Author's response to reviews

Title: Association of Fascin-1 with Mortality, Disease Progression and Metastasis in Carcinomas: a Systematic Review and Meta-analysis

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Author's response to reviews: see over
1.10.12
To: Dr. S. Alam, Editor-in-Chief, BMC Medicine, London.

Re: Association of Fascin-1 with Mortality, Disease progression and Metastasis in Carcinomas: a Systematic Review and Meta-analysis
First submitted as BMC Medicine MS: 1846584776729662

Dear Dr. Alam,

Please find here submitted our revised manuscript entitled “Association of Fascin-1 with Mortality, Disease progression and Metastasis in Carcinomas: a Systematic Review and Meta-analysis”. We would like to thank the reviewers for their constructive comments on the original submission. Full responses to reviewers' comments are given in the “Responses to the Reviewers” that follow this letter. We believe that we have carefully addressed the comments of the reviewers in full. Our responses include two changes to our analyses. We have pooled the 19 studies which presented mortality outcomes under the term “Mortality”, irrespective of the provision of a clear statement of whether or not mortality was cancer-specific. In addition, we have changed the term “disease-free survival” to “time-to-disease progression,” as only time-to-event data were extracted in our analyses. These changes are reflected in the new title. If any of our responses are not clear or comprehensive, we would be happy to consider further comments.

We confirm that this manuscript is not under consideration elsewhere and that all authors have seen and approved the final revised version. There are no conflicts of interest to declare. We hope that with the included revisions and additions our study will now be appropriate for publication in BMC Medicine, and look forward to hearing from you in the near future.

Yours sincerely,

Josephine C. Adams and Richard M. Martin, on behalf of the co-authors
Response to Reviewers comments on BMC Medicine MS: 1846584776729662

Revised title: Association of Fascin-1 with Mortality, Disease progression and Metastasis in Carcinomas: a Systematic Review and Meta-analysis

Responses to Reviewer 1: Chaker Adra

1) Minor Essential Revisions: in page 9, please be more clear in the text about which studies’ data were included and used.

Response: The details of the data extracted from each study are presented in the Methods section (page 7) and Additional file 1. The details of which studies were included in the meta-analysis are presented in the ‘Results section’ for each carcinoma type.

2) Discretionary Revisions: Widening of the cancer types would strengthen the paper. However, I recommend the publication of this manuscript.

Response: We appreciate the reviewer’s comment. The reason why we chose to restrict our analysis to breast, colorectal, gastric, lung and oesophageal carcinomas is because these are the most common carcinomas which have been extensively studied with regard to fascin-1. For many other carcinoma types there is only one published study. For tumour types other than carcinoma, eg, glioblastoma, there are few studies and fascin-1 is of less interest as a biomarker because it is expressed by normal astrocytes and microglia. In response to the reviewer’s comments, we have made changes to the sentence on page 5-6 as follows “We focused on these carcinomas because they are the most common carcinomas which have been extensively studied and a previous narrative review [2] identified at least two published studies for each tissue, allowing pooling of effect estimates.”

Responses to Reviewer 2: Cord Langner

We thank the reviewer Cord Langner for the thoughtful comments.

Major Compulsory Revisions

1) The authors should make clear already in the methods section, that significance is achieved only when p-values are below 0.05. Thus, there is no statistically significant association, when p-values are 0.05 or even higher. I would like to give an example: The use of the term “weak association” (already present in the abstract) for p-values of 0.09 or even 0.24 is discouraged. This is “no association”.

Response: A p-value measures the strength of the evidence against the null hypothesis. Although it has been common practice to arbitrarily categorise results into ‘significant’ when a p value of <0.05 is obtained and ‘not significant’ when the p value is >0.05, this was not the intention of the founders of statistical inference and most epidemiologists are now resisting this potentially misleading simplification of probability. Hence, we did not report our results as ‘significant’ or ‘non-significant’ but rather we present both the confidence interval and the exact p values, and use p-values to indicate the strength of evidence against the null-hypothesis, as recommended by Sterne et al (Sterne et al Sifting the evidence—what's wrong with significance tests? BMJ 2001;322 (7280):226-31). As we present exact p-values, readers can very easily apply their own judgements about the data, but we refrain from providing readers with potentially misleading conclusions about the ‘significance’ of any result.
2) The authors should more clearly sum-up the limitations of their analysis at the end of the discussion. Patient numbers are relatively small for meta-analyses, and the dataset is deeply influenced by case (and study) selection.

Response: We appreciate this point and have made changes to the ‘Conclusions’ (page 24-25) as follows “Due to limitations of the individual studies to date (issues of methodological quality due to retrospective study designs, inadequate sample size or power justification, possible biases due to selective reporting and heterogeneity in study methodologies), adequately powered prospective studies, particularly in breast, colorectal, gastric and oesophageal carcinomas, will be needed to fully determine the relative independent prognostic impact of fascin-1.”

2) The authors comment upon tumor heterogeneity only regarding different histological subtypes of breast cancer and different stages of colorectal cancer. Anyhow, these are all adenocarcinomas. There are possibly, however, much more important implications regarding the other types of cancer, even if no heterogeneity between the studies was noted (this might merely confer that Fascin-1 acts independent from morphological subtype, tumor histogenesis and molecular background): In the esophagus we have squamous cell carcinoma and Barrett’s adenocarcinoma, and there is no basis to lump these together. In the lungs, we have small cell and non-small cell carcinomas, and in the stomach, we have diffuse cancer related to E-cadherin inactivation/mutation and the intestinal subtype, all of these having different genetic backgrounds. Thus, pooling data of these biologically different tumors which by some chance arise within the same organ is a matter of debate and should be commented upon in appropriate way.

Response: For the lung carcinoma studies, we found that heterogeneity was evident between the studies and discuss this in the Discussion section (page 23) as follows “Heterogeneity was also evident between the lung carcinoma studies (I² = 41.9%). All the studies in our dataset had analysed only non-small cell lung carcinomas, which include both squamous cell carcinomas and adenocarcinomas. Patients with pulmonary squamous cell carcinoma have a higher mortality rate than those with adenocarcinomas, which might be attributable to confounding factors such as smoking status and age-related co-morbidities [79,80]. Adenocarcinomas include different subtypes such as acinar, papillary, bronchioalveolar carcinoma and adenocarcinomas with mixed subtypes, which could account for the histologic heterogeneity. One study reported that immunostaining of fascin-1 was found to be more common in adenocarcinomas with prevalent invasive components of the acinar, papillary and solid types compared to adenocarcinomas with a prevalent bronchioalveolar component [24]. Thus, the observed heterogeneity in these studies could be due to differences in fascin-1 immunostaining among different subtypes of adenocarcinomas.”

For the oesophageal carcinoma studies, all studies included had analysed squamous cell carcinomas only (Additional file 1), so the heterogeneity is not due to pooling of results across biologically different tumours within the same organ. We have added this point to the Discussion (page 23) as follows “In contrast, all the oesophageal carcinoma studies had analysed squamous cell carcinomas (Additional file 1); thus the between study heterogeneity is not due to pooling of results across biologically different tumours within the same organ.”

3) “We assumed that “death” referred to cancer-specific mortality and not to all-cause mortality” (on page 7). I do not agree. Every investigator who has ever related morphological findings in cancer tissues to outcome knows that it is comparably easy to find out whether a patient is dead or still alive. But if he/she is dead (perhaps years ago) it is difficult to assess the reason. Thus, if the authors of a given paper do not report on “cancer-specific mortality” (if they do, this is improved quality) they most probably only checked the overall status, dead or alive.

Response: We have now pooled the results of all the 19 studies which presented mortality outcomes, irrespective of the provision of a clear statement of whether or not they were
cancer-specific (see page 16 and Figure 2). In addition, we have carried out a sensitivity analysis for the 10 studies which provided a clear definition that mortality referred to death from cancer and for the 8 studies which did not have a clear definition of the cause of death. The results of the sensitivity analyses are presented in the ‘Results section’ (page 17-18) and in Table 1B and show that there is strong evidence that fascin-1 is associated with increased risk of cancer-specific mortality (RR=1.49 (1.29-1.72); p<0.001), also that the RR is consistent with the analysis of all mortality outcomes.

Minor Essential Revisions

1) Please do not use the abbreviated names as you currently do it. The abbreviated terms should only be given in brackets, e.g., one of us (XYZ). In addition, reference [2] is by Hashimoto and colleagues not only by “JCA” (last line of page 5).

Response: The narrative review has two co-first authors and one corresponding author. To avoid further complexity of wording we have changed the sentence on page 5 to read “We focused on these carcinomas…. as a previous narrative review [2] identified at least two published studies for each tissue, allowing pooling of effect estimates.”

2) The authors should go into more detail, whether the reported studies were performed on full sections or (only) on TMAs (I am sorry, I cannot open the supplemental material due to some technical problems and do therefore not know whether this information is provided there, but brief data on this topic are needed already within the main text).

Response: We have added the details of whether the studies were performed on tissue microarrays (TMA) or on full tissue sections to Additional file 1.

3) Likewise we need at least some overview data on the length of follow-up in the assessed studies, as this should be a criterion to include or exclude a specific investigation.

Response: The length of follow-up in each of the assessed studies was included in Additional file 1 in the first version of the manuscript.

Responses to Reviewer 3: Konstantinos Tsilidis
We thank the reviewer for the positive comments.

Major Compulsory Revisions

1) Some subgroup meta-analyses would be very helpful to explain some of your results for all cancers combined, even though I realize that you may end up with few studies in some of those subgroups. In addition, could you also perform a sub-analysis by whether the results of the individual studies were adjusted or crude? To what extent do you believe that the prognostic role of fascin-1 is independent of stage and grade of cancer?

Responses: In the first version of the manuscript, we had carried out subgroup analyses by carcinoma type. Figures 2-5 show the analyses stratified by each carcinoma type and the results pooled across all carcinoma types for each outcome. We are therefore unclear whether the reviewer is requesting any additional analyses. We would be happy to provide further clarification of the reviewer if needed.

In addition, we have now carried out subgroup analysis by a) strata of methodological quality scores (≥6 points , 5 points ,<5 points) (page16 and Table 1Ai) and b) by whether the results of the individual studies were adjusted or unadjusted (page17 and Table 1Aii). Subgroup
analysis of studies which presented adjusted results shows that fascin-1 protein was associated with a 44% increased risk of mortality. Ideally, meta-analysis would be based on the data of individual patients to allow for standardised control for confounding across all studies. As the hazard ratios from different studies have been adjusted for different confounders, we were unable to assess the independent role of fascin-1 as a new marker over existing markers such as stage and grade. This is stated in the Discussion.

2) You stated in the Methods that you stratified the results according to the method of scoring fascin-1 staining, but I didn’t see the analysis in the Results.

Response: The stratified results for “high versus low” and “positive versus negative” are presented in the Results section under each carcinoma type and in Figures 2-5.

3) I am not quite sure why you haven’t performed any formal assessment for publication bias. It seems that you have info on relative risk (95% CIs) and number of participants in each study. You could alternatively use the Harbord test if you have got information from a 2x2 table. I am afraid that this literature, as most basic/clinical science literature, will have lots of it.

Response: We did not include formal assessment of publication bias in the first version because our main focus was on individual carcinomas, for which there are a small number of studies for each carcinoma. In response to the comment, publication bias has been assessed for each outcome by inspection of funnel plots, with Egger’s test, as presented in Figure 6 and in the results section (page 18). The data show that publication bias is unlikely to be an explanation for our findings.

3) Alternatively, you could also perform a formal test for excess significance bias for your all-cancer endpoint. This test examines whether there are too many reported statistically significant results in single studies based on what would be expected under different assumptions about the plausible effect size of each association. This test can supplement the tests for publication bias above, and may even be better, because the publication bias tests may not be very sensitive or specific for detecting such bias, especially when a limited number of studies is included in a meta-analysis. Check for more info at: Ioannidis JP, Trikalinos TA. An exploratory test for an excess of significant findings. Clin Trials. 2007;4(3):245-253.

Response: We thank the reviewer for pointing out this new test. As it is an exploratory test, which is non-standard, and with which we have no experience, we would prefer not to undertake this, but rather focus on the funnel plot and Egger analyses, which is standard and very well understood.

4) It would be great if the authors could also provide some more details (and discussion about them) of the overall design and quality of the individual studies included in the meta-analysis. How were the subjects selected? Consecutive, random, other? Were the pathologists blinded to the outcome of the cancer cases? Was the same pathologist used for all plates in a study?

Response: We have now carried out a risk of bias assessment using the Newcastle-Ottawa scale to assess the quality of the studies which had presented results for mortality. The results of the risk of bias assessment are presented in Additional file 2 and are described in the ‘Results section’ (page 16). The results of our analysis show that for the mortality outcome, only two studies were found to be of high methodological quality with Newcastle Ottawa scores of above 6 points. Six studies had a score of 5 points which is just below the threshold for high methodological quality. Eleven studies had a score of less than 5 points (see
Additional file 2 for results of the risk of bias analysis). For our meta-analysis, only one study involved a prospectively assembled cohort (page 11). The remaining studies were retrospective in design using convenience samples based on the availability of specimens with interpretable cores (which may have been non-random) and available clinical histories, rather than specifying and recruiting a truly representative sample from a clearly defined target population. No studies provided an appropriate justification of the sample size or a power calculation (page 20). The pathologists were blinded to the outcome of the carcinoma cases for most of the studies and we did not have specific information from all papers on whether the same pathologist was used for all plates in a study.

What were the characteristics of the study population: race/ethnicity, stage and grade of cancer at diagnosis, cancer treatment? This information could be added to Table 1.

Response: We have added details of the relationship between fascin-1 and study characteristics such as stage and grade to Additional file 1. Details of the type of cancer treatment were not included, as few studies presented data on this. Race/ethnicity was not included as this was not fully reported by the individual studies, but the geographical regions of the studies are stated in Additional file 1.

In complement to the above, the authors could elaborate a bit more on the issues of retrospective study design, selective reporting and heterogeneity that they mention in the Conclusions.

Response: We have elaborated more on the issues of retrospective study design and selective reporting in the ‘Discussion section’ as follows:

-Page 20: “Only one cohort [25] was prospectively assembled. The remaining studies were retrospective in design using samples based on the availability of specimens with interpretable cores (which may have been non-random) and clinical histories, rather than specifying and recruiting a truly representative sample from a clearly defined target population. No studies provided an appropriate justification of the sample size or a power calculation.”

-Pg 19-20: “Publication bias could result from selective reporting of results by individual studies, because statistically insignificant results are often not published. We identified several studies which did not report all their data, but many authors did not respond to requests for information or clarification. However, funnel plot analyses were not generally indicative of any strong publication bias as visual inspection of funnel plots did not show asymmetry (Figure 6).”

Minor Essential Revisions

1) The total number of participants and by case/control status (e.g., dead case vs. alive case) for all 25 included studies should be provided in the Abstract.

Response: We are unable to include details of the total number of participants and the case/control status for all 26 included studies in the abstract due to the word limitations of the abstract. The total number of participants for each carcinoma type is included in the results section and details of the case/control status are presented in Additional file 1.
2) In several instances in the Methods, you stated that the authors of the original papers were contacted and asked for additional information. Could you be more explicit and state which authors provided what additional info? This could be part of the supplemental material if it is too long.

**Response:** We have indicated which results or information was provided by the authors in Additional file 1.

3) The wording in the Results when you are evaluating the heterogeneity of the literature is not consistent. In one instance, I-sqr is 55.3% and is deemed moderate heterogeneity, while when I-sqr is 45.3 it is strong evidence of heterogeneity.

**Response:** We have revised the wording in the Results section to ensure a more consistent approach. The $I^2$ value measures the percentage of total variation across studies that is due to heterogeneity rather than chance. The $I^2$ value is used as a guide in judging consistency of evidence; however, its values are not absolute. To make the evaluation of our meta-analysis more consistent across the paper, we have defined an $I^2$ value of 0 to be considered as no heterogeneity, $I^2=1-25\%$ as low heterogeneity, $I^2=25-75\%$ as moderate heterogeneity and $I^2=75-100\%$ as high heterogeneity. However, only two studies were included in the time-to-progression meta-analysis of colorectal carcinoma studies and there was evidence of heterogeneity between the two studies ($I^2=73\%$; p=0.06) (page 12). In line with our definitions, we have described an $I^2$ value of 73% as moderate-to-high heterogeneity.

4) In Figure 1, please list the number of exclusions next to the reason for exclusion.

**Response:** We have added the reference and number of studies excluded next to the reason for exclusion in Figure 1.

5) In Figures 2-5, could you list the number of cases/controls instead of the total number of participants?

**Response:** We have listed the number of cases/controls in Figures 2-5.

**Response to Reviewer 4: Sarah Donegan**

**Major compulsory revisions**

1. Risk of bias: There is no assessment of risk of bias or quality of these studies. This is an essential part of a systematic review. Cohort studies can be assessed using the Newcastle-Ottawa scale or other appropriate methods. The methods and results of the assessment should be presented.

**Response:** In response to the reviewer’s comment, we have now included a risk of bias assessment using the Newcastle-Ottawa scale. Details of the method are presented in the Methods section (page 9). The results are presented in the Additional file 2 and discussed in the results section (page 16).

2. Meta-analysis of risk ratios and hazard ratios (in ‘Statistical analysis’ section). Risk ratios and hazard ratios can’t be combined together in meta-analysis. Hazard ratios should be combined together using the generic inverse variance method to give a pooled hazard ratio. Risk ratios can be combined together using the generic inverse variance approach (or event rates can be combined can be combined using the Mantel-Haenszel method) to give a pooled risk ratio. Rephrase the methods text and correct the analyses.
For our meta-analysis, we chose to combine the risk ratios and hazard ratios for mortality as a relative risk. Hazard ratios are a measure of the relative risk over time and describe how many times more likely the exposed cases will have the outcome at a particular point in time compared to the unexposed controls. Although there are subtle differences between relative risks and hazard ratios, for our analysis, the hazard ratios have been calculated using the Cox proportional hazards model which assumes that the hazard in the exposed group is a constant proportion of the hazard in the other group over time.

3. Choosing data for the meta-analysis (in ‘Statistical analysis’ section).

Response: For our meta-analysis of the association of fascin-1 with mortality, we extracted multivariable analysis results from most studies, except one which presented univariable analysis results, one which presented two-year mortality rates, and seven studies which presented results as univariable Kaplan-Meier curves. For our primary analysis, we have pooled both adjusted hazard ratios and crude hazard ratios together. In the ‘Statistical Analysis’ section, we have changed the sentence on page 9 to “We used the metan command in Stata (StataCorp. 2009. Stata Statistical Software: release 11.2 College Station, TX: StataCorp LP) to calculate summary RR estimates by combining fully adjusted hazard ratios, crude hazard ratios and/or risk ratios.” The details of whether the results are adjusted hazard ratios or crude hazard ratios for each study are presented in Additional file 1. To take into account the effect of potential confounders, we have also now carried out subgroup analysis by whether the results of the individual studies were adjusted or crude estimates. The results of the subgroup analysis are presented in Table 1Aii and in the ‘Results section’ (page 17). The subgroup analysis of studies that presented multivariable analysis results showed that fascin-1 protein was associated with a 44% increased risk of mortality.

4. Forest plots: The comparison ‘positive versus negative’ and the comparison ‘high versus low’ should be on separate plots. Data from the two comparisons should not be combined.

Response: We have provided the information as fully as possible in the figures i.e. with the comparisons presented separately and in combination. Thus the reader can see the data presented in both ways, and can choose to look only at the stratified results if they wish.

5. Assessment of heterogeneity (in ‘Statistical analysis’ section). In addition to the I square statistic, test for heterogeneity by comparing the overlap of confidence intervals of the study specific estimates and using the chi-square test. Describe these methods in the statistical analysis section.

Response: We have described the methods in the ‘Statistical analysis’ section (page 9) as follows “Heterogeneity was assessed by performing the Cochran’s Q test”. The I-square
statistic already incorporates formal information about the overlap of confidence intervals of the study specific estimates.

6. Subgroup analyses, sensitivity analyses or publication bias (in ‘Statistical analysis’ section). In the methods, describe how possible causes of heterogeneity were explored using subgroup analyses or meta-regression? Which covariates were considered?

Response: Possible causes of heterogeneity have been explored using subgroup analysis for studies which had presented adjusted hazard ratios from multivariable models and for those which presented unadjusted results and by each strata of methodological quality scores (>6 points, 5 points, <5 points). In the first version, we had stratified our results by method of fascin-1 scoring to explore possible causes of heterogeneity in meta-analysis. This is described in the ‘Statistical analysis’ section (page 9) as follows “For our meta-analysis, we stratified the results by method of scoring (i.e low versus high or positive versus negative) and by type of carcinoma.” Although there was qualitative evidence of heterogeneity between the individual studies, due the small number of available studies for each carcinoma type, it was not possible to investigate this statistically using meta-regression. This is stated on page 21.

Also, how was the robustness of the results explored with regard to biases etc?

Response: The robustness of the results has been explored by carrying out sensitivity analysis based on studies which had a clear definition that mortality referred to death from cancer. In addition, for colorectal carcinoma studies, sensitivity analysis was carried out for studies which had included only stage III/IV patients in their analysis (page 18 and Table 1B). These data show that for patients with stage III/IV colorectal carcinomas, there is strong evidence that fascin-1 expression is associated with a 73% increased risk of mortality. This result is consistent with the analysis of all stages of colorectal carcinoma.

Was publication bias explored using funnel plots or other methods?

Response: Publication bias has been assessed for each outcome by inspection of the funnel plots and using Egger’s test. The results are shown in Figure 6 and discussed in the Results (page 18). The data show that publication bias is unlikely to be present in our meta-analysis.

7. Choice of carcinomas (in ‘Data sources’ section): I think that the decision regarding which carcinomas to include should be based on whether the question is of interest rather than the number of available studies. One large kidney study may provide valuable information but it is excluded from this review because a previous review included less than two kidney studies. Previous reviews may be outdated. The review authors could change the inclusion criteria or provide alternative justification.

Response: The reviewer may have mis-understood our criteria. The reason why we chose to restrict our analysis to breast, colorectal, gastric, lung and oesophageal carcinomas is because they are the most common carcinomas for which there is more than one published study on fascin-1 to analyse up to April 2012. We have made this more explicit in the ‘Data sources’ section (page 5-6) as follows “We focused on these carcinomas because they are the most common carcinomas which have been extensively studied for fascin-1 and a previous narrative review [2] had identified at least two published studies for each tissue, allowing pooling of effect estimates.”
8. Clarity of the eligibility criteria (page 6): I think the criteria needs to more clearly stated. Specifically, eligible study designs (e.g. randomised controlled trials, non-randomised studies) should be stated with a brief summary of the study design if it is a non-conventional design.

Response: We have clarified the eligibility criteria as follows (page 6): “Studies had to meet the following inclusion criteria: either randomised controlled trials, cohort or case-control studies that detected fascin-1 by immunohistochemistry in at least one of the selected carcinomas (breast, colorectal, gastric, lung and oesophageal).”

Furthermore, the comparison of interest needs to be made clear i.e. low versus high and negative versus positive. It would also be helpful to define ‘high’, ‘low’, ‘negative’ and ‘positive’ with regard to staining. Perhaps an explanation why are these comparisons of interest could be given in the background.

Response: We have added details of the fascin-1 comparison to page 6 as follows “Within the included set, different scoring systems have been used to assess the immunohistochemical data on fascin-1. Some studies measured the intensity of fascin-1 staining and/or the percentage of immunoreactive cells, while others studies multiplied the intensity of fascin-1 staining by the percentage of immunoreactive cells. Some studies categorised fascin-1 expression as high versus low, others categorised expression as positive versus negative, and others categorised fascin-1 as negative, low, or high.” In addition, we have changed the sentence in the Background (page 5) as follows “Immunohistochemistry is a complex metric for meta-analysis, due to the use of different scoring systems to assess the extent of fascin-1 staining in tumour specimens, yet studies of fascin-1 have the advantage that almost all publications to date have used the same two antibodies to fascin-1.”

Minor essential revision

9. In the abstract, I disagree with the statement that the PRISMA guidelines have been followed.

Response: We have checked and verified that our systematic review and meta-analysis was conducted in accordance to the PRISMA guidelines.

10. In the data sources section, describe whether organisations were contacted to obtain unpublished results; whether authors of conference abstracts and on-going studies were contacted for the published or unpublished report.

Response: We did not contact any organisations to obtain unpublished results and we did not identify any on-going studies from our systematic review. In the data extraction section (page 9), we have changed the sentence to “For papers or conference abstracts where data were missing or not clear, authors were contacted requesting further information that would enable us to include their data in our meta-analysis.”

11. Also, in the data sources section, describe whether the study authors were written to if eligibility was unclear; clarify whether two review authors independently assess the titles and abstracts; and state whether the full report was assessed when eligibility was unclear based on the abstract.

Response: We have added the following sentences to the ‘Data sources section’ (page 7): “The title and abstracts of all retrieved papers were then assessed using pre-specified inclusion criteria (see below) by one author (VYT). Where abstracts were not available or when eligibility was unclear based on the abstract, the full papers were obtained and assessed.”
12. Lastly, in the data sources section, I would rephrase ‘For multiple study publications from the same research group with the same outcomes, we chose the study with the largest number of cases.’ The same research group may publish similar studies that are different; on the other hand, different research groups may publish the same study as separate articles (e.g. if it is a collaborative project).

Response: The sentence has been amended as follows “For multiple study publications from the same patient cohort reporting on similar outcomes, we chose the study with the largest number of cases. For studies that presented different outcomes, we extracted outcomes from both publications” (page 7).

13. Regarding the eligible outcomes (page 6), please clarify whether: ‘lymph-node or distal metastasis’ are one or two outcomes. Also, choose either ‘survival’ or ‘mortality’ as an outcome rather than both because I think that they are the same. Clarify whether mortality is this ‘cancer-specific mortality’, ‘all-cause mortality’ or whether you looked at both separately.

Response: In our meta-analysis, lymph node metastasis and distant metastasis are two separate outcomes. We have explained this further on page 6 as follows: “Some studies categorised fascin-1 expression as high versus low, others categorised expression as positive versus negative, and others categorised fascin-1 as negative, low, or high. Only studies which presented categories of either high versus low fascin-1 staining, negative versus positive fascin-1 staining, or negative, low and high fascin-1 staining in relation at least one of the clinical outcomes of lymph-node or distal metastasis, time-to-disease progression, or mortality, in at least one of the selected carcinomas, were included.”

For our meta-analysis, we have pooled the results of all the 19 studies which presented mortality outcomes, irrespective of the provision of a clear statement of whether or not they were cancer-specific (see page 16 and Figure 2). In addition, we have carried out sensitivity analyses for the 10 studies which provided a clear definition that mortality referred to death from cancer and for the 8 studies which did not have a clear definition of the cause of death. The result of the sensitivity analysis is presented in Table 1B and in the ‘Results section’ (page 17-18) and shows that there is strong evidence that fascin-1 is associated with increased risk of cancer-specific mortality (RR=1.49 (1.29-1.72); p<0.001). Please see response to reviewer 2, comment 3.

14. Also, with respect to the outcomes, if only time to event data will be extracted, I would rephrase the outcomes, such as, ‘time to lymph-node metastasis’, ‘time to distal metastasis’, ‘time to mortality’, ‘time to disease free’. If dichotomous data, at different time points will also be extracted, I would state ‘lymph-node metastasis, distal metastasis, mortality’, ‘disease-free’.

Response: For our meta-analysis, dichotomous data were extracted for lymph-node metastasis and distant metastasis. Only one study had presented adjusted hazard ratios and 95% CIs for distant metastasis as an outcome. Mortality data included both time-to-event data and dichotomous data. We have changed the term ‘disease-free’ to “time-to-disease progression” throughout the text as only time-to-event data were extracted.

15. Refer to figure 1 in the results, rather than the methods.

Response: We refer to Figure 1 in the results (page 11).
16. In data extraction section, describe whether hazard ratios were extracted that were unadjusted for confounders, or adjusted hazard ratios, or both.

Response: We have changed the sentence in the Data extraction section (page 8) as follows “For mortality outcomes, both adjusted and unadjusted hazard ratios (HRs) and 95% confidence intervals (CIs) for fascin-1 in relation to mortality were extracted from the published tables or the results section.”

17. In data extraction section, if the analysis is a time to event analysis, replace ‘relative risk’ with ‘hazard ratio’.

Response: We prefer to use relative risk as an accepted general term for risk, rate and hazard ratios (see Kirkwood and Sterne, Essentials of Medical Statistics).

18. Was a sensitivity analysis done excluding the studies that did not clearly define mortality? Rather than assuming that the outcome is cancer mortality, my preference would be to write to authors to ask for the definition of mortality. If no reply is received, I would treat these studies separately using the outcome ‘mortality’.

Response: Please see responses to reviewer 2, comment 3 and reviewer 4, comment 14.

19. In data extraction section, please clarify the disease-free survival analysis. To my understanding, initially the patients were diseased. If interest is in the time from surgery to date of disease progression, this is an analysis of ‘time to disease progression’, rather than ‘time to disease free’.

Response: In the ‘Data extraction section’ (page 8), we have changed the definition as follows “For studies that did not provide a clear definition of their outcomes for time-to-disease progression analysis, we assumed that time-to-disease progression was calculated from the date of surgery to the date of disease progression. For time-to-disease progression analysis, a positive relative risk (RR) implies an increased risk of disease progression.”

20. In data extraction section, please clarify which ‘results’ were extracted for metastases outcomes and for the mortality outcomes. Were dichotomous data (i.e. number of people with a particular event and the total number of people, or risk ratios and confidence intervals) extracted? Were time to event data (i.e. hazard ratios and confidence intervals) extracted? Were measures of effect extracted that were adjusted for confounders or just the unadjusted measures of effect?

Response: We have clarified this in on page 8 as follows: 1) “For mortality outcomes, both adjusted and unadjusted hazard ratios (HRs) and 95% confidence intervals (CIs) for fascin-1 in relation to mortality were extracted from the published tables or the results section.” 2) “Relative risks and 95% CIs for fascin-1 in relation to metastasis were estimated from extracted dichotomous data reported in published tables or results sections. For the study by Oh et al [29], adjusted hazard ratios and 95% CIs for distant metastasis outcome were extracted from the published table.”

21. Relative risks and risk ratios are different names for the same measure of effect. Use one name consistently.

Response: We have relabelled the measure of effect consistently as relative risk.
22. In the statistical analysis section, simply state that the analyses were stratified by comparison (i.e. low versus high, positive versus negative) and by type of cancer (i.e. lung, gastric,...). Delete ‘We stratified the results according to method of scoring fascin-1 staining’ and ‘For our meta-analysis, we stratified the results by method of scoring to determine if the different scoring methodologies might lead to heterogeneity in our effect-estimates.’

Response: We have changed the Statistical analysis section (page 9) as follows “For our meta-analysis, we stratified the results by method of scoring (i.e. low versus high or positive versus negative) and by type of carcinoma.”

23. I would move the description of staining from the statistical analysis section (lines 5-7) to the eligibility section.

Response: We have moved the description of staining to the eligibility section (page 6).

24. Numbers in the results text and figure 1 are inconsistent (e.g. 48/49 full papers for review).

Response: We have checked all the numbers in the Results text and Figure 1 and have corrected the typo.

25. In the results section, describe how many studies reported each outcome. If no studies reported the outcome, state this.

Response: The number of studies which had reported each outcome was included in the ‘Results section’ of the original version of the manuscript under each carcinoma subtype.

Discretionary revisions

26. I would describe the inclusion/exclusion criteria before describing the search strategy (i.e. ‘data sources’).

Response: We thank the reviewer for the comment. However, we have chosen to describe the search strategy first before the inclusion/exclusion criteria because the first step of our systematic review and meta-analysis was to identify all the published literature before we assessed the eligibility of the studies using the inclusion/exclusion criteria.

27. In the data sources section, consider rephrasing ‘A systematic review of all published literature, including papers, conference abstracts and reviews’ because it is not clear whether this is a review of studies or a review of reviews or a combination of both. In any case, this sentence seems redundant to me and could be moved to the background.

Response: We have changed the sentence in page 5 to “A systematic review of all published literature on the association of fascin-1 protein expression with carcinoma progression in breast, colorectal, gastric, lung and oesophageal carcinomas was carried out.”

28. In the data sources section, I would describe the selection of studies under a different heading i.e. from ‘The title and abstracts of all ....’. Then the data sources section will describe the search strategy alone.

Response: We have made alterations on page 5-6 and have described the selection of the studies under the ‘Inclusion and exclusion criteria section’.
29. I found the eligibility criteria on page 6 difficult to follow. To clarify the criteria, I suggest that: sub-headings (i.e. study design, participants, interventions, outcomes) could be used; or sentences could be shortened.

Response: We have made changes to the eligibility criteria by shortening the sentences in the ‘Inclusion and exclusion criteria section’ in page 6.

30. It would be useful to have the number of studies that were excluded for each individual reason in the flow diagram (fig 1).

Response: The details of the number of studies that were excluded for each individual reason have been added to Figure 1.

31. I think that the results text should have three distinct sections: description of the characteristics of the included studies; risk of bias results; results of the analysis in this review.

Response: Other reviewers had no problem with the organisation and we think that the Results section is very clear. If the Journal Editor requests this change we would be happy to make it.

32. Avoid describing the included studies in the methods, that is, delete the first four lines of the statistical analysis section. Also, I would avoid providing references to specific included studies in the data extraction section. Instead, I would write ‘When studies did not ...’.

Response: In response to the questions of several referees, the Statistical analysis section has been altered. This has included removing the description of the included studies. We have kept some references for clarity, for example “For studies [20,21,24,29-31] that did not provide a clear definition of their outcomes for time-to-disease progression analysis, we assumed that time-to-disease progression was calculated from the date of surgery to the date of disease progression” (page 8).