Reply to Problematic methodology in a systematic review and meta-analysis of DecisionDx-Melanoma

To the Editor: Marchetti et al[1] comment on the systematic review and meta-analysis study titled "Molecular risk prediction in cutaneous melanoma: a meta-analysis of the 31-gene expression profile prognostic test in 1,479 patients," by Greenhaw et al.[2]

In response to their comments regarding the lack of a prespecified protocol or inclusion/exclusion criteria for specified studies, the systematic review was performed according to a detailed protocol, described in the methods and outlined in Supplemental Fig 1. The protocol included performing both the systematic review and meta-analysis according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses statement and a description of the prespecified search terms used to identify relevant articles. The protocol was established before our study started and the systematic review was performed on January 11, 2019 (as specified in the methods), so the report by Keller et al[3] was not considered for inclusion because of its publication date, March 2019. As stated in the methods section, "Studies were excluded if they contained cases that overlapped with larger data sets identified during the search and included in the analysis" and, as detailed in Supplemental Table I, the cases described in the study by Gerami et al[4] were excluded because they were included in the study by Gastman et al.[5]

Marchetti et al comment that the meta-analysis may be biased by the exclusion of clinicopathologic factors such as sex, anatomic site, and mitotic index. We agree that these features are associated with outcomes; however, they are not included in the American Joint Committee on Cancer Eighth Edition staging criteria[6] and because of that, their use for risk assessment is not consistently applied. The analysis in the study by Greenhaw et al[2] focused on those features that are currently included in clinically applied staging criteria, such as those included by the American Joint Committee on Cancer (age was added at the request of reviewers). We have, however, revisited the meta-analysis for the end point of recurrence-free survival to assess the effect of the 31 gene expression profile when mitotic index and anatomic location were included along with the clinicopathologic features listed in the original analysis. Sex as a data point was collected as part of the study by Greenhaw et al[2] only and therefore was not included. This additional analysis demonstrated no significant influence on the 31-GEP effect when mitotic index and site were included, with a hazard ratio of 2.75 (95% confidence interval [CI] 1.90-3.98) for a fixed-effects model and 2.77 (95% CI 1.89-4.06) in the random-effects model (Fig 1), very close to the hazard ratio of 2.90 (95% CI 2.01-4.19) for both models reported in Greenhaw et al.[2]

Marchetti et al also comment that the study by Greenhaw et al[2] is potentially biased because of the exclusion of the novel cohort from bias assessment using the Quality in Prognosis Studies tool. The study design of the novel cohort followed the study design described by Gastman et al, and the results of the bias

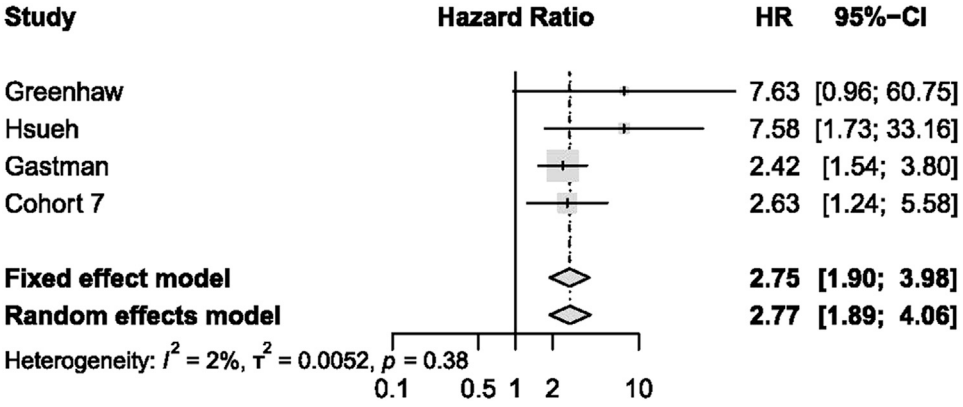

Fig 1. Comparison of hazards of recurrence associated with 31-GEP test and clinicopathologic features, including thickness, ulceration, age, SLNB, mitotic rate, and anatomic location. Gray boxes reflect the weight of the study in the aggregated estimate (diamonds) based on study error and effect size (where error is inversely related to weighting), vertical lines represent hazard ratio, and horizontal lines represent 95% confidence interval. Dotted vertical line and center of the diamond represent the aggregated hazard ratios of fixed- and random-effect models, and diamond width indicates the overall confidence interval in both fixed- and random-effects models. *CI*, Confidence interval; *HR*, hazard ratio.

analysis were similar. Using data presented in Fig 1 of Greenhaw et al,[2] Marchetti and colleagues performed a regression test, which showed evidence of small study effects. Performing an Egger test, we found that the greatest outlier was the study by Greenhaw et al.[2] To address outliers within the analysis we performed a trim-and-fill procedure, which identifies studies with significant bias and generates an opposing study with opposite-effect magnitude to compensate.[7] Inclusion of a bias-correcting study resulted in a model with a hazard ratio equal to 2.81 (95% CI 1.96-4.03), well within the range estimated without correction (hazard ratio 2.90; 95% CI 2.01-4.19). Additionally, using this new model that includes mitotic index and anatomic site, we repeated the Egger test and found no significant asymmetry in the funnel plot ($P = .06$) and are therefore reassured about the design and result for this aspect of our study.

Marchetti et al note that past studies have shown that financial conflicts held by study coauthors can influence bias assessment, and as a result, alignment of results and stated conclusions can be compromised. However, they do not provide instances in our article in which our conclusions do not match the results. During the peer review process, neither the editors nor the reviewers noted or commented on a discordance between our results and conclusions, but instead the review commented that "the conclusions were proper."

Finally, we agree that clinical validity should not be interpreted as clinical utility. The issue of clinical utility has been addressed previously.[8] In the study by Greenhaw et al,[2] we demonstrated the prognostic accuracy and clinical validity of the 31-GEP test in the context of current staging factors. The American Academy of Dermatology Guidelines of Care for Melanoma recognize that staging tests are typically validated "on the basis of their sensitivity and specificity," as has previously been applied to sentinel lymph node biopsy.[9] This meta-analysis assessed accuracy metrics, using sound methodology, to determine recurrence risk with the 31-GEP, and demonstrated that the 31-GEP test is an independent predictor of risk that augments American Joint Committee on Cancer Eighth Edition staging.

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Reprints not available from the authors.

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