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A Th1/IFNγ Gene Signature Is Prognostic in the Adjuvant Setting of Resectable High-Risk Melanoma but Not in Non–Small Cell Lung Cancer

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ABSTRACT

Purpose: Immune components of the tumor microenvironment (TME) have been associated with disease outcome. We prospectively evaluated the association of an immune-related gene signature (GS) with clinical outcome in melanoma and non–small cell lung cancer (NSCLC) tumor samples from two phase III studies.

Experimental Design: The GS was prospectively validated using an adaptive signature design to optimize it for the sample type and technology used in phase III studies. One-third of the samples were used as "training set"; the remaining two thirds, constituting the "test set," were used for the prospective validation of the GS.

Results: In the melanoma training set, the expression level of eight Th1/IFNγ-related genes in tumor-positive lymph node tissue predicted the duration of disease-free survival (DFS) and overall survival (OS) in the placebo arm. This GS was prospectively and independently validated as prognostic in the test set. Building a multivariate Cox model in the test set placebo patients from clinical covariates and the GS score, an increased number of melanoma-involved lymph nodes and the GS were associated with DFS and OS. This GS was not associated with DFS in NSCLC, although expression of the Th1/IFNγ-related genes was associated with the presence of lymphocytes in tumor samples in both indications.

Conclusions: These findings provide evidence that expression of *Th1/IFNγ* genes in the TME, as measured by this GS, is associated with clinical outcome in melanoma. This suggests that, using this GS, patients with stage IIIB/C melanoma can be classified into different risk groups.

Introduction

Immuno-oncology drugs have become part of the standard therapy for many tumor types including melanoma and non–small cell lung cancer (NSCLC). Blocking antibodies against CTL-associated antigen 4 (CTLA-4) and programmed cell death protein-1 (PD-1) or its ligand (PD-L1) have been approved for treatment of melanoma and NSCLC [nivolumab, pembrolizumab, atezolizumab (NSCLC; refs. 1–4)], among other indications. However, clinical responses and survival benefit have been observed in only a subset of patients (3, 5, 6), highlighting the need for biomarkers that can predict the likelihood of patients to respond to a given immunotherapy. Furthermore, identification of prognostic biomarkers informative of the patient's overall cancer outcome regardless of therapy would guide selection of patients for adjuvant systemic treatment.

The tumor microenvironment (TME) is composed of multiple cellular components, including cells of the immune system (7). The immune component of the TME plays a key role in clinical outcome ("natural"/prognostic or in response to treatments/predictive) in many tumor types (7–9).

Among the immune cells within TME, tumor-infiltrating lymphocytes (TIL) such as cytotoxic $CD8^+$ memory T cells ($CD8^+CD45RO^+$), and $CD4^+$ Th1 cells producing IL2 and IFNγ, have been shown to correlate with improved prognosis in terms of disease-free survival (DFS) and overall survival (OS) in various cancer types (7, 8, 10).

Gene expression profiling has been used to establish molecular gene signatures (GS) for classifying subtypes of primary tumors and predicting clinical outcome of multiple cancers including melanoma and lung cancer (11–14). An 84-gene Th1/IFNγ GS was identified in a previous study (15) using gene expression profiling of metastatic melanoma and NSCLC samples from two phase II studies: MEL-PhII and NSCLC-PhII, respectively (16, 17). The GS was associated with clinical benefit following immunization with MAGE-A3 antigen combined with the GSK proprietary immunostimulant AS15 (MAGE-A3 immunotherapeutic). Of note, given the absence of a control group

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Translational Relevance

Immune features of the tumor microenvironment have been shown to be associated with the natural course of disease (prognostic) in different cancer settings, mostly in retrospective studies using archived tumor samples. Here, we report the prospective validation of a prespecified Th1/IFNγ gene signature (GS) as prognostic using an adaptive signature design in the adjuvant setting of resectable high-risk melanoma; of note, the same GS was not prognostic in non–small cell lung cancer (NSCLC). This GS identifies, independently of known prognostic factors, patients with stage IIIB/C melanoma who were previously considered a homogeneous high-risk population and can now be classified into different risk groups, which potentially benefit from different treatments. The lack of association with clinical outcome in the nontreated arm of the study in the NSCLC adjuvant setting suggests differences in the capacity of a natural immune response to control tumor in different disease settings.

in MEL-PhII, the prognostic value could not be established (16). In NSCLC-PhII, the GS appeared to be predictive of the treatment effect, without a strong prognostic effect (17).

Here, we report the results of exploratory analyses prospectively evaluating the association of a Th1/IFNγ GS with clinical outcome in melanoma and NSCLC tumor samples from the phase III studies DERMA (18) and MAGRIT (19), respectively.

Materials and Methods

Study design and participants

The DERMA study (ClinicalTrials.gov NCT00796445; ref. 18) was a double-blind, randomized, placebo-controlled phase III study that included adult patients with histologically proven, resected stage IIIB–C cutaneous MAGE-A3–positive melanoma with macroscopic lymph node involvement defined according to the TNM staging system and AJCC classification (sixth edition). In this study, the stage III T_undefined is composed of the patients for whom the primary tumor was not identified, and of the patients with known primary tumor location but with unknown Breslow thickness and/or ulceration. Macroscopic lymph node involvement was defined as clinically detectable lymph node metastases confirmed by pathologic examination following therapeutic lymphadenectomy. Patients' lymph node metastases had to show expression of the *MAGE-A3* gene per quantitative MAGE-A3 gene expression determined by RT-PCR analysis on formalin-fixed paraffin-embedded (FFPE) tissue. A total of 1,345 patients received a maximum of 13 doses of MAGE-A3 immunotherapeutic (893 patients) or placebo (452 patients) over a 27-month period: 5 doses at 3-weekly intervals, followed by 8 doses at 12-weekly intervals.

The MAGRIT study (ClinicalTrials.gov NCT00480025; ref. 19) was a randomized, double-blind, placebo-controlled phase III trial that included adult patients with histologically proven, completely resected stage IB, II, or IIIA, MAGE-A3–positive NSCLC defined according to the sixth edition of the TNM staging system, and with mediastinal lymph node removal (the extent of lymph node resection being left up to standard of care), either directly after surgery or after surgery and adjuvant chemotherapy. A total of 2,312 patients received a maximum of 13 doses of MAGE-A3 immunotherapeutic (1,515 patients) or placebo (757 patients) during 27 months.

The demographic and disease characteristics of the participants from the training and test sets of the DERMA and MAGRIT studies are provided in Supplementary Tables S1 and S2, respectively; no imbalance between training and test sets were observed for the different covariates.

In the respective studies, all patients gave informed consent for study participation. Both studies included the assessment of DFS in the overall population and prospective validation of a potentially predictive GS as coprimary objectives. Clinical data in this report originate from the final analysis of each study, conducted in 2013–2014 after a median follow-up of 28 months (DERMA) or 39 months (MAGRIT). Both studies were conducted according to the ethical guidelines [Good Clinical Practice, the Declaration of Helsinki, US FDA Code of Federal Regulations [title 21 part 50 and 56), and all applicable regulatory requirements; refs. 18, 19]. Both protocols were approved by national, regional, or investigational center institutional review boards or ethics committees. Each study was monitored by an independent data monitoring committee (IDMC) that reviewed study endpoints and safety data. The GS testing in both studies was done as part of the GS coprimary efficacy endpoints (18, 19). The adjustment of the efficacy analysis with the prognostic GS requiring testing of the prognostic GS in the DERMA test set samples was included in an amendment to the protocol and was approved by the ethics committees. Anonymized individual participant data and study documents can be requested for further research from www.clinicalstudydatarequest.com.

Because of the change in sample type (fresh-frozen to FFPE samples) and technology (microarrays to qRT-PCR) for measuring gene expression between the phase II and phase III studies, requiring optimization of the initial GS, the prospective clinical validation of the GS was performed using the "split-sample approach" as previously described in the adaptive signature design (ASD; refs. 20, 21). The GS classifier was fine-tuned on samples collected from one-third of the available patients ("training set"). Samples collected from the remaining two-thirds of patients constituted the "test set," allowing for the prospective clinical validation of the elaborated classifier. The GS assay was performed on the same tumor samples used for MAGE-A3 expression testing. Details on the ASD in these studies as well as description of tumor samples, RNA purification, GS genes, and qRT-PCR assay are described in a separate communication (18).

Data analysis: global gene expression patterns and classifier definition in the training set

The full GS assay detected the expression of 55 genes (for one gene, immunoglobin kappa constant, results were not valid in NSCLC samples). However, only eight of these genes were used in the GS described in this study. These eight genes were selected on the basis of the melanoma phase II study gene expression data (15), as the classifier representative of the Th1/IFNγ pathway. The classifier score is the average of these gene expression values after scaling (z-score) based on parameters from independent cohorts of melanoma-involved lymph nodes and primary NSCLC samples to account for differences in tissue type (fresh-frozen vs. FFPE samples). The Th1/IFNγ GS classifier score is the average of the negative normalized mRNA expression level of genes determined using qRT-PCR. The threshold originally set up for this classifier as potentially predictive to MAGE-A3 immunotherapeutic was kept. Thus, the GS based on these predefined parameters was first applied to the training set and then to the test set of the phase III studies. All classifier parameters are provided in the Supplementary Materials and Supplementary Table S3. The data analyses were performed using R version 2.15.3.

Histopathologic evaluation

Histopathologic evaluation of TILs was done on tumor tissue sections stained with hematoxylin and eosin. The intratumoral area was scribed by the pathologist for tissue microdissection for GS expression testing. This area was defined as a tumor area with at least 50% neoplastic cell content. Each sample was evaluated by one of three pathologists as "No," "Low," "Moderate," or "Abundant" infiltration based on the immune cell content. This scoring system was agreed on and standardized among the three pathologists by examining a subset of the samples. The pathologists were blinded to the gene expression results.

The association between GS score and the degree of TIL was assessed by linear regression models where the outcome is the prognostic score and the predictor is the degree of tumor immune infiltration (modeled as integer).

Statistical analysis

In the MAGRIT and DERMA studies, nonparametric estimates of median time-to-event endpoints were generated using Kaplan–Meier methodology with confidence intervals (CI) calculated using the Brookmeyer and Crowley method (22). Estimates of the HRs were obtained by Cox proportional regression modeling, with two-sided *P* values originating from the Wald test.

In the DERMA study, exploratory univariate and multivariate Cox models were developed on the placebo patients from the test set to determine factors associated with DFS and OS and validate the prognostic classifier. Starting from the significant variables (at level 0.05) in the univariate analysis, multivariate models were built using either a stepwise approach (only significant variables were kept in the final models) or a full model (including all variables significant at the univariate level). Simple forward and backward selection procedures were also tested to check for consistency. Two-sided *P* values originated from the Wald test.

The statistical analyses were performed using SAS version 9.2.

Results

Of the 366 patients allocated to the DERMA training set, valid results for the gene expression testing were obtained for 357 patients (**Fig. 1A**; ref. 18). Of note, 356 patients were analyzed because of exclusion of 1 patient with invalid informed consent. The stage III T_undefined was composed of approximately 80% of patients for whom the primary tumor was not identified and of approximately 20% of patients with known primary tumor location but unknown Breslow thickness and/or ulceration (data not shown).

On the basis of the biological pathways and correlation among genes observed in the MEL-PhII study with MAGE-A3 immunotherapeutic, we selected eight genes (*CCL4, CCL5, CXCL10, CXCL9, GBP4, GBP5, PSMB10, TAP1*) as hallmarks of the Th1/IFNγ response and features of potential predictive classifier to treatment response to MAGE-A3 immunotherapeutic. The Th1-/IFNγ GS classifier score was defined as the average of the negative normalized mRNA expression level of these eight genes. Thus, the Th1/IFNγ GS score is inversely correlated with the expression of the eight genes in the classifier. Using the prespecified cutoff aimed at showing a predictive effect, this classifier yielded 139/356 (39%) Th1-/IFNγ GS^+, below the threshold, that is, high expression of the *Th1/IFNγ* genes. **Figure 2** shows the heatmap of the eight genes expression in the samples in the DERMA training set ordered by prognostic score; as expected, the expression pattern of these genes is highly correlated.

The classifier was highly prognostic with little or no predictive effect in the training set: higher expression (lower GS score) of the immune-related genes implies a better outcome, independent of treatment. The median DFS in placebo GS^+ and GS^- patients were 44.2 and 5.5 months, respectively (**Fig. 3A**). The median OS in placebo GS^+ and GS^- patients were "not reached" and 30.4 months, respectively (**Fig. 3B**).

Given the lack of predictive effect and the unexpected strong prognostic effect observed for this GS in the training set, we sought to further validate its prognostic value in the placebo arm of the DERMA study test set (**Fig. 1B**) as a *post hoc* exploratory analysis. Applying the prespecified classifier and cutoff to the test set yielded 106/242 patients (44%) GS^+ in the placebo arm of the test set. In the univariate analysis, the DFS HR for the GS^- versus GS^+ patients was 2.37 (95% CI, 1.68–3.37; $P < 0.0001$; **Fig. 4A**), whereas the OS HR was 2.17 (1.37–3.45; $P = 0.0010$; **Fig. 4B**). The median DFS in the GS^- population was 5.6 months (95% CI, 3.3–11.1) and it was "not reached" for the GS^+ patients (16.6–not reached). Of note, as observed in the training set, the Th1/IFNγ GS was also associated with clinical outcome in the MAGE-A3 immunotherapeutic-treated arm (Supplementary Fig. S1).

To assess (i) the additional information brought by the GS in presence of other known clinical covariates and (ii) the prognostic effect across the range of GS score values (e.g., without cutoff), the GS score was used as a continuous variable. Patients with stage IIIA and IV tumors, representing only very few patients (one and ten, respectively) and even though ineligible stages, were included in this analysis as they were part of the primary analysis (intent-to-treat population; ref. 18). The results of the univariate analysis for all covariates examined in the test set are shown in Supplementary Table S4. Building a multivariate Cox model using a stepwise approach in the test set "placebo patients" from clinical covariates based on the seven baseline variables, which were significant (level 0.05) at the univariate level, an increased number of melanoma-involved lymph nodes and a higher GS score as continuous variable were associated with reduced DFS; the same variables were associated with OS (**Table 1**). The full model including all variables proving significant at the univariate level is presented in Supplementary Table S5.

The pooling of categories of lymph node involvement and GS status elicit similar prognoses allowing definition of three risk groups: (i) one or two melanoma-involved lymph nodes, GS^+ (low risk), (ii) three or more lymph nodes melanoma-involved/matted nodes and GS^+ or one or two melanoma-involved lymph nodes and GS^- (medium risk), and (iii) three or more melanoma-involved lymph nodes/matted nodes and GS^- (high risk). Kaplan–Meier curves for DFS and OS by risk group are shown in **Fig. 5**.

The outcome differences between the GS^+ and GS^- subpopulations were observed regardless of the number of melanoma-involved lymph nodes, providing additional information on the patient's clinical outcome on top of known prognostic clinical covariates including number of melanoma-involved lymph nodes (Supplementary Fig. S2).

We also applied the prespecified Th1/IFNγ prognostic GS (eight-gene classifier) to the placebo arm of the MAGRIT training set (**Fig. 1C**). This classifier yielded 75/205 patients (37%) in the MAGRIT training set placebo arm classified as GS^+ (higher expression of immune genes). The DFS HR for the GS^- patients versus GS^+ patients was 0.91 (95% CI, 0.58–1.44; *P*: 0.69) whereas the OS HR was 0.67 (0.38–1.21; *P*: 0.18; **Fig. 6**), indicating that the eight-gene GS was not prognostic in NSCLC. Given these results and the termination of the MAGRIT study due to lack of clinical efficacy, the classifier was not further applied to the MAGRIT test set.

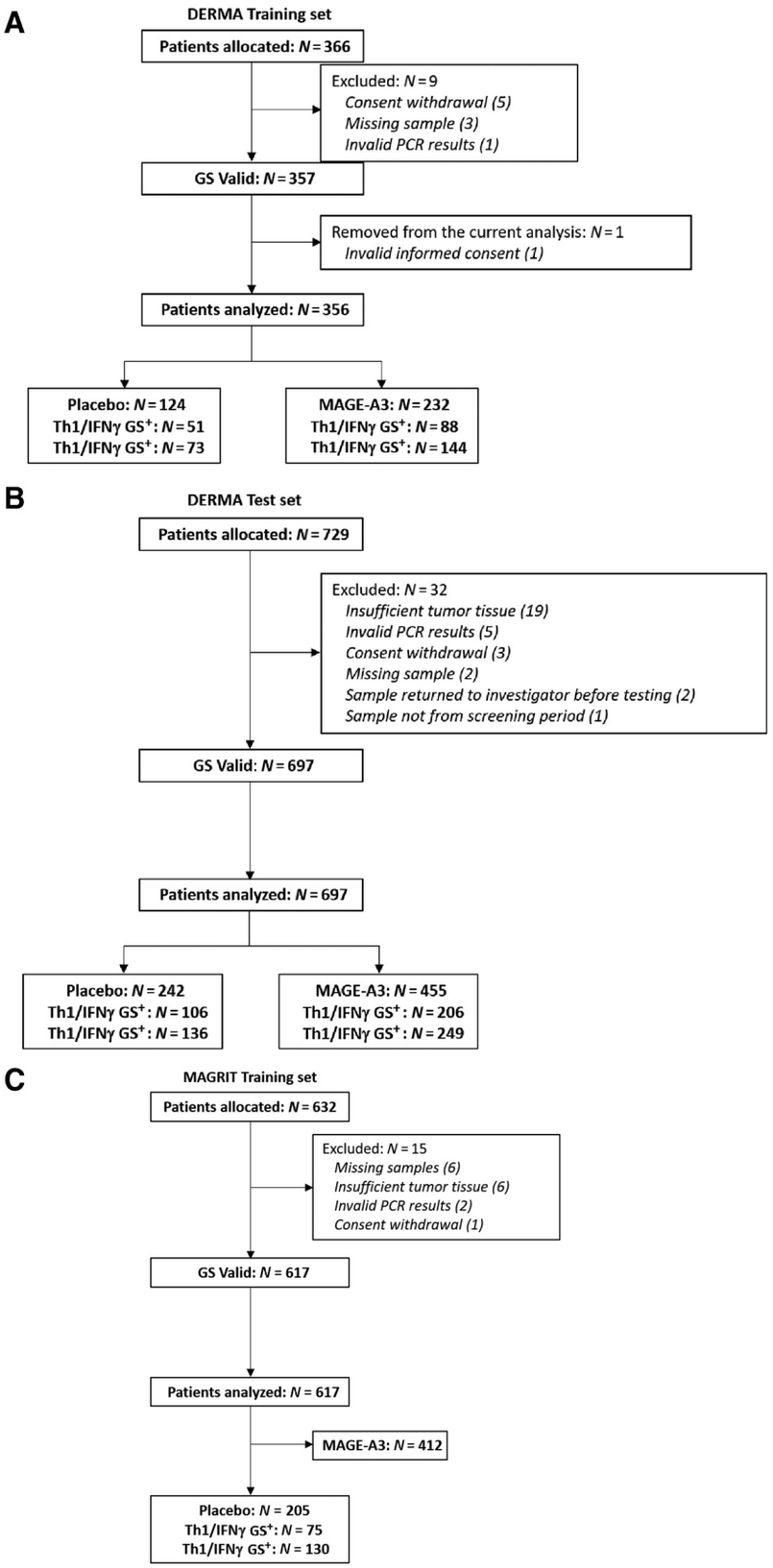

Figure 1.
Flow diagram of the patient samples obtained from two randomized clinical trials: DERMA training set (**A**) and test set (**B**) and MAGRIT training set (**C**). N, number of patients; PCR, polymerase chain reaction; GS, gene signature.

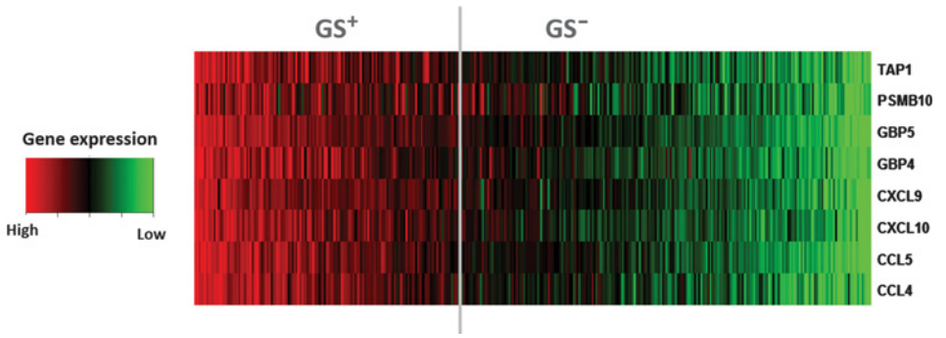

Figure 2.
Heatmap of the eight genes expressed in the DERMA training set, ordered by prognostic score. GS, gene signature.

We then investigated the association of the GS with immune histopathologic features of the tumor samples (shown in Supplementary Fig. S3). The degree of TILs was associated with the prognostic score defined by the expression of *Th1/IFNγ* genes in both melanoma and NSCLC datasets as shown in Supplementary Fig. S4.

Discussion

The potentially predictive eight-gene Th1/IFNγ GS based on the findings from the phase II studies with MAGE-A3 immunotherapeutic was not associated with treatment effect in the phase III studies DERMA and MAGRIT. The lack-of-treatment effect in the overall population in these studies [DFS HR = 1.01 (95% CI, 0.88–1.17, $P = 0.86$) and 1.02 (95% CI, 0.89–1.18, $P = 0.74$) in DERMA and MAGRIT, respectively] demonstrated that very few, if any, patients with melanoma and NSCLC benefited from this treatment (18, 19); thus, validation of the GS to identify a subpopulation in which a statistically significant treatment effect could be demonstrated was not achieved. Unexpectedly and despite the relatively short follow-up period (28 months), which is one of the DERMA limitations, we found a strong association of the Th1/IFNγ GS with clinical outcome in the placebo arm of the training set in the DERMA study, which was prospectively validated in the test set of this study. To our knowledge, this is the first prospective validation of a GS in randomized phase III studies using a completely predefined assay and GS classifier. The multivariate analyses (stepwise and full model) showed that the Th1/IFNγ GS provides information on the patient's clinical outcome additionally to the known prognostic clinical covariates including the number of melanoma-involved lymph nodes. Of note, different to the stepwise model, the number of lymph node was not significant in the full model, this is probably due to interactions between the variables number of lymph node, stage, and N category, which are likely correlated to a large extent. This observation further emphasizes the prognostic importance of the GS prognostic score. Furthermore, we have confirmed the association of the Th1/IFNγ GS with TILs. These findings support the hypothesis that a Th1/IFNγ–immune TME is associated with clinical outcome of melanoma and add to the body of evidence that the immune context of the TME can be associated with tumor progression (7, 8, 10).

TILs and their functional orientation have been associated with disease outcome in different tumor types. In settings where immune infiltration was associated with clinical outcome, upregulation of IFNγ signaling through STAT-1/IRF-1 transcription factors and expression of Th1 chemokines has been described previously (8). High levels of certain chemokines and adhesion molecules' mRNA in tumors have also been associated with prolonged DFS (23). Furthermore, the distribution of T-cell infiltrates is not homogenous within the tumor, with zones of more or less dense infiltrations, and varies also by tumor type (7, 10). Understanding the mechanisms by which a favorable immune contexture might be created and maintained is essential for guiding therapies and rational treatment combinations.

One limitation of the DERMA study was the lack of information on primary tumor (such as nonidentified; T_undefined, location, Breslow thickness, ulceration) for 10% of the patients in the test set placebo patients. These patients were included in the stage III T_undefined group for analysis purposes. In addition, a small number of patients with stage IIIA and IV tumors, although ineligible but wrongly randomized, were grouped and included in this analysis as they were part of the primary analysis (intent-to-treat population; ref. 18). Thus, further validation of the findings in a more standardized population might be needed. Given that the DERMA study included only patients with MAGE-A3–positive melanoma, further studies will be needed to assess the association of this GS with the outcome in other patient populations; however, previous studies have suggested association of this type of GS and TILs with clinical outcome in nonselected patients with melanoma. These studies were performed retrospectively in analyses from samples in tumor banks (12, 14, 24, 25). In one of these studies, a 53-gene immune GS was associated with disease-specific survival and recurrence-free survival in stage II–III resected melanoma (14). Network analysis showed that this gene set is related to the Th1 signaling pathways. Another study reported a 46-gene GS with strong overexpression of immune response genes predictive of better survival in patients with resectable macroscopic stage III melanoma (12).

These prognostic immune GSs reported in the literature were derived from samples at different stages or locations of the disease, for example, primary (14) or as in this study, nodal metastases (12). These observations, together with the DERMA study results reported in this manuscript, suggest that in melanoma, immune TME features associated with prognosis might be detectable early in the course of disease and be preserved in lymph node metastasis.

In contrast, the same eight-gene Th1/IFNγ GS shown to be prognostic in the adjuvant setting of metastatic melanoma was not associated with clinical outcome in NSCLC among treated or nontreated arms in the MAGRIT training set, despite its association with TILs. Similarly, the lack of prognostic effect was previously observed in the NSCLC-PhII study (15). Tertiary lymphoid structures [identified by mature dendritic cells (mDC)] and TILs have been reported as prognostic in NSCLC (26). The presence of mDCs showed stronger association with prognosis than $CD8^+$ T cells, and mDCs presence still showed difference in survival even in $CD8^+$-high tumors (27). Compared with the Th1/IFNγ GS, it is possible that IHC detection of mDCs assesses more functional characteristics of the tumor immune infiltration or that it better quantifies immune infiltration, especially in lung tissue, which

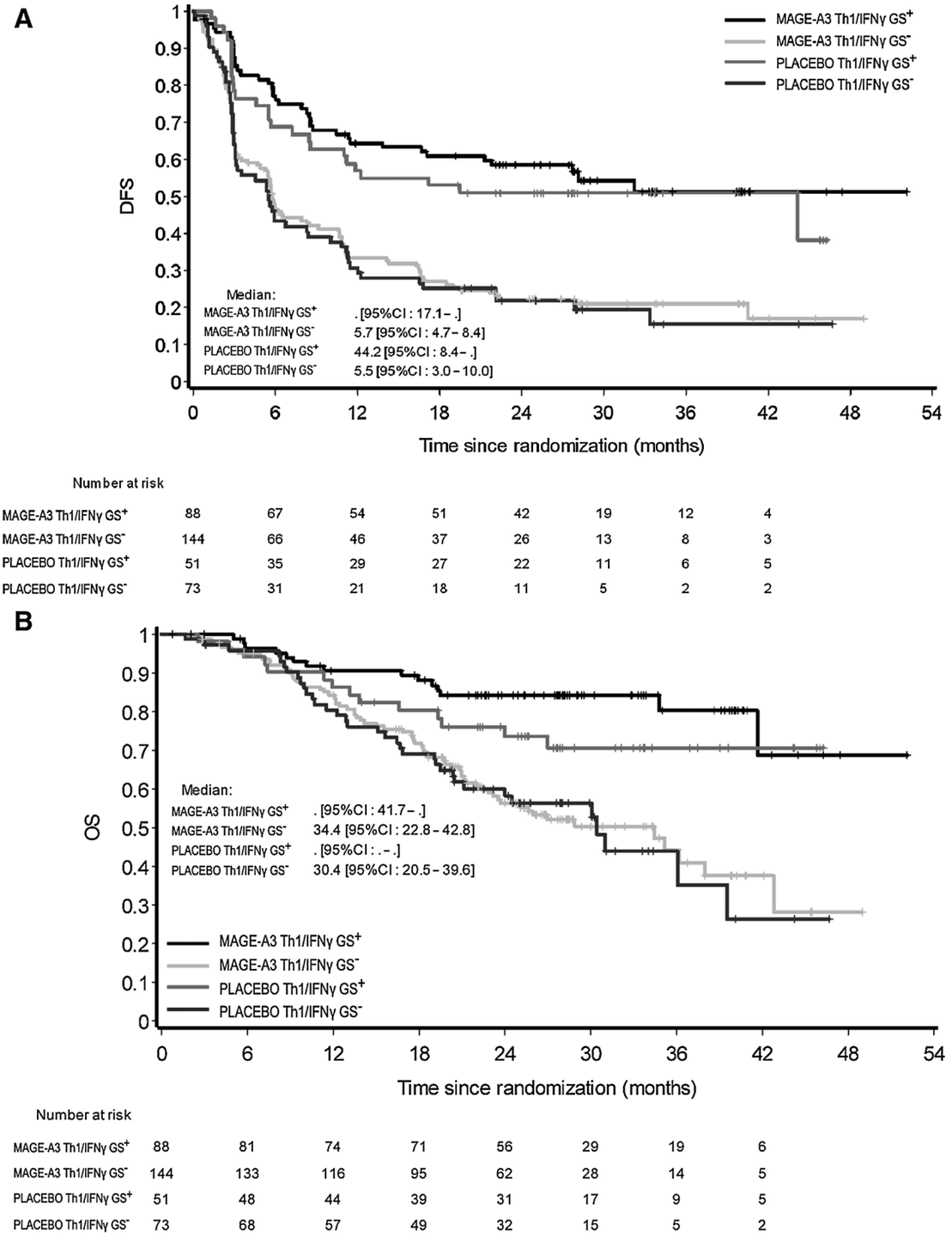

Figure 3.

DFS (**A**) and OS (**B**) in the DERMA training set with the Th1/IFNγ classifier. MAGE-A3 Th1/IFNγ GS$^+$, patients treated with MAGE-A3 immunotherapeutic, positive for Th1/IFNγ GS. MAGE-A3 Th1/IFNγ GS$^-$, patients treated with MAGE-A3 immunotherapeutic, negative for Th1/IFNγ GS. PLACEBO Th1/IFNγ GS$^+$, patients treated with placebo, positive for Th1/IFNγ GS. PLACEBO Th1/IFNγ GS$^-$, patients treated with placebo, negative for Th1/IFNγ GS. DFS, disease-free survival; OS, overall survival; GS, gene signature; CI, confidence interval.

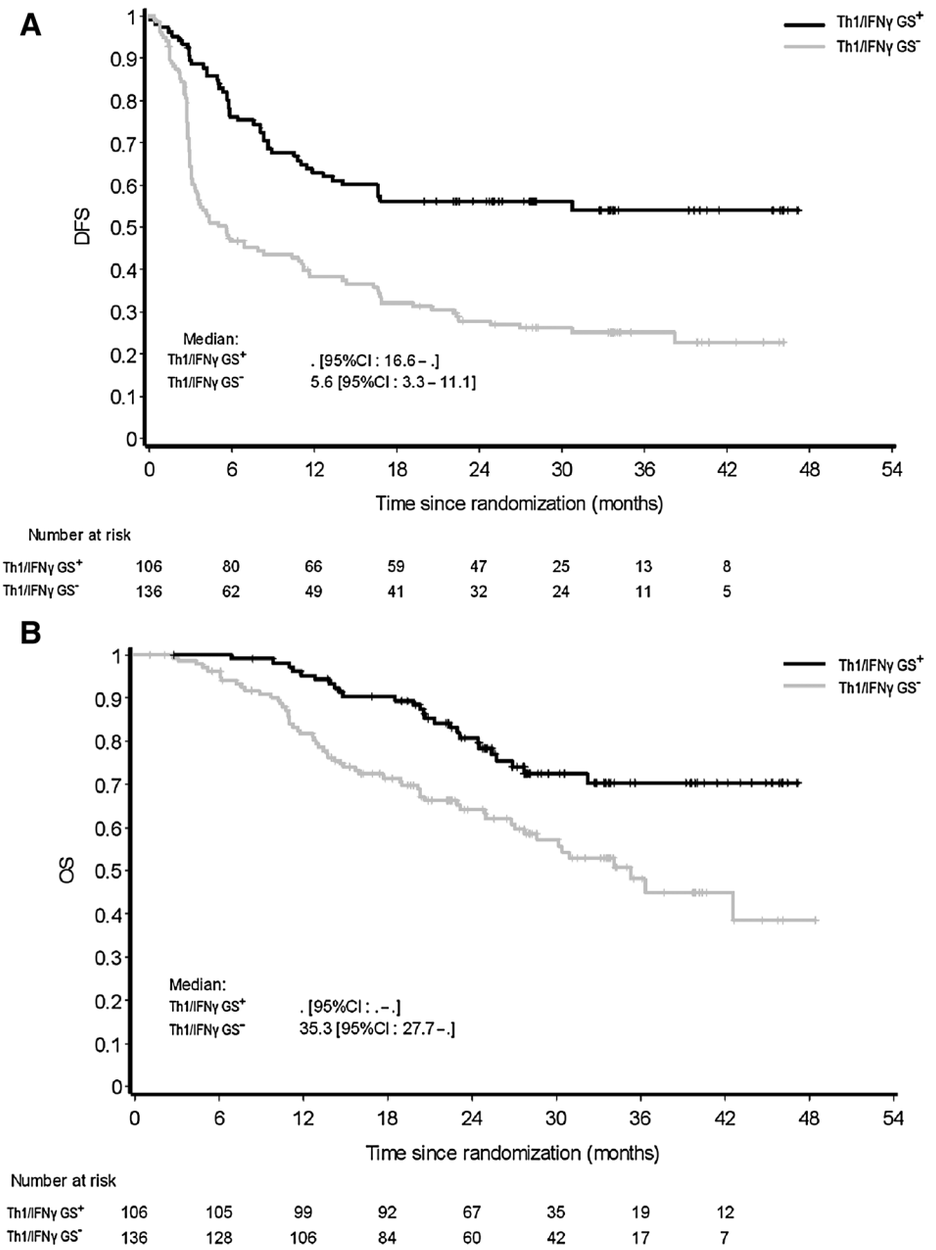

Figure 4.
DFS (**A**) and OS (**B**) in the placebo arm of the DERMA test set with the Th1/IFNγ classifier. Th1/IFNγ GS$^+$, patients positive for Th1/IFNγ GS. Th1/IFNγ GS$^-$, patients negative for Th1/IFNγ GS. DFS, disease-free survival; OS, overall survival; GS, gene signature; CI, confidence interval.

Table 1. Cox model for DFS and OS in the test set placebo patients (stepwise approach) based on baseline variables, which were significant (level 0.05) at the univariate level.

	DFS			OS		
Parameter	**Parameter estimate**	**HR (95% confidence limits)**	***P***	**Parameter estimate**	**HR (95% confidence limits)**	***P***
Number of melanoma-involved lymph nodes (ordered)	0.35212	1.422 (1.250–1.618)	<0.0001	0.28197	1.326 (1.120–1.569)	0.0010
GS prognostic score (continuous)	0.46735	1.596 (1.341–1.899)	<0.0001	0.48714	1.628 (1.283–2.065)	<0.0001

Abbreviations: HR, hazard ratio coming from a Cox regression model, with Efron method to handle ties.
P value, two-sided *P* value from a Wald test.

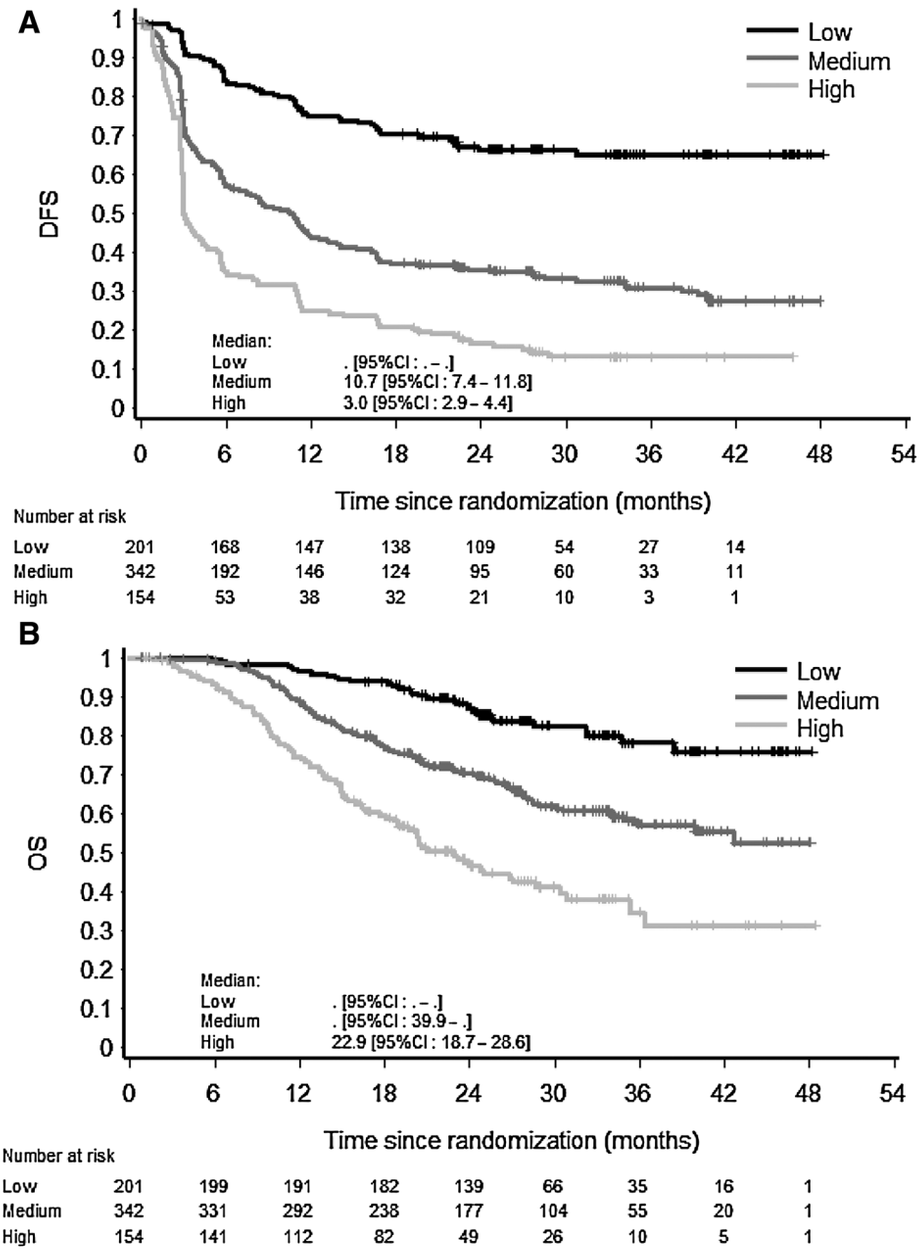

Figure 5.
DFS (**A**) and OS (**B**) in the DERMA test set population by risk group (total treated population). Low, low risk: one or two lymph nodes involved, GS^+. Medium, medium risk: 3 or more lymph nodes involved/matted nodes and GS^+. High, high risk: 3 or more lymph nodes involved/matted nodes and GS^-. DFS, disease-free survival; CI, confidence interval; OS, overall survival.

already contains immune cells. In addition, in the MAGRIT study, the survival of patients with NSCLC on adjuvant therapy was improved compared with the NSCLC-PhII study. The role of mDCs, other immune cells, and GSs in prognosis of survival in cohorts with more recent standard of care remains unclear. In addition, further prospective studies developed under the AJCC 8th edition classification would be of added value and support the validation of the prognostic gene signature. Encouragingly, an attempt to preclassify the patients in the DERMA study using the AJCC 8th edition showed that approximately 80% of the patient population would not have changed stage (data not shown). Importantly, the study population that would have been reclassified was in the stage IIIB/C category. Furthermore, using the number of involved lymph node as variable, instead of stage subgroup, or N category, also limits the effect of reclassification, leading us to expect that the findings would be validated under the most recent classification guidelines.

The strong prognostic effect of the GS, which adds information beyond known clinical covariates, also suggests that this parameter might be considered to adjust for heterogeneity in future clinical studies in this melanoma population. The association of an immune-related GS with outcome of anti–CTLA-4–treated patients has been previously reported (28, 29). For anti–PD-1 check-point blockade, there are reports suggestive of higher response rates in patients with melanoma with the "inflamed" tumor phenotype (30–32). Recently, an IFNγ-related GS identified in metastatic melanoma has been shown to be predictive of anti–PD-1 check-point blockade clinical outcome in different indications (33). These tumors

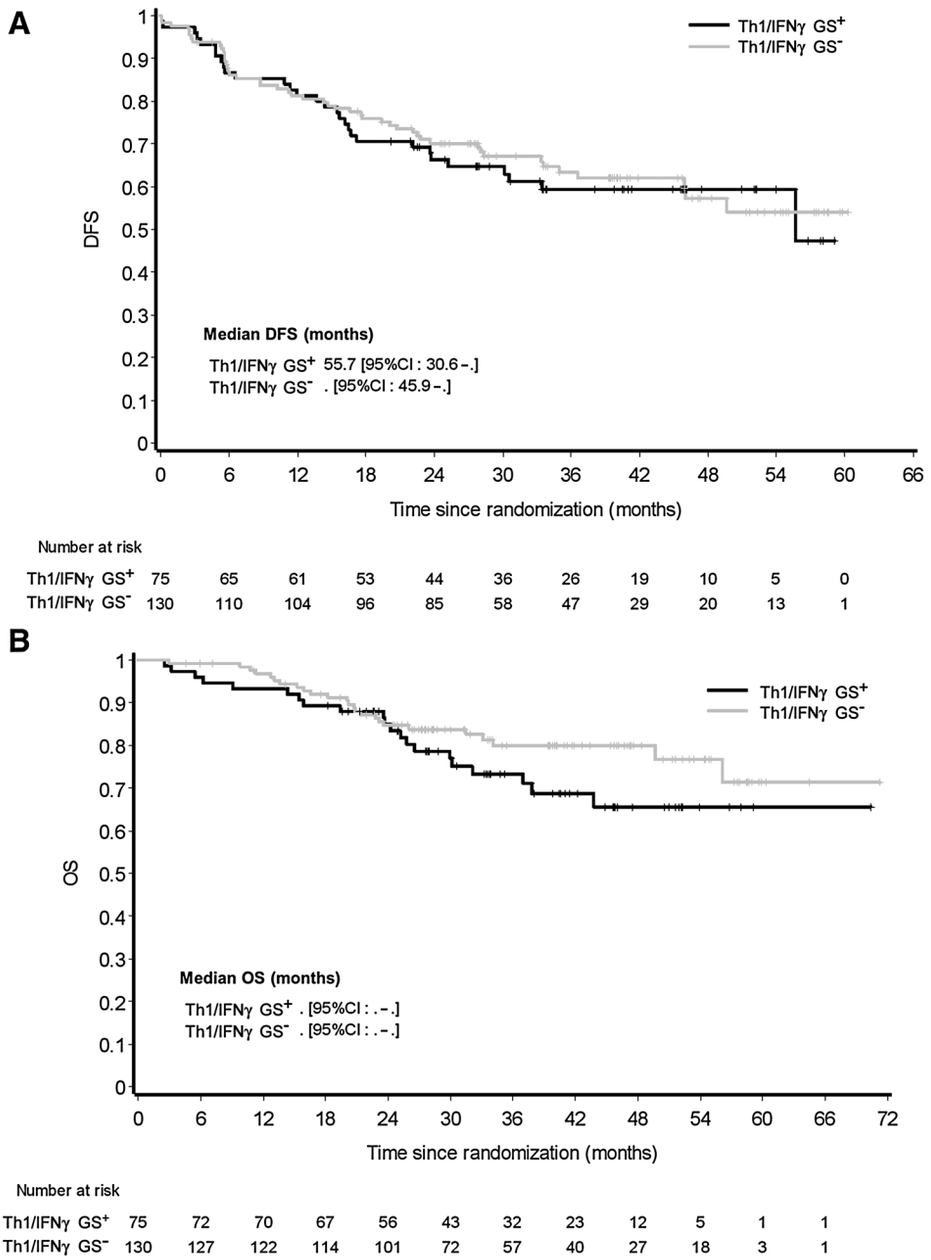

Figure 6.

DFS (**A**) and OS (**B**) in the placebo arm of the MAGRIT training set with the Th1/IFNγ classifier. Th1/IFNγ GS$^+$, patients positive for Th1/IFNγ GS. Th1/IFNγ GS$^-$, patients negative for Th1/IFNγ GS. DFS, disease-free survival; CI, confidence interval; OS, overall survival.

show presence of CD8$^+$ and CD4$^+$ T cells in the tumor parenchyma and mRNA expression of proinflammatory and effector cytokines (30). Not all patients with this phenotype respond, thus, these features seem necessary but not sufficient for treatment response. A similar observation was made with the GS in the previous clinical study with MAGE-A3 immunotherapeutic (15). Of note, the majority of studies assessing the association of immune-related TME features with treatment outcomes were performed in unresectable melanoma in trials without a placebo arm.

The differences in the association between Th1/IFNγ GS signal measured with these eight genes and clinical outcome in two different tumor types (melanoma vs. NSCLC) suggest fundamental differences in the immune response mechanisms elicited by these two solid tumors that both show response to anti–PD-1 interventions. The proportion of tumor subsets with the "immune excluded" phenotype might be different in these solid tumor types; this phenotype is characterized by the presence of abundant immune cells in the stroma surrounding nests of tumor cells (30). It is unclear whether the eight-gene GS described here can discriminate between the "inflamed" and "immune excluded" tumor phenotypes and additional investigation of these findings could provide information for designing combination therapies for each indication and subpopulations within each indication (5).

In summary, we have prospectively validated the association of the Th1/IFNγ GS with prognosis in the adjuvant setting of resectable high-risk melanoma, indicating that this GS identifies patients with stage IIIB/C melanoma who were previously considered a homogeneous high-risk population, and can now be classified into

different risk groups, which potentially benefit from different treatments. The same GS was not associated with clinical outcome in the nontreated arm of the study on NSCLC in the adjuvant setting, even though it was associated with TILs, suggesting differences in the capacity of a natural immune response to control the tumor in different disease settings.

Disclosure of Potential Conflicts of Interest

B. Dizier is an employee/paid consultant for and holds ownership interest (including patents) in the GSK group of companies and UCB Biopharma. A. Callegaro is an employee/paid consultant for and holds ownership interest (including patents) in the GSK group of companies. M. Debois is an employee/paid consultant for and holds ownership interest (including patents) in the GSK group of companies. B. Dreno holds ownership interest (including patents) in the GSK group of companies, Roche, Bristol-Myers Squibb, and Regeneron. H.J. Gogas reports receiving speakers bureau honoraria from MSD, Bristol-Myers Squibb, Novartis, Pierre-Fabre, and Amgen. J.M. Kirkwood is an employee/paid consultant for and holds ownership interest (including patents) in Novartis, Bristol-Myers Squibb, Amgen, Array, Merck, and Immunocore, and reports receiving commercial research grants from Bristol-Myers Squibb, Iovance, and Merck. L.V. Sequist is an employee/paid consultant for AstraZeneca, Janssen, Blueprint Medicines, Merrimack Pharmaceuticals, and Genentech, and reports receiving commercial research grants from AstraZeneca, Novartis, Boehringer Ingelheim, Genentech, Merrimack Pharmaceuticals, Blueprint and LOXO, and holds ownership interest (including patents) in Blueprint. C. Debruyne is an employee/paid consultant for the GSK group of companies. B. Spiessens is an employee/paid consultant for Janssen Pharmaceuticals R&D, and holds ownership interest (including patents) in Janssen Pharmaceuticals R&D and the GSK group of companies. J. Louahed is an employee/paid consultant for the GSK group of companies. F. Ulloa-Montoya is an employee/paid consultant for and holds ownership interest (including patents) in the GSK group of companies. No potential conflicts of interest were disclosed by the other authors.

Disclaimer

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