the effects of lovastatin on atypical nevi. Two groups of patients were randomized to treatment with lovastatin or placebo. Each patient in the first group had two nevi matching in size and clinical degree of atypia; each patient in the second group had one large clinically atypical nevus at least 8mm diameter, with a second atypical nevus that could be followed photographically. The goals of the trial were to analyze clinical, microscopic, and molecular endpoint biomarkers in atypical nevi pre- and post-treatment, to quantify potential chemopreventive effects of lovastatin, and to obtain data needed for subsequent trials.

MATERIALS AND METHODS

Protocol Design

The study was a randomized, double-blind placebo-controlled phase II clinical trial of lovastatin in patients with atypical nevi. The trial involved four clinical sites in the United States with subjects on trial from 12/2007 through 4/2011. Human subjects committees at each site approved the study protocol. The study was conducted according to the Declaration of Helsinki principles. Written informed consent was obtained from all subjects. The trial has been registered at ClinicalTrials.Gov, registration number NCT00462280.

Recruitment and Study Population

Patients older than 18 years of age were eligible for study entry if they had two atypical nevi with the following characteristics: Group 1-*either* two nevi matching in size and clinical degree of atypia, *or* Group 2-one large clinically atypical nevus ≥ 8mm diameter, with a second atypical nevus that could be followed photographically. This design with these two different groups was chosen to enable comparison of these two different groups with respect to evaluation of study endpoints and reproducibility. It may be argued that biopsies of different nevi (Group 1), despite being clinically matched, may yield such variability in the biomarkers as to be less desirable. Conversely, biopsy of a portion of a large nevus both pre and post treatment (Group 2) may lead to errors due to variability within different portions of the nevus, or more importantly, the inflammatory and healing process from the initial biopsy may lead to exposure to cytokines and effects from the inflammatory and healing process might alter the endpoint biomarkers independent of the agent being tested.

All target lesions were clinically atypical nevi (16), but must not have had a level of clinical atypia that required a biopsy to rule out melanoma. Subjects were ineligible if they were currently on lipid lowering agents, had been on lipid lowering agents of any type within the last three months, or had a history of coronary artery disease or stroke. Females were ineligible if they were pregnant, breast feeding, or of child bearing age and were not using a reliable method of contraception, as use of lovastatin is contraindicated in pregnancy.

Randomization, Assignment, and Dose Regimen

Eligible subjects were randomized to receive either lovastatin or placebo in a 1:1 ratio. Stratified randomization was used to balance the treatment arms with respect to clinical center because clinical site variability is the largest source of variation in multi-center

clinical trials (17). Subjects were stratified into groups: Group 1 =Two Matched Atypical Nevi and Group 2 =One Large, >8mm Atypical Nevus + Another Atypical Nevus. The groups were placed in blocks of fours alternating with blocks of twos. A random number generator was used to determine the randomization assignments. A Randomization Table was established for each site. The statistician created the random allocation sequences. The allocations were presented as a sequence of numbers specific for each site, e.g. 1011001, 1011002, 1011003, 1011004, 1021005, 1021006, etc. Site study teams enrolled the subjects and contacted staff at the Central Site UC Irvine to obtain the appropriate randomization number. In this way, the staff could verify enrollment criteria had been met.

Study treatments were dispensed to trial participants as encapsulated tablets in labeled bottles. Initially, participants were given a 40 mg capsule of lovastatin or placebo orally on a daily basis. If the 40 mg dose was tolerated and lab results were acceptable, at 6 weeks the dose was increased to 80 mg (two 40 mg capsules) given orally on a daily basis. The monitoring and dosage adjustment guidelines followed in this study were within the normal range for clinical use of this drug. Duration of study participation and study drug was 6 months.

Masking

The study was carried out in an entirely double blinded manner. The study statistician generated the random allocation sequence. Investigators at each of the 4 sites enrolled subjects and obtained allocations from the study pharmacist in a blinded fashion. All subjects were given identical over-encapsulated pills and were completely blinded to their treatment arm for the duration of the study. Similarly, all evaluations were carried out in a blinded fashion by evaluators completely blinded as to which arm the study subjects were on.

Participant Flow and Follow-up

Adherence and Compliance—Participant compliance was monitored at the two week post randomization visit and at each subsequent visit (1 mo, 3 mo and 6 mo) and telephone monitoring was performed 6, 8 and 16 weeks post randomization.

Clinical Assessments—During the randomization visit, the target atypical nevi were identified, mapped, and photographed; the randomly designated nevus of the matched pair of nevi or a portion of the ≥8mm nevus were biopsied. Each participant then received study medication and was started on the study. Follow-up visits with laboratory assays were performed at intervals of a few weeks during the time the subject was on study. For the 50 subjects with two matched atypical nevi, the nevi were designated nevus A and nevus B respectively.

Final visits and off-study monitoring—Participants returned to the clinic 24 weeks (± 2 week) post-randomization for off-study monitoring that included complete skin examination for atypical nevi; photos of remaining intact target nevi; photos of entire back from shoulders to natal cleft; biopsy of matched remaining atypical nevi or a section of the ≥8mm designated study nevus; monitoring of any adverse events, and received a lab order

for test including a complete blood count (CBC), Chem 20, CPK, fasting lipid panel, C-reactive protein, and, for females of child-bearing potential, a urine pregnancy test. The 'dosing calendar' was reviewed and collected with returned medication containers for pill counts. Study subjects were seen two weeks later for suture removal and to follow up on laboratory tests.

Trial Endpoints and Analysis

Histopathologic Analysis of Biopsied Atypical Nevi: The primary endpoint was histopathologic regression of atypical nevi in response to treatment. Standard histopathologic evaluation of all or portions of atypical nevi was done pre- and post-treatment by two dermatopathologists nationally recognized in pigmented lesion/melanoma evaluation (RJB & AJC). Atypia in nevomelanocytic lesions is characterized by cytologic atypia, architectural atypia or disorder, and host response. Cytologic atypia consists of one of more of an increase in the nuclear/cytoplasmic ratio, prominent nucleoli, an irregular chromatin pattern, variations in thickness of the nuclear membrane, or finely distributed melanin pigment in the cytoplasm. Architectural atypia consists of one or more of asymmetry, bridging of theques between rete ridges, nevus cells at the shoulders of rete ridges, a lentigenous distribution of nevus cells at the dermo-epidermal junction, or nevus cells present above the basal layer of the epidermis. Host response is characterized by a lymphocytic infiltrate, fibroplasia, capillary/endothelial hyperplasia, and/or incontinence of pigment. All of these factors are considered together while evaluating sufficient multiple microscopic fields and sections of the histopathologic specimen to arrive at a diagnosis with a corresponding assigned level of atypia (Supplementary Figure S1) (see (18) for details on the description and grading of levels of atypia). The level of atypia was graded in a standard fashion on a discrete scale of seven levels of atypia, with zero for no atypia and six for melanoma. The grading system was as follows: 0 = no atypia/normal nevus, 1 = mild atypia, 2 = mild to moderate atypia, 3 = moderate atypia, 4 = moderate to severe atypia, 5 = severe atypia, 6 = melanoma. For each patient, the change from baseline in the level of atypia was calculated.

Clinical Analysis of Photography of Target Nevi.: Secondary endpoints included clinical regression of atypical nevi and change in number of nevi on the back. Pre- and post-treatment macroscopic photos were taken of target atypical nevi and evaluated by a panel of three physicians who are clinically active in the pigmented lesion clinics at their respective institutions. The physicians, blinded to treatment arm and pre- or post-treatment status, assigned a grade to each pair of photos (Supplementary Figure S2). The grading system was as follows: 1= left photo shows a complete resolution of atypia relative to right photo, 2 = left photo shows a strong lessening of atypia relative to right photo, 3 = left photo shows a mild lessening of atypia relative to right photo, 4 = left and right photos show same degree of atypia, 5 = right photo shows a mild lessening of atypia relative to left photo, 6 = right photo shows a strong lessening of atypia relative to left photo, 7 = right photo shows a complete resolution of atypia relative to left photo. After unblinding of pre- or post-treatment status for photos, an ordinal variable was created representing clinical regression of atypical grade.

To determine change in number of nevi on the back, photos of the subjects' backs, superiorly from the horizontal line formed by the shoulders and inferiorly to the top of the natal cleft, were obtained both pre- and post-therapy, identifiers were removed, and photos were assessed in the similar blinded fashion by study clinicians (Supplementary Figure S3). The grading system was as follows: 1= moles apparent in the left photo that are not present in the right photo, 2 = both photos, left and right, show the same nevi, 3 = moles apparent in the right photo that are not present in the left photo. An ordinal categorical variable was generated after the pre- or post-treatment status was un-blinded. The back is the anatomic region with the highest number of atypical nevi on average, and is the anatomic region with the highest incidence of melanoma.

Molecular biomarkers: Molecular biomarkers selected for evaluation were candidates along the nevomelanocytic carcinogenic pathway and could be measured on standard formalin-fixed, paraffin-embedded sections. Biomarkers were measured pre- and post-study and included measures of angiogenesis, proliferation, p21 (WAF1/CIP1) protein, RelA, and expression of ecadherin and n-cadherin.

Please see the Supplement for details on the immunohistochemical staining and analysis.

Angiogenesis is closely linked with carcinogenesis. As a biomarker associated with angiogenesis, VEGF expression was measured because it has been shown to correlate with level of atypia (19) and a reduction in VEGF expression may indicate change towards a more benign phenotype. In addition, modulation of angiogenesis has been associated with statins (20). We also analyzed the expression of HIF1alpha, an important regulatory protein in angiogenesis.

Increased proliferation is a hallmark of carcinogenesis progression. Several studies have shown that Ki-67 expression correlates with level of atypia in the nevomelanocytic system (21-25). However, the main increases are seen in the transition from atypical nevus to melanoma, with further increases occurring with invasive and metastatic potential within melanomas.

Another protein involved in proliferation inhibition, p21 (WAF1/CIP1), was measured since there is evidence that p21 may be affected by HMG-CoA reductase inhibitors (9, 26).

RelA was measured because we and co-workers have identified RelA as a biomarker that varies with the level of atypia of nevomelanocytic lesions (27).

Expression of e-cadherin and n-cadherin were measured because differences in expression patterns of these proteins has been demonstrated between benign nevomelanocytic cells and melanoma (28).

Serum components: An objective was to evaluate the correlation between serum markers known to be affected by lovastatin and the tissue endpoints. To this end, blood was collected both pre- and post-study. A standard lipid panel was performed and C-reactive protein was measured. C-reactive protein is involved in immune and inflammatory process modulation and could possibly impact carcinogenesis in targets such as nevomelanocytic precursors. If

melanoma incidence is indeed decreased by oral statins, a key question, which we have not seen asked, is whether this is due to a direct effect of the statins intracellularly on the molecular machinery of the nevomelanocytic cells, or is the effect a secondary one brought about by changes in the extracellular milieu that then impact the nevomelanocytic cells, or some combination of these two pathways. It is conceivable that chemoprevention of melanoma by statins could be due to effects of statins on serum components, or on the stroma, rather than the statins directly acting on the nevomelanocytic cells themselves.

Statistical considerations—The study was designed with adequate power to detect a significant reduction in atypia in the lovastatin arm, should such a reduction occur. Power calculations for a Wilcoxon rank sum test were performed using nQuery Advisor 5.0 (Statistical Solutions, Ltd., 2002). Let μ_1 be the mean change in histopathologic grade from baseline for the placebo group and let u_2 be the mean change for patients treated with Lovastatin. Assuming a common standard deviation, σ, the effect size is $\delta = (u_1 - u_2)/\sigma$. Because statistical comparisons were to be made for each of the two study groups, the Bonferroni multiple comparisons procedure was applied to achieve an overall significance level of 0.05. With 25 subjects in each treatment arm, and a 0.025 significance level, the two-sided Wilcoxon rank sum test would have 80 percent power to detect an effect size of -0.972 (29). The accrual goal was 120 subjects, with at least 2 current atypical nevi in locations that could be easily biopsied, to assure 100 evaluable patients, one group of 50 subjects with two matched atypical nevi and a second group of 50 patients with one large, ≥8mm atypical nevus and another atypical nevus.

The Wilcoxon rank sum test was applied to compare treatment arms with regard to change from baseline in histopathologic score after treatment. Values were determined by subtracting the histopathologic score after treatment from that obtained before treatment. A categorical variable was created representing regression, no change, or increase in level of atypia after treatment; the chi-square test was applied to assess the association between change in histopathologic score and treatment group.

For analysis of the secondary endpoint, clinical regression of atypical nevi, the Wilcoxon rank sum test was applied to compare the change in clinical grade between treatment groups. A categorical variable was created to indicate no change, decrease or increase in number of nevi after treatment; Fisher's exact test was applied to assess the association between change in number of nevi on the back and treatment group. Analyses of clinical secondary endpoints were exploratory and intended for hypothesis generation. No adjustment for multiple comparisons was made.

Seven biomarkers were analyzed in terms of the changes in percent of staining intensity from baseline and the mean change from baseline on immunostained histology slides. The estimated mean changes and 95% confidence intervals for the means were reported by treatment group and pathologist. Independent two-sample *t*-tests were applied to compare mean changes from baseline between treatment arms, adjusted for multiple comparisons (30). Similarly, independent sample *t*-test were used to compare mean changes from baseline in total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides and in 16 additional laboratory tests, adjusted for multiple comparisons.

Data were combined for the group with two matching nevi and the group with one large clinically atypical nevus. Fisher's exact test was applied to test for an association between treatment arm and occurrence of at least one adverse event.

RESULTS

Accrual

Eighty subjects total were randomized. All were at least partially evaluable, and were included in one or more of the analyses. Sixty-six subjects with two matched nevi were randomized to treatment with lovastatin (n=34) or placebo (n=32). Ten subjects in the lovastatin arm and seven in the placebo arm discontinued the trial early. Thus, there were 49 evaluable subjects in Group 1, 24 in the lovastatin arm and 25 in the placebo arm (Figure 1). Accrual for Group 2 with one large atypical nevus, partially biopsied pre- and post-study, was slow and was halted after accrual of 14 subjects (Supplementary Figure S4. Subjects in this group were not included in the analysis of primary endpoints or biomarkers. However, for this group, the results of photographic secondary endpoints, adverse events, and serum lipid measurements are included.

Demographics of Participants

Baseline variables were similar across the two treatment groups (Table 1).

Primary Endpoint

Figure 2 displays the change from baseline in the level of histopathologic atypia for the treatment groups as determined by two pathologists. Based on atypia grades determined by pathologist 1, the lovastatin group showed a mean increase in the grade of atypia of 0.50 compared to a mean decrease of 0.12 for the group treated with placebo (Wilcoxon rank sum test p=0.048). Based on grades determined by pathologist 2, the lovastatin and placebo groups showed a mean increase in the grade of atypia of 0.17 and 0.04, respectively; these means did not differ significantly (p=0.919).

As determined by pathologist 1, 12.5% of 24 patients who took lovastatin showed regression of atypia, 29.2% showed no change, and 58.3% showed an increase in level of atypia after treatment. In comparison, for 25 patients taking placebo these percentages were 40.0%, 28.0%, and 32.0% respectively. The association between change in histopathologic score and treatment group was marginal (Chi-square test p= 0.068). Based on values determined by pathologist 2, 37.5% of 24 patients who took lovastatin showed a regression of atypia, 25.0% showed no change, and 37.5% showed an increase in level of atypia after treatment, compared to 36.0%, 20.0%, and 44.0%, respectively, for 25 patients who took placebo. There was no significant association between change in histopathologic score and treatment group (Chi-square test p= 0.873).

Concordance between the two pathologist's evaluations were analyzed (see Supplement, Supplementary Figure S5).

Secondary Endpoints

Nevi grade—Clinical analysis of target nevi was performed by a panel of three physicians expert in the evaluation of pigmented lesions, who examined pre- and post-treatment photographs of nevi (Supplementary Figure S2). For each participant, the mean of changes recorded by three reviewers was computed. The mean difference between treatment arms was 0.06 (-0.11, 0.23). No significant difference in the clinical regression of atypical grades was found between treatment arms (p=0.61) (Supplementary Figure S6).

Number of nevi—From photos taken pre- and post-treatment, the three physicians determined the number of nevi on the backs of 23 patients who took lovastatin and 26 patients who took placebo (Supplementary Figure S3). Data from the three physicians were combined. There were 6% of photographs from the lovastatin group found to have fewer nevi after the treatment, 93% with the same number of nevi, and 1% with more nevi after the treatment compared to 4%, 87%, and 9%, respectively in the placebo group. No significant association was found between treatment arm and change category overall (Fisher's exact test p=0.13) or for any of the three evaluators (Fisher's exact test p=1.00 for evaluator 1, p=0.51 for evaluator 2 and p=0.49 for evaluator 3).

Molecular Biomarkers

As a secondary endpoint, changes in molecular biomarkers based on immunostaining intensity on histopathologic slides were evaluated by two pathologists in a blinded fashion (Figure 3). Molecular biomarkers examined included HIF1alpha, e-cadherin, n-cadherin, VEGF, RelA, p21, and Ki-67. Based on assessments by pathologists 3 and 4, none of the molecular biomarkers showed a significant difference between treatment arms with regard to mean change from baseline (two-sample *t*-test).

Concordance analyses for the two pathologists were performed (See Supplement, Supplementary Table S1, Supplementary Figures S7-S8).

Serum Components and Adverse Events

The lovastatin arm showed a statistically significant decrease in total cholesterol and LDL cholesterol post-study compared to the placebo arm (Figure 4, Supplementary Table S2). This confirms expectations that lovastatin would lower cholesterol levels, including LDL, and is a reassuring check on subject compliance with taking the study medications. Evaluation of 16 additional laboratory tests, including liver enzymes and CPK (U/L), showed no significant difference between the lovastatin and placebo arms at the end of study, corrected for multiple comparisons (Supplementary Table S2).

Safety and Adverse Events

In considering safety and adverse events, 34% of 41 participants taking lovastatin reported at least one study-related adverse event during the study compared to 28% of 39 participants taking placebo. These observed differences were not significant (Fisher's exact test p=0.48).

Discussion

This study showed no beneficial changes in the primary end point which was change in histopathologic atypia of the target atypical nevi for lovastatin compared to placebo, nor any of the secondary endpoints including clinical atypia, nevi numbers, or biomarkers for lovastatin compared to placebo. Only the expected beneficial changes in lipids in the lovastatin arm were seen. It is concluded that if lovastatin were to lower the incidence of melanoma, it would appear not to be doing so by reversing atypia of precursor atypical nevi over the six month time frame studied.

It can always be argued that the length of time subjects were on the trial was insufficient to manifest an effect, and a positive result with subjects on study medication for a longer period of time cannot be ruled out. The length of time on medication is always a compromise of resources and effort devoted to the trial, and study subjects' willingness to participate (recruitment and compliance) impacting choice of trial length. Length of time on study medication for future trials will be dependent on understanding the proposed mechanism of action, along with any further information that is developed for the progression of precancerous changes in the formation of melanoma.

It may also be the case that if lovastatin were to lower the incidence of melanoma, it may act through a mechanism other than reversing atypia in precursor atypical nevi.. Though around 25% of cutaneous melanomas arise from a preexisting nevus, 75% are thought to arise denovo from isolated skin melanocytes. Perhaps if statins did have an effect, they could be acting on reducing melanomas arising in this fashion. Also, perhaps they could be acting at a later stage, after the formation of the melanoma, but through slowing its progression, as previously discussed (7).

This study highlights the difficulty in designing a chemoprevention trial for melanoma: If the primary endpoint of the trial is the reduction in the incidence of new melanomas, the trial must encompass many thousands of subjects followed over several years. This would be a costly and resource intensive endeavor which would require a considerable degree of pre-study evidence that the huge effort would be worthwhile. On the other hand, atypical nevi, known precursors to melanoma, if used as a surrogate endpoint biomarker, are difficult to evaluate in a reproducible fashion, particularly in regard to quantitation of the level of atypia. Also, there are currently no well characterized biomarkers for progression of atypical nevi to melanoma. This makes study of putative chemoprevention agents for melanoma problematic at this time.

Despite these difficulties, pursuit of chemoprevention agents for melanoma is a desirable goal given the high degree of morbidity and mortality currently associated with this disease. Further pre-clinical work on understanding the developmental pathways of melanoma and in characterizing potential biomarkers is needed for future clinical trials testing potential chemopreventive agents.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

LDL	Low Density Lipoprotein
CBC	Complete Blood Count
CPK	Creatine Phosphokinase
VEGF	Vascular Endothelial Growth Factor

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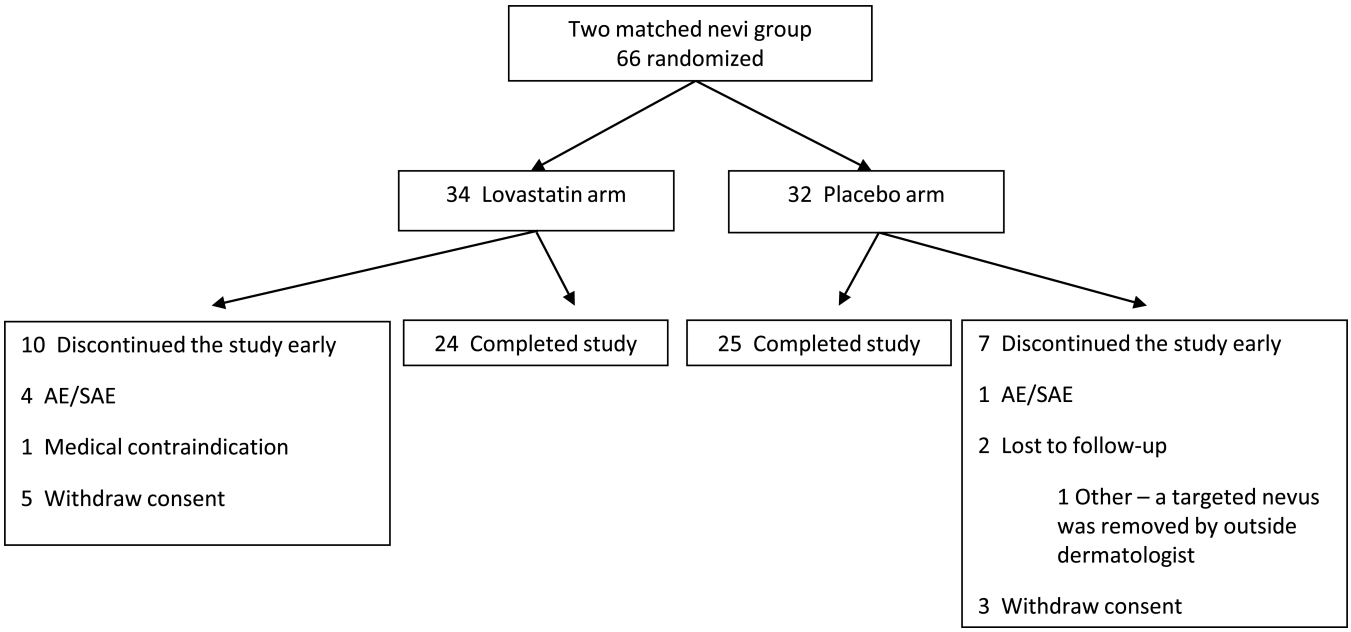

Figure 1.
Study diagram for two matched nevi group.

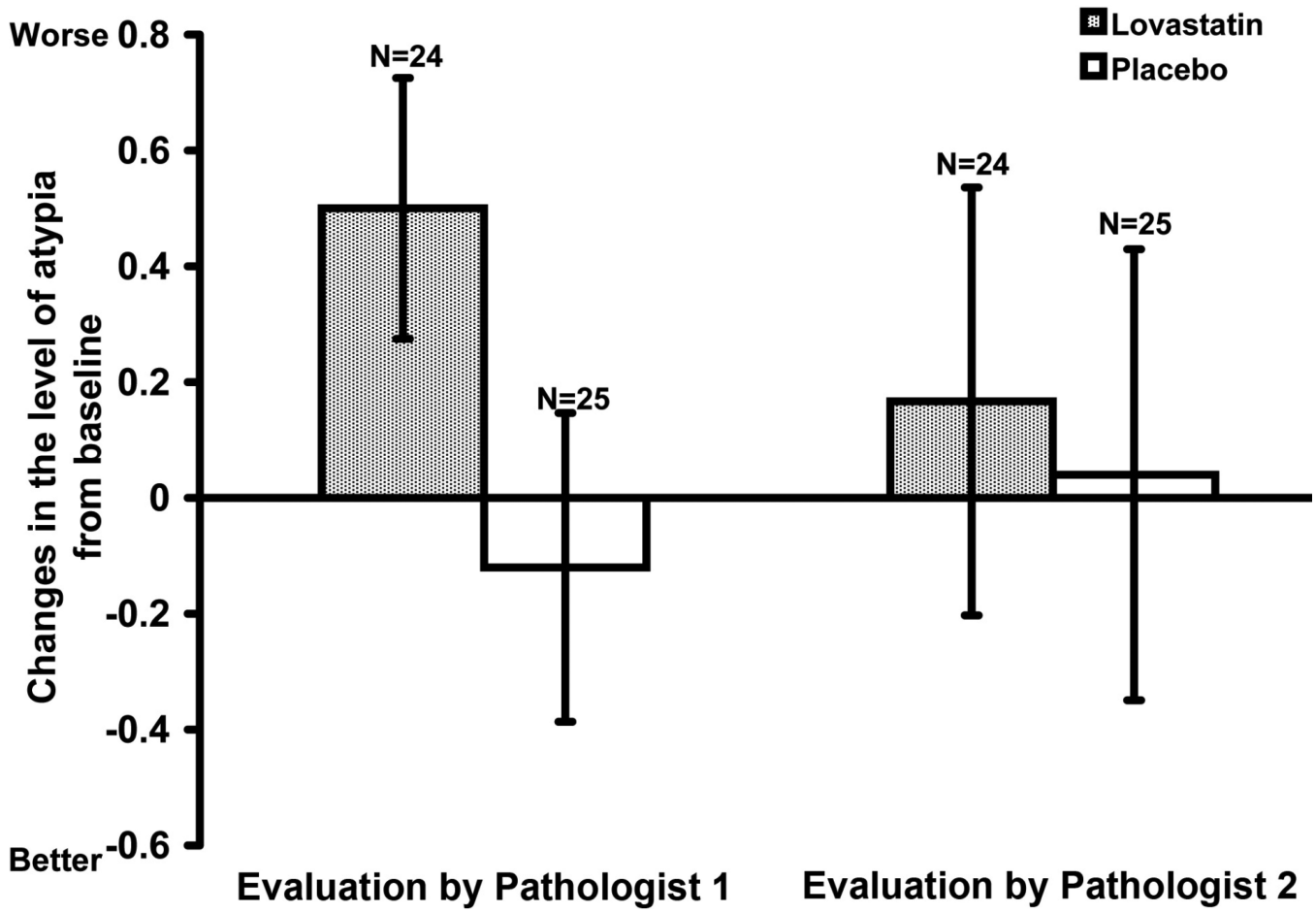

Figure 2. Histogram of changes in the level of atypia from baseline, mean +/− SEM, by treatment arm and pathologist

The level of atypia was graded using a seven point scale in a standard fashion as detailed in the Materials and Methods. Based on Wilcoxon rank sum test, there is a borderline significant increase in the level of atypia from baseline between lovastatin and placebo (p=0.048) evaluated by pathologist 1. However, there is no significant difference in changes in the level of atypia from baseline between lovastatin and placebo (p=0.919) evaluated by pathologist 2.

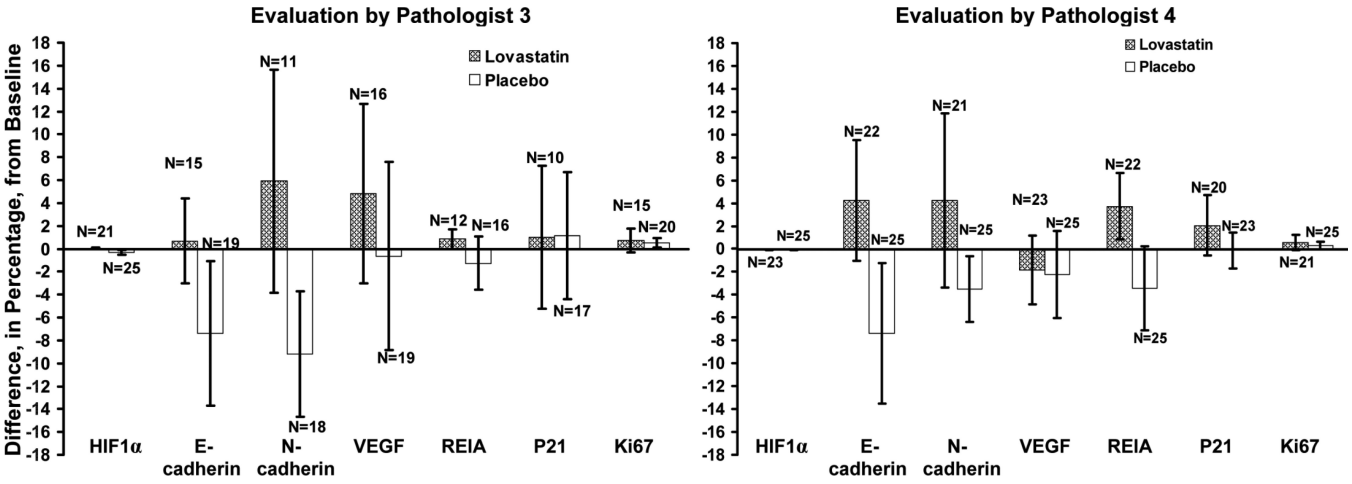

Figure 3. Differences in percent of staining intensity from baseline (mean +/− SEM) for 7 biomarkers by treatment arm and pathologist

None of the biomarkers showed a significant difference between lovastatin and placebo for either of the two reviewing pathologists.

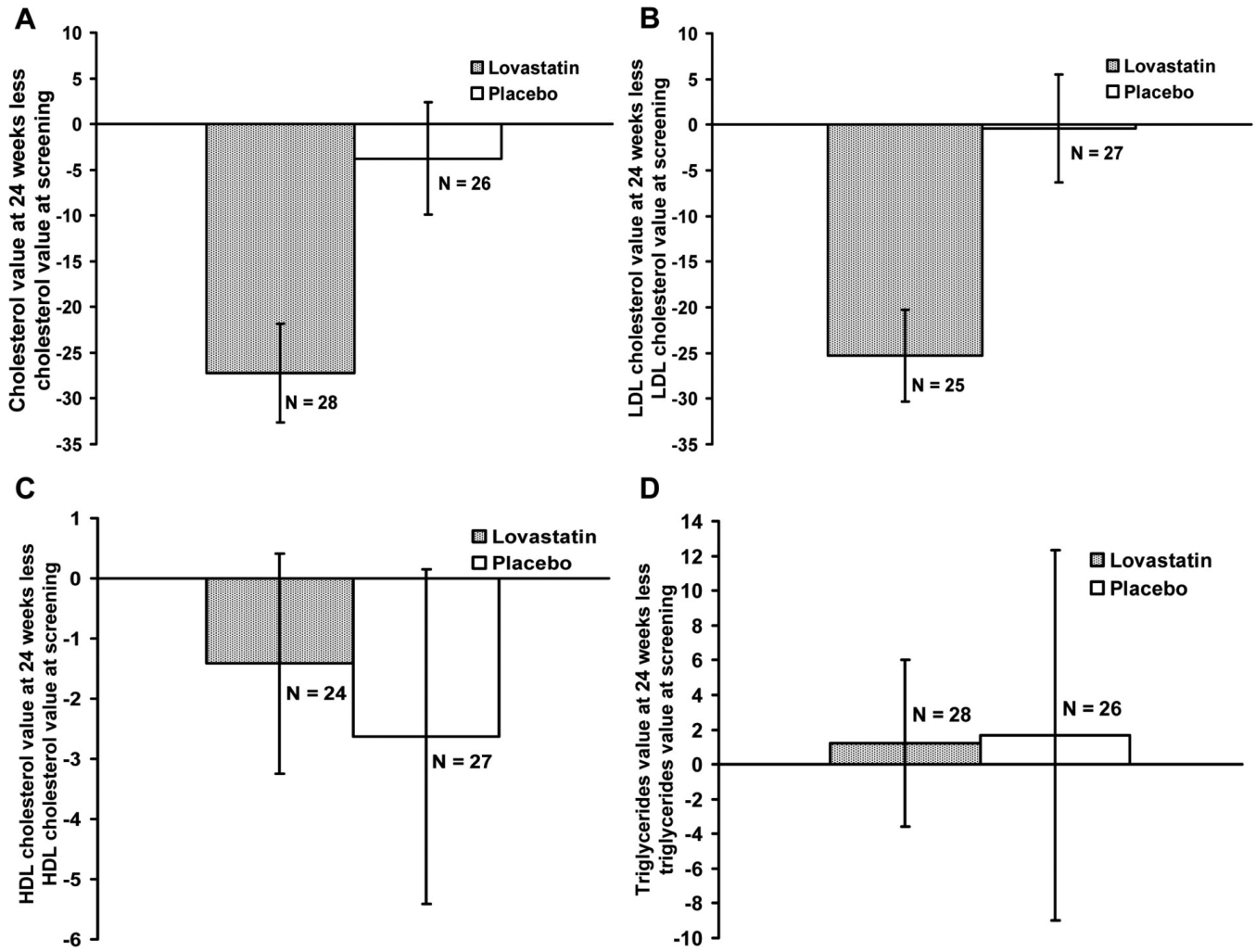

Figure 4. Histogram of changes in serum lipids from baseline (mean +/− SEM) by treatment arm

The lovastatin arm showed a statistically significant decrease in total cholesterol and LDL cholesterol post-study compared to the placebo arm (See Supplementary Table S2 for details).

Table 1

Baseline characteristics of study participants with two matched nevi.

Characteristic[2]	Lovastatin[1] (N = 34)	Placebo[1] (N = 32)
Demographics		
Male, n (%)	12/34 (35)	12/32 (38)
Race n (%)		
Caucasian	34/34 (100)	32/32 (100)
Ethnicity n (%)		
Hispanic or Latino	1/34 (3)	0/32 (0)
Age at enrolled (y)	42.8 (10.96)	42.2 (11.28)
Height (cm)	171.5 (9.42)	172.3 (10.63)
Weight (kg)	74.6 (16.12)	80.0 (20.30)
BMI	25.2 (4.58)	26.8 (5.66)
Blood Pressure, Systolic	125.1 (13.98)	122.4 (13.71)
Blood Pressure, Diastolic	78.6 (12.31)	78.0 (8.91)
Laboratory results at baseline		
Cholesterol	194.3 (37.25)	210.0 (39.02)
HDL cholesterol	56.2 (17.72)	55.3 (19.09)
LDL cholesterol	111.2 (29.98)	130.3 (32.18)
Triglycerides	108.6 (109.8)	124.1 (76.61)
CPK	100.6 (83.16)	94.3 (81.32)
Albumin	4.3 (0.33)	4.3 (0.26)
Alkaline phosphatase	64.2 (19.28)	72.0 (16.68)
ALT (U/L)	18.1 (7.30)	23.5 (16.33)
AST (U/L)	27.0 (9.2)	29.8 (11.56)
Bilirubin, Total	0.6 (0.30)	0.6 (0.37)

[1] Values are count and column percentage for categorical variables, mean +/− SD for continuous variables.

[2] Depending on availability of data, sample sizes varied from 32 to 33 for the Lovastatin arm and from 31 to 32 for the Placebo arm.