The REMARK checklist

Item to be reported		Reported on Page Number/Line Number	Reported on Section/Paragraph
INTRODUCTION			
1	State the marker examined, the study objectives, and any pre-specified hypotheses.	Page 3-4 / Line 94-104	#Introduction
MATERIALS AND METHODS			
Patients			
2	Describe the characteristics (e.g., disease stage or co-morbidities) of the study patients, including their source and inclusion and exclusion criteria.	Page 4 / Line108-111	##Patients and samples
3	Describe treatments received and how chosen (e.g., randomized or rule-based).	Page 4 / Line108-112	##Patients and samples
Specimen characteristics			
4	Describe type of biological material used (including control samples) and methods of preservation and storage.	Page 4 / Line 111-113	##Patients and samples
Assay methods			
5	Specify the assay method used and provide (or reference) a detailed protocol, including specific reagents or kits used, quality control procedures, reproducibility assessments, quantitation methods, and scoring and reporting protocols. Specify whether and how assays were performed blinded to the study endpoint.	Page 7 / Line 205-214	##qRT-PCR
Study design			
6	State the method of case selection, including whether prospective or retrospective and whether stratification or matching (e.g., by stage of disease or age) was used. Specify the time period from which cases were taken, the end of the follow-up period, and the median follow-up time.	Page 4 / Line108-116	##Patients and samples
7	Precisely define all clinical endpoints examined.	Page 6/ Line 191-199	##Survival Analysis
8	List all candidate variables initially examined or considered for inclusion in models.	Page 4 6/ Line 119-126	##Microarray Data
9	Give rationale for sample size; if the study was designed to detect a specified effect size, give the target power and effect size.	Page 4 6 / Line 119-126	##Microarray Data
Statistical analysis methods			
10	Specify all statistical methods, including details of any variable selection procedures and other model-building issues, how model assumptions were verified, and how missing data were handled.	Page 5-6 / Line 143-144 152-153 162-164 194-195	#Methods
11	Clarify how marker values were handled in the analyses; if relevant, describe methods used for cutpoint determination.	Page 4-5 / Line 119-126	#Methods

RESULTS			
Data			
12	Describe the flow of patients through the study, including the number of patients included in each stage of the analysis (a diagram may be helpful) and reasons for dropout. Specifically, both overall and for each subgroup extensively examined report the numbers of patients and the number of events.	Page 7 / Line 218-221 236-240 244-252 266-268	#Results Fig.1
13	Report distributions of basic demographic characteristics (at least age and sex), standard (disease-specific) prognostic variables, and tumor marker, including numbers of missing values.	Page 6-8 / Line 197-199 255-261	##Survival Analysis Table 1
Analysis and presentation			
14	Show the relation of the marker to standard prognostic variables.	Page 8 / Line 255-261	#Results ##Survival
15	Present univariable analyses showing the relation between the marker and outcome, with the estimated effect (e.g., hazard ratio and survival probability). Preferably provide similar analyses for all other variables being analyzed. For the effect of a tumor marker on a time-to-event outcome, a Kaplan-Meier plot is recommended.	Page 8 / Line 256-259	#Results ##Survival analysis
16	For key multivariable analyses, report estimated effects (e.g., hazard ratio) with confidence intervals for the marker and, at least for the final model, all other variables in the model.	Page 8 / Line 260-261	#Results ##Survival analysis
17	Among reported results, provide estimated effects with confidence intervals from an analysis in which the marker and standard prognostic variables are included, regardless of their statistical significance.	Page 7-8 / Line 225-233 239-241 245-252 256-261	#Results
18	If done, report results of further investigations, such as checking assumptions, sensitivity analyses, and internal validation.	Page 8 / Line 266-268	##Expression of
DISCUSSION			
19	Interpret the results in the context of the pre-specified hypotheses and other relevant studies; include a discussion of limitations of the study.	Page 8-10 / Line 271-319	#Discussion
20	Discuss implications for future research and clinical value.	Page10 / Line 320-323	#Discussion

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*As the checklist was provided upon initial submission, the page number/line number reported may be changed due to copyediting and may not be referable in the published version. In this case, the section/paragraph may be used as an alternative reference.