Cyclin D1 and Cancer Development in Laryngeal Premalignancy Patients

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Abstract In a previous trial, we found that combined 13-*cis*-retinoic acid, IFN-α, and α-tocopherol more effectively reversed advanced premalignant lesions of the larynx than of the oral cavity and that *cyclin D1* (*CD1*) G/A870 single nucleotide polymorphism correlated with cancer risk. We conducted the present trial primarily to confirm the clinical activity of the combination in advanced laryngeal premalignancy and to confirm and extend our findings on CD1, both genotype and protein expression, in association with cancer risk in this setting. Twenty-seven moderate-to-severe laryngeal dysplasia patients underwent induction with combined 13-*cis*-retinoic acid daily, α-IFN twice weekly, and α-tocopherol daily for 1 year; 14 nonprogressing patients then were randomized to maintenance fenretinide or placebo for 2 years. During induction, two patients had pathologic complete responses, six had partial responses (30% overall response rate), and five developed laryngeal cancer. There were no significant differences between maintenance fenretinide and placebo in response or cancer rates. Ten patients developed cancer overall. Twenty-four patients were evaluated for the *CD1* G/A870 genotype, and 23 for pretreatment and posttreatment CD1 protein expression. Consistent with our earlier report, shorter cancer-free survival was associated with the *CD1* AA/AG genotype ($P = 0.05$). Extending our earlier work, high CD1 expression was associated with worse cancer-free survival overall ($P = 0.04$) and within each *CD1* genotype group. These findings support CD1 genotype and protein expression as important risk markers for laryngeal cancer and suggest future trials targeting upstream regulators of *CD1* transcription.

Premalignant lesions of the head and neck are a useful model for evaluating strategies for chemoprevention of upper aerodigestive tract cancers (1). These lesions most often develop in association with exposure to tobacco and alcohol and frequently precede the development of invasive carcinoma (2). Although early premalignant head and neck lesions (hyperplasia and mild dysplasia) are commonly responsive to single-agent retinoid therapy (3, 4), advanced premalignant head and neck lesions (moderate-to-severe dysplasia) are resistant to retinoid monotherapy (3–5). The combination of retinoids and IFNs (biochemoprevention) enhances the induction of cell differentiation and suppression of cell proliferation (6–10). A clinical trial of combined 13-*cis*-retinoic acid (13-cRA), IFN-α, and α-tocopherol [based on possible enhancement of activity and attenuation of retinoid toxicity (11, 12)] for 12 months in patients with oral and laryngeal premalignant lesions produced a high response rate of laryngeal lesions (13). In the original study, we also examined cyclin D1 (CD1) G/A870 single nucleotide polymorphism, gene amplification, and protein expression. CD1 is key regulatory protein of the cell cycle and tissue homeostasis, and alterations of both its gene copy number and protein expression are frequently found in premalignancy and neoplasia. The CD1 G/A870 single nucleotide polymorphism is associated with two different splice variant transcripts: CD1a and CD1b. CD1a encodes for the full-length native form of the CD1 protein, which has a short nuclear half-life, tightly regulated by phosphorylation of residues in exon 5, followed by nuclear export and ubiquitination (14). CD1b encodes for a truncated alternate CD1 protein, which lacks exon 5 and is a constitutively nuclear protein with enhanced oncogenic properties (14). The major translational findings were a correlation between *CD1* AA or AG genotype and increased cancer risk (versus *CD1* GG genotype) and a trend toward an association between modulation of CD1 protein expression and lesion response (15).

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We designed the present randomized trial in two phases: induction with combined 13-cRA, IFN-α, and α-tocopherol to confirm the clinical/histologic activity of this regimen in patients with advanced premalignant lesions of the larynx followed by maintenance with fenretinide (versus placebo) to prolong the chemopreventive effects. We chose the synthetic retinoid fenretinide for the maintenance phase because it has a different mechanism of action from that of the related compound 13-cRA, the potential to reverse persistent lesions (16–18), and a favorable toxicity profile (19). The major translational goal of this study was to prospectively confirm and extend the CD1 findings of our earlier trial.

Materials and Methods

Patient eligibility

All patients were required to provide signed informed consent before entering this randomized, double-blind, placebo-controlled, single-institution trial conducted at M. D. Anderson Cancer Center, where the trial protocol was approved by the Institutional Review Board. Eligibility included the following criteria: histologic evidence of moderate or severe dysplasia or carcinoma *in situ* of the larynx, no retinoid therapy within the 3 mo before study entry, no prior history of invasive cancer for the 3 y preceding entry into the study, adequate bone marrow, hepatic, renal function, and triglyceride level ≤2.5 the upper limit of normal. Patients with gastroesophageal or laryngopharyngeal reflux were treated with anti-reflux measures and esomeprazole or omeprazole for 2 mo. Only patients that showed no signs of clinical improvement of their laryngeal lesion(s) along with symptomatic reflux improvement were eligible for inclusion in the study. Exclusion criteria included use of oral anticoagulants and severe intercurrent illness.

Evaluation and treatment plan

Pretreatment evaluation consisted of complete history and physical examination and detailed information on history of gastroesophageal reflux disease and a dietary questionnaire. Detailed alcohol and tobacco consumption information was obtained at baseline and at months 6, 12, 24, and 36. All patients were counseled about smoking cessation and a proactive smoking cessation program was offered. Suspension laryngoscopy with videographic recording evaluation and biopsy was done at baseline, months 12 and 36, and at any time patients developed symptoms (hoarseness) or clinical evidence of progression on laryngeal examination. The postbiopsy appearance of the vocal folds was recorded and stored such that the precise localization of the biopsy sites could be determined. Complete history and physical examination was done every 3 mo during the active intervention.

All patients received induction therapy consisting of 2 million units (MU)/m^2 IFN-α s.c. twice weekly, daily 13-cRA (80 mg/m^2 orally), and daily α-tocopherol (1,200 IU orally). Patients unable to tolerate the starting dose (toxicity grade ≥3) had the dose reduced to level minus 1 (IFN-α, 1.5 MU/m^2 twice weekly; 13-cRA, 60 mg/m^2/d). Two additional dose reductions were implemented for recurrent toxicity grade ≥3: level minus 2 (IFN-α, 1 MU/m^2 twice weekly; 13-cRA, 40 mg/m^2) and level minus 3 (IFN-α, 0.75 MU/m^2 twice weekly; 13-cRA, 20 mg/m^2). After 12 mo of induction therapy, responders and patients with stable disease (SD) were randomized (1:1) to daily fenretinide (200 mg orally) or placebo for 24 mo (maintenance phase). In case of grade ≥3 toxicity, drug would be stopped and the patient was permanently taken off treatment; no dose reductions were implemented during the maintenance therapy phase. Patients were followed every 6 mo for a minimum of 2 y after study completion.

Criteria for response and toxicity

Histologic response definitions. Complete response (CR) was defined as complete disappearance of dysplastic features with no clinically evident lesion, partial response (PR) as downgrading of dysplastic features from severe to moderate or from moderate to mild, SD as persistence of the same level of dysplasia after treatment, and progressive disease (PD) as worsening of the degree of dysplasia from moderate to severe or from mild to moderate, or progression to invasive laryngeal cancer.

Clinical response definitions. CR was defined as disappearance of all measurable and evaluable lesions for at least one cycle of therapy or 4 wk. PR was defined as 50% or greater decrease in the sum of the products of diameters of all measured lesions for at least 4 wk; SD, stabilization of all existing lesions with no new lesions developing, no PD, or less than PR; PD, any increase of >25% in the sum of the products of diameters of any measurable lesion or in estimated size of nonmeasurable lesions or the appearance of an unequivocal new lesion or progression to invasive carcinoma. Clinical response was assessed by a speech pathologist (J. Lewin) doing the videostroboscopy, in conjunction with a medical oncologist (V. Papadimitrakopoulou) and head and neck surgeon (J. Myers) in a joint review of the videographic recording (Fig. 1) resulting in a consensus decision on response.

Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria version 2.0 as well as previously published definitions of toxicities associated with treatment with retinoids (20).

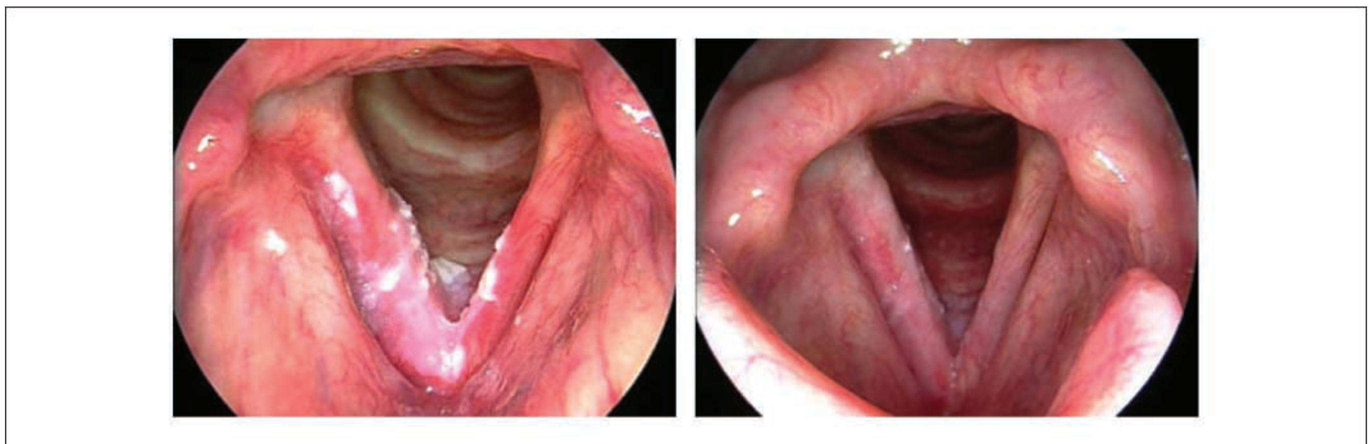

Fig. 1. Left, baseline laryngeal dysplasia involving both true vocal folds and immediate subglottis; right, near-complete resolution of laryngeal dysplasia after treatment.

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Histopathologic evaluation

All biopsies (baseline and months 12 and 36) were evaluated for pathologic diagnosis from H&E-stained slides. Unstained slides were prepared from formalin-fixed paraffin-embedded blocks, cut into 4-μm-thick sections, and used for CD1 assessment. Histopathologic changes in tissue in response to the therapy were evaluated by a pathologist (A. El-Naggar) according to previously described criteria (13, 21).

CD1 genotype and protein assessments

Genomic DNA samples and target tissue lesions, including baseline and 12-mo biopsies, were available for CD1 evaluation in 24 patients.

CD1 genotyping was done using genomic DNA derived from peripheral blood lymphocytes using proteinase K followed by isopropanol extraction and ethanol precipitation. The 870A genotype was assessed using the Taqman single nucleotide polymorphism assay. The probes were labeled fluorescently with either 6-FAM or VIC on the 5′ end and a nonfluorescent minor grove binder quencher on the 3′ end (Applied Biosystems). Primers and amplification conditions were previously described (22). The reactions were read using the ABI Prism 7900HT Sequence Detection system, and the analyzed fluorescence results were further called into genotypes (i.e., AA, AG, and GG) using the built-in software.

CD1 protein expression was evaluated using immunohistochemistry on formalin-fixed paraffin-embedded sections with a mouse monoclonal antibody (clone P2D11F11; Novocastra) as previously reported (23). Only nuclear immunoreactivity was considered positive and the intensity of the staining was evaluated as follows: 0 = no staining, 1 = weak, 2 = moderate, or 3 = strong. Positive controls were placed on each section and negative controls were included in each immunohistochemistry batch. For comparison with patient characteristics and clinical outcome, the results were expressed as the labeling index (LI; i.e., the fraction of CD1-positive cells expressing a staining intensity ≥2) and the weighted mean index [WMI; i.e., the sum of the intensity scale values (0-3) of each cell divided by the total number of evaluated cells; refs. 15, 23]. Two investigators (J.G. Izzo and T.L. Ceron) evaluated CD1 immunolabeling in a blinded fashion and without knowledge of genotype and clinical data. The final scores of each target tissue were calculated as the average score between the individual scores of each investigator. When the individual scores varied by >0.05, the cases were considered discrepant and were recounted, and a final score was determined based on consensus of the two investigators.

Statistical analysis

Histologic and clinical end points and analysis plan. The primary end point was histologic response at 12 mo. Secondary end points included clinical response, toxicity, comparing fenretinide with placebo in maintaining histologic response, clinical responses in the induction and maintenance phases, and laryngeal cancer development.

We planned to enroll 100 eligible patients in the induction phase based on an anticipated rate of response and SD of 80% and a SE of 4%; thus, 80 patients were expected to remain in progression-free status (i.e., with improved or stabilized histology of premalignant lesions) at 12 mo and therefore eligible for the maintenance therapy. This sample size would be sufficient to detect a 30% difference in the relapse rate (histologic PD after initial response or SD; 10% in the 4-HPR arm and 40% in the placebo arm) between the two groups with 80% power. The sample size calculation assumes a two-sided 5% type I error rate and a 5% lost-to-follow-up rate. The original study design included 3 y of accrual, 3 y of treatment, and additional 2 y of follow-up.

CD1 analysis plan. To examine the relationship between *CD1* genotype and clinical correlates, the analysis was conducted for two genotype forms (i.e., combined AA/AG and GG group) because of the biological functional dominant effect of the A allele (24). Fisher's exact test and Wilcoxon rank sum tests were done to examine the associations between clinical characteristics, *CD1* genotype, CD1 expression, and response to treatment, when appropriate. To examine the relationship between CD1 protein expression and clinical correlates, CD1 expression was dichotomized into high and low expression based on the median of baseline LI and WMI values (CD1 expression versus response to intervention) and also on the mean of the baseline WMI values (CD1 expression and time to cancer). The cancer-free survival by *CD1* genotypes (AA/AG versus GG) and CD1 WMI groups (high versus low) was estimated by Kaplan-Meier method and the difference was tested by Wald test when the robust sandwich estimate for covariance was used or log-rank test, when appropriate. Multicovariate Cox proportional hazards model was used to evaluate the effect of *CD1* genotype and CD1 expression on cancer-free survival. P values of ≤0.05 (two sided) were considered to be statistically significant. Patients were pooled for these analyses as they all received uniform treatment during the induction period and the effect of fenretinide in the small subgroup of patients receiving fenretinide is considered negligible.

Results

Patients and treatment characteristics

We registered 40 patients from November 1, 1998 to May 30, 2004. Accrual was slow and did not allow for the projected number of patients to be entered into the randomized maintenance phase within the funding period of the trial. Thirty of the 40 registered patients were eligible for the trial, of which 27 began the 1-year induction phase and 3 refused to start treatment. As summarized in Table 1, patient characteristics included a predominance of males (59.3%) and current or former smokers (77.7%), no alcohol consumption in 66.7%, and gastroesophageal reflux in 96% of the patients.

Eighteen patients completed the full 12-month course of induction therapy; 9 discontinued therapy because of disease progression (4 patients), patient preference (3), or toxicity (2). Seventeen induction patients required dose reductions: 12 to dose level minus 1, 4 to minus 2, and 1 to minus 3. Fifteen induction patients were eligible (responded or had SD) to proceed to the maintenance phase, and 14 were randomized to placebo (n = 7) or fenretinide (n = 7); 5 discontinued maintenance treatment because of disease progression (2 on placebo and 3 on fenretinide).

Histologic and clinical outcomes

Induction results are based on all 27 patients who entered the induction phase (Tables 2 and 3). Two induction patients had histologic CRs and six had PRs, for an overall response rate of 30%. Eight patients (30%) had SD and seven (26%) had PD (five invasive laryngeal cancer and two worsening dysplasia). Four patients (15%) were inevaluable for the primary end point [early dropouts before response evaluation due to patient's preference (2) or toxicity (2); Table 2]. There were no statistically significant differences in induction response rates according to gender, smoking status, or baseline histology (Table 3). Of the 23 evaluable patients, 2 experienced clinical CR, 6 PR, 9 SD, and 6 PD.

The following histologic responses occurred during maintenance therapy (Table 2): two CRs, two SDs, and three PDs (one to invasive cancer and two to worsening dysplasia) in the placebo arm (n = 7); one CR, one SD, and four PDs (three to invasive cancer and one to worsening dysplasia); and one patient was inevaluable due to poor quality of the biopsy obtained (clinical response: PR) in the fenretinide arm (n = 7). Clinical responses during the maintenance phase were

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Table 1. Participant characteristics (n = 27 patients who started induction therapy)

Gender	
Female	11 (40.7%)
Male	16 (59.3%)
Age	
Mean ± SD (range)	56.6 ± 10.7 (30.5-75.3)
Race	
White	24 (88.9%)
Hispanic	2 (7.4%)
Asian	1 (3.7%)
Cancer history	
No	22 (81.5%)
Yes	5 (18.5%)
Previous treatment for cancer	
Radiation	1
Surgery	3
Radiation and surgery	1
Baseline histology	
Moderate dysplasia	14 (51.8%)
Severe dysplasia	13 (48.1%)
Smoking status	
None	6 (22.2%)
Former	12 (44.4%)
Current	9 (33.3%)
Packs-years, mean ± SD (range)	
Former	39.9 ± 26.2 (5.0-90.0)
Current	44.6 ± 18.5 (22.5-80.0)
Smoking quit years for former smokers	
Mean ± SD (range)	14.2 ± 8.8 (2.2-26.4)
Alcohol usage	
None	18 (66.7%)
Beer	2 (7.4%)
Wine	3 (11.1%)
Liquor	3 (11.1%)
Unknown	1 (3.7%)
Reflux	
Present and treated	26 (96.3%)
No reflux	1 (3.7%)

two CRs, two SDs, and three PDs in the placebo arm and one CR, one PR, one SD, and four PDs in the fenretinide arm.

Overall (induction and maintenance), PD occurred in 14 of 27 patients, and 9 patients (33%) developed cancer, whereas 1 cancer developed after the treatment period. After a median follow-up of 5.1 years, the cancer-free survival rates are 84% [95% confidence interval (95% CI), 71-99.7%] at 1 year and 63% (95% CI, 46.9-85.7%) at 2 years; median progression-free survival is 2.74 years (95% CI, 1.33-NA); and progression-free survival rates are 76% (95% CI, 61.1-94.8%) at 1 year, 55% (95% CI, 38.7-79.1%) at 2 years, and 46% (95% CI, 29.8-71.4%) at 3 years. Median cancer-free survival has not yet been reached.

Table 3. Histologic responses to induction therapy by patient characteristics (n = 23, evaluable)

Characteristic	Response, n (%)			P
	CR/PR	SD	PD	
Gender				
Female	4 (40)	3 (30)	3 (30)	1.00
Male	4 (30.8)	5 (38.5)	4 (30.8)	
Smoking status				
None	1 (16.7)	1 (16.7)	4 (66.7)	0.058
Former	2 (20)	5 (50)	3 (30)	
Current	5 (71.4)	2 (28.6)	0 (0)	
Alcohol usage				
None	3 (20)	6 (40)	6 (40)	0.12
Beer	0 (0)	1 (50)	1 (50)	
Wine	3 (100)	0 (0)	0 (0)	
Liquor	1 (50)	1 (50)	0 (0)	
Baseline histology				
Moderate dysplasia	3 (25)	6 (50)	3 (25)	0.35
Severe dysplasia	5 (45.5)	2 (18.2)	4 (36.4)	

Toxicity

The highest-grade adverse event during the induction phase was grade 2 in 8 patients, grade 3 in 17 patients, and grade 4 in 1 patient; the most common adverse events were mucocutaneous toxicities related to retinoids (i.e., cheilitis, conjunctivitis, and dry skin), hypertriglyceridemia, and IFN-related arthralgia, fatigue, myalgia, anorexia, fever, chills, and injection site reactions (Table 4). The maintenance phase was better tolerated in both placebo and fenretinide groups, with three patients experiencing at least one grade 2 adverse event in each arm. No grade 3 or 4 toxicities were observed.

Table 2. Histologic response rates

Response	Induction phase, n (%)	Maintenance phase, n (%)	
	n = 27	Placebo (n = 7)	Fenretinide (n = 7)
CR	2 (7)	2 (29)	1 (14)
PR	6 (22)	0 (0)	0 (0)
SD	8 (30)	2 (29)	1 (14)
PD	7 (26)	3 (43)	4 (57)
INE	4 (15)	0 (0)	1 (0)

Abbreviation: INE, inevaluable.

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CD1 genotype and protein expression at baseline

CD1 G/A870 genotype and protein expression were evaluated in 24 of the 27 patients that initiated induction therapy. Two patients carried the AA (8.3%) genotype, 13 carried the AA/AG (54.2%) genotype, and 9 patients carried the GG (37.5%) genotype. The calculated allele frequency distributions were 35.4% for the A allele and 64.6% for the G allele. Because the AA and AG genotypes harbor similar clinical phenotypes (15, 23), the AA and AG genotypes were combined for clinical analyses. There were no statistically significant differences in the distribution of CD1 genotype (AA/AG versus GG) by gender, smoking status, and baseline degree of dysplasia. The percentages of CD1-positive cells and the levels of nuclear CD1 expression detected in the target tissues were similar in both genotype groups (LI: mean ± SD, 0.21 ± 0.21 for AA/AG versus 0.21 ± 0.21 for GG, $P = 0.77$; WMI: mean ± SD, 0.48 ± 0.58 for AA/AG versus 0.32 ± 0.44 for GG, $P = 0.95$, Wilcoxon rank sum test; Fig. 2).

CD1 genotype, protein expression, and response to intervention

None of the *CD1* genotypes was associated with response to induction therapy ($P = 0.19$, Fisher's exact test); similarly, neither the degree nor the level of CD1 expression at baseline was predictive of histologic response ($P = 0.61$ for both LI and WMI, Wilcoxon rank sum test).

Table 4. Selected adverse events by participants—induction phase ($n = 27$)

Toxicity	Grade (no. participants)			
	1	2	3	4
Cheilitis	6	19	2	0
Dry skin	13	14	0	0
Conjunctivitis	8	14	0	0
Epistaxis	11	0	0	0
Hair changes	5	1	0	0
Hypertriglyceridemia	11	9	5	1
Pancreatitis	0	0	1	0
Fatigue	7	17	2	0
Myalgia	11	10	2	0
Arthralgia	9	9	6	0
Injection site reaction	17	3	0	0
Fever	13	2	0	0
Chills	13	4	0	0
Headache	8	2	1	0
Dizziness	3	2	1	0
Depression	3	3	0	0
Memory loss	11	5	0	0
Alopecia	16	1	0	0
Hyperglycemia	9	5	2	0
Leukopenia	12	3	0	0
Granulocytopenia	6	7	2	0
Anemia	10	1	0	0
Diarrhea	5	3	1	0
Nausea	12	2	0	0
Anorexia	13	4	0	0
Elevated alkaline phosphatase	8	1	0	0
Elevated alanine aminotransferase	8	1	0	0

CD1 genotype, protein expression, and cancer development

In univariate analysis, the CD1 AA/AG genotype was a significant predictor for shorter cancer-free survival compared with the GG group (hazard ratio, 7.21; 95% CI, 1.00-52.06; $P = 0.05$). As shown in Fig. 3A, the median time to cancer for patients with the AA or AG genotype was 1.84 years, whereas the median for the GG genotype has not been reached. High level of nuclear CD1 expression at baseline (cutoff value of 0.41; i.e., baseline CD1 WMI mean) was similarly a significant predictor of shorter cancer-free survival (hazard ratio, 4.58; 95% CI, 1.10-19.14; $P = 0.04$) in univariate analysis. The median time to cancer for patients with baseline CD1 WMI ≥0.41 was 1.45 years, whereas it was not reached for patients with baseline CD1 WMI <0.41 (Fig. 3B). Notably, high CD1 expression was also associated with worse outcome within each genotype group (Fig. 3C). In the AA/AG high-cancer risk group, six of the seven (85.7%) patients with baseline CD1 WMI ≥0.41 developed cancer compared with three of the eight (37.5%) patients with <0.41. Similarly, in the GG group, one of the two (50%) patients with baseline CD1 WMI ≥0.41 developed cancer compared with none with CD1 WMI <0.41.

In a multicovariate analysis evaluating the effect of both CD1 genotype and baseline protein expression on time to cancer, similar to the univariate analysis, trends were observed; the hazard ratio for the level of CD1 nuclear expression ≥0.41 was 3.51 (95% CI, 0.78-15.83; $P = 0.10$) and for CD1 AA/AG genotype was 5.54 (95% CI, 0.83-37.09; $P = 0.08$).

Discussion

The overall 30% response rate (regression of laryngeal lesions) during induction was substantially lower than that of the laryngeal lesions in our earlier trial of this regimen (64% at 12 months; ref. 13). This difference may reflect the exclusion of approximately one third of the patients screened for the study because their gastroesophageal reflux improved with anti-reflux measures and medications. In fact, several observational studies have described reversal of carcinoma *in situ* with treatment of reflux (25, 26) as well as regression of premalignant changes and decreased incidence of cancer recurrences after initiating H2 receptor antagonists (27), and a meta-analysis suggests that GERD may be a factor in the pathogenesis of laryngeal cancer (28). Our previous trial could have been biased by including patients with lesions responsive to gastroesophageal reflux control alone. Therefore, future trials in laryngeal premalignancy patients should control for a concurrent diagnosis and/or treatment of gastroesophageal reflux.

Cancers developed in 37% of the patients of the present study, close to the expected (historical) incidence of cancer in this setting (27, 29–31). Half of the cancers occurred during induction and half occurred after, suggesting little or no effect of induction or maintenance on laryngeal cancer development in these patients. In sum, the two-phase regimen did

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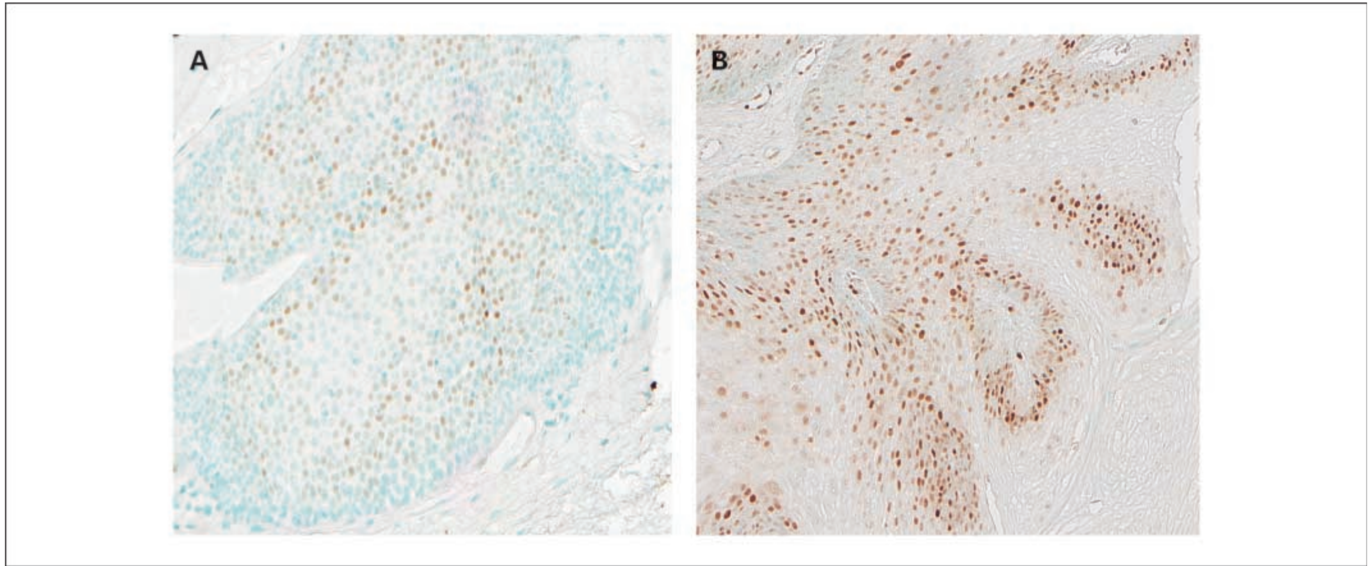

Fig. 2. CD1 protein expression detected by immunohistochemistry. *A,* GG genotype: low nuclear expression. *B,* AG genotype: high nuclear expression.

not prevent laryngeal cancer despite objective responses of premalignant laryngeal lesions. This result contrasts with results of a phase II trial of combined 13-cRA, IFN-α, and α-tocopherol to prevent new cancer in curatively treated stage III to IV head and neck squamous cell carcinoma patients (32); the regimen produced remarkable 5-year rates of overall survival (79%) and disease-free survival (80%) confirmed by longer-term follow-up (33).

We believe that the present findings with the greatest implications for head and neck cancer prevention involve CD1 genotype and protein expression. The current study confirmed the increased risk of cancer in patients with the *CD1* AA and AG genotype (versus the GG genotype) detected in the earlier study but also added important new findings. Baseline expression of CD1 protein correlated with cancer risk within both the unfavorable and favorable *CD1* genotypes. Interestingly, neither the level of CD1 protein expression nor the genotype correlated with lesion response. This finding raises again the issue of the validity of premalignant lesion response as a surrogate for cancer development as has been recently4 observed in a large chemoprevention trial in oral premalignancy (34).

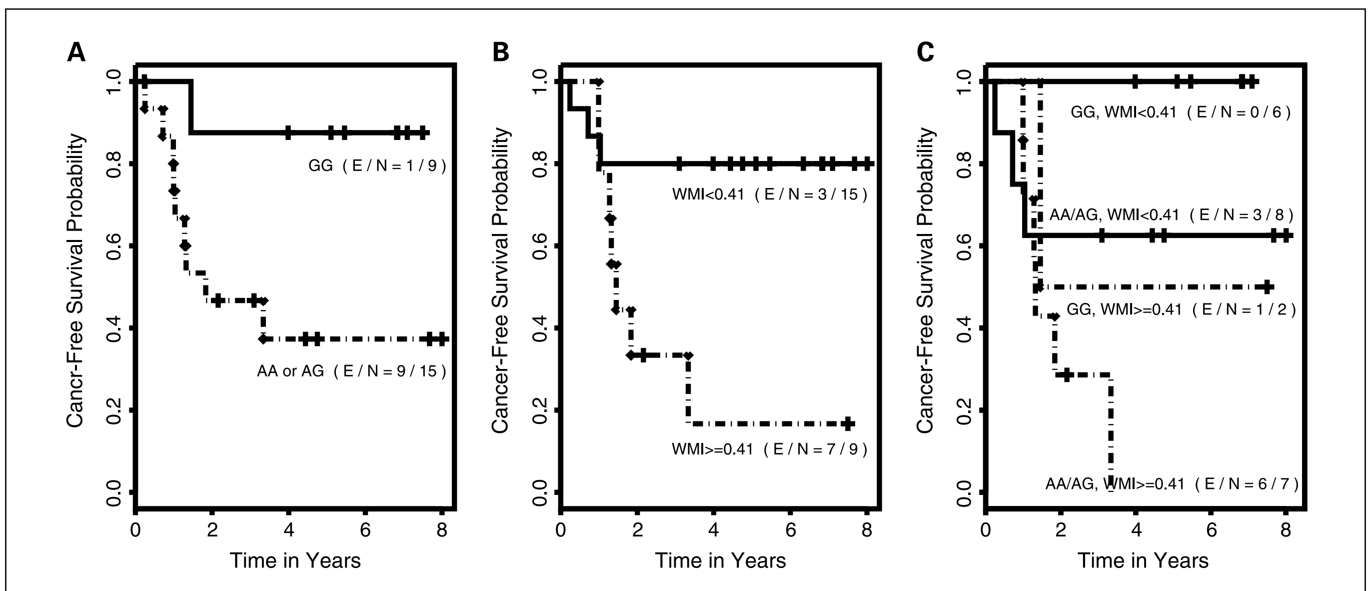

Fig. 3. *A,* cancer-free survival by *CD1* 870A genotype. The difference between the groups AA or AG versus GG was tested by Wald test ($P = 0.05$). E, number of events; N, total number of patients per arm. *B,* cancer-free survival by CD1 protein expression using WMI cutoff of 0.41 ($P = 0.04$, Wald test). *C,* cancer-free survival by CD1 G870A genotype stratified by CD1 protein expression using WMI cutoff of 0.41 (log-rank test comparing CD1 ≥0.41 versus <0.41; $P = 0.08$, for GG group; $P = 0.17$, for AA/AG group; $P = 0.04$, overall log-rank test).

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The *CD1* G/A870 polymorphism is functionally important because it is occurring at a splicing donor site, resulting in two different splice variant transcripts: CD1a and CD1b. CD1a encodes for the full-length native form of the CD1 protein, which has a short nuclear half-life and is tightly regulated by phosphorylation of residues in exon 5, following which it undergoes nuclear export and ubiquitination. CD1b encodes for a truncated alternate CD1 protein, which lacks exon 5 and has a longer nuclear half-life (14). Although the mechanisms underlying the regulation of splicing at the 870 site are not fully characterized, the A870 allele impairs the normal splicing and enhances the production of CD1b, which seems to hold oncogenic properties (14). At the time of the present study, there were no specific antibodies to CD1b. Therefore, we used an antibody that detects both CD1 forms and presented our data in two different ways, LI and WMI, the latter reflecting more accurately the levels of protein expression. As in our previous report (15), the A870 (i.e., AA and AG genotypes) background was associated with a significantly shorter cancer-free survival. The novel finding of the present study, however, is the prognostic significance of high baseline CD1 expression, which seems to have the strongest association with an increased risk of cancer and affects both *CD1* genotype groups. Although limited by a small sample size, our results suggest that baseline nuclear CD1 levels may be useful in identifying the shortest cancer-free survival within high-risk patients having *CD1* AA or AG genotypes. A recent case-control study also found that *CD1* genotype correlated with head and neck cancer risk (35). Validation of the findings in a larger cohort and using an antibody detecting the oncogenic CD1b is certainly warranted.

In conclusion, the combination of 13-cRA, α-IFN, and α-tocopherol had modest activity in advanced laryngeal premalignancy. Our current CD1 findings add to our understanding of the biology of head and neck carcinogenesis and cancer risk and should be integrated along with other high-risk molecular characteristics [e.g., loss of heterozygosity at 9p and 3p (36) and chromosome instability (37)] in the screening of patients suitable for intervention. We believe that the present results warrant future molecular-targeted prevention trials of an agent or combination that can target upstream regulators of CD1 transcription and protein expression in patients selected for a high risk of head and neck cancer based on their CD1 genotype and protein profile. With the personalizing aspects of high risk (38) and medicine targeting a component of this risk, such a trial promises to advance personalized cancer prevention in the head and neck.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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