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Comment on: The prognostic significance of tumour-stroma ratio in oestrogen receptor-positive breast cancer

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Sir,

It has been demonstrated that reactive stromal formation in solid tumours is associated with disease progression and poor outcome. Genes have been identified that are involved in biological processes such as angiogenesis and alterations in the extracellular matrix, including desmoplasia. (Gao *et al*, 2011; Planche *et al*, 2011; Berdiel-Acer *et al*, 2014; Dakhova *et al*, 2014; Duss *et al*, 2014).

The presence of stromal cells located in the interior of the tumour, surrounded by small groups or nests of tumour cells, is partly determinative of its (pre) metastatic capacity. Over the last decade, the tumour-stroma ratio (TSR) has gained significant interest in the disease prediction of patients with breast, colon, oesophageal, lung and cervical cancer. The elegance of the parameter is the use of conventional Hematoxylin & Eosin-stained slides for histopathological microscopy analysis. The use of a simple cut-off value, proven to be applicable for multiple solid tumour types, distinguishes between stroma-high and stroma-low tumours, of which the stroma-high tumours are independently associated with a relatively worse prognosis. (Mesker *et al*, 2007, 2009; Courrech Staal *et al*, 2010; West *et al*, 2010; Beck *et al*, 2011; Courrech Staal *et al*, 2011; de Kruijf *et al*, 2011; Ahn *et al*, 2012; Huijbers *et al*, 2012; Moorman *et al*, 2012; Wang *et al*, 2012, 2013; Dekker *et al*, 2013; Liu *et al*, 2014; Park *et al*, 2014).

The TSR has demonstrated the highest prognostic power when looking at the population of triple-negative breast tumours. For this group of patients, the prognostic hazard ratio (HR) for disease recurrence was reported as high as 4.12 ($P = 0.006$) and 3.0 ($P = 0.0034$) for patients harbouring stroma-rich tumours. (10,13). Furthermore, within our own data set, oestrogen receptor-positive patients also show a significant relapse-free survival (RFS) difference in the disadvantage for stroma-producing tumours (RFS $P = 0.001$, HR 1.8). Similar results were observed in the POP study (de Kruijf *et al*, 2011; Dekker *et al*, 2013).

Now, Downey *et al* present the analysis of 118 female breast cancers with stromal formation resulting in a relatively favourable prognosis. These data are in contrast with formerly obtained data on breast and other solid cancers scoring the TSR parameter as described by our group (and subsequently validated by others). (Mesker *et al*, 2007, 2009; Courrech Staal *et al*, 2010; West *et al*, 2010; Beck *et al*, 2011; Courrech Staal *et al*, 2011; de Kruijf *et al*, 2011; Ahn *et al*, 2012; Huijbers *et al*, 2012; Moorman *et al*, 2012; Wang *et al*, 2012, 2013; Dekker *et al*, 2013; Liu *et al*, 2014; Park *et al*, 2014). In the rest of this letter, we will describe methodological differences between the method used by Downey *et al* and previously published reports, which might underlie the differences in results.

First, Downey *et al* evaluate only one 9 mm^2 area at the tumour's leading or non-leading edge. This area was selected with the emphasis that the advancing 'front' of a tumour may be more proliferative and the metabolic activity of tumour cells in this area is not compromised by a potential lack of nutrients. This method of TSR scoring of a given tumour underestimates the heterogeneity within the tumour concerning stromal production (Zhang *et al*, 2014). It is our personal experience that a solid tumour can be very heterogeneous for desmoplastic characteristics. Estimation of the TSR as indicated by our group entails evaluation of the complete tumour area after which the TSR is determined based on the intratumoural area with the highest degree of stromal formation (Mesker *et al*, 2007, 2009; Courrech Staal *et al*, 2010; de Kruijf *et al*, 2011; Courrech Staal *et al*, 2011; Ahn *et al*, 2012; Huijbers *et al*, 2012; Moorman *et al*, 2012; Wang *et al*, 2012, 2013; Dekker *et al*, 2013; Liu *et al*, 2014; Park *et al*, 2014). For colorectal cancer, it has been shown that the deepest invasive part of the tumour is the most determinative for tumour progression and almost invariably demonstrates the highest stromal formation (if any). For breast cancer this is not applicable, possibly in part because these tumours do not progress through adjacent, consecutive tissue layers as is the case in colorectal tumours (mucosa, submucosa, muscle layers and so on). As such, the area with the highest amount of desmoplasia cannot be predicted and thus warrants evaluation of all available microscopic slides. Also for cervical, non-small lung and oesophageal cancer confirmatory data was observed (Courrech Staal *et al*, 2010, 2011; Wang *et al*, 2012, 2013; Liu *et al*, 2014).

Downey's study also did not indicate whether patients were pre-treated with radio, chemo or endocrine therapy. The studies by Moorman *et al* (2012) and de Kruijf *et al* (2011) excluded patients with neoadjuvant therapy as therapy influences the tissue arrangement including desmoplasia. Furthermore, no clinical-pathological data with respect to the proportion of stroma was provided, and neither were data for univariate analysis.

West *et al* (2010) used an identical approach as Downey for colorectal cancer, for this study an area of the luminal region was selected, resulting in a comparable cut-off points and survival data within stages I–III as given for the conventional TSR scoring. For this study no patients with pre-operative chemo or radiotherapy were included.

In the current setting we do not think that the reported method of Downey *et al* validates the approach of the TSR as it was only based on a subselected tumour area. The previously described TSR by our group is determined on the distribution of the stroma within the complete tumour including areas with heterogeneity and highly aggressive stromal formation. These contrasting reports illustrate the use of stringent criteria for scoring intratumoural stromal formation in order to reliably integrate the TSR into clinical decision-making.

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Reponse to: comment on, 'Tumour-stroma ratio (TSR) in oestrogen-positive breast cancer patients'

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Sir,

We thank Dr Mesker *et al* for their comments on our study, (Downey *et al*, 2014) recognising their significant work promoting the concept of using tumour-stroma ratio (TSR) to determine the outcome in cancer (Mesker *et al*, 2007, 2009; Courrech Stall *et al*, 2010, 2011; de Kruijf *et al*, 2011; Dekker *et al*, 2013; Huijbers *et al*, 2013).

None of our ER-positive cohort (118 female, 62 males; Downey *et al*, 2014) received neoadjuvant therapy of any type. Neoadjuvant treatment induces pathological changes in the tumour, hence would render samples unsuitable for TSR analysis. We were limited in the amount of information that could be supplied in a short communication, however univariate and multivariate outcomes were provided.

We found high stromal content was related to better survival across genders in ER-positive disease (Downey *et al*, 2014), contrasting data in triple-negative breast cancer (de Kruijf *et al*, 2011) and, as highlighted by Mesker *et al*, their own work on ER-positive cases (de Kruijf *et al*, 2011; Dekker *et al*, 2013). As breast cancer is heterogeneous, subtle differences in stromal biology may exist between breast cancer subtypes, potentially impacting on outcome. Notably, tubular carcinoma, a type of invasive breast ductal carcinoma with an abundant stroma (Figure 1), is almost always ER-positive and has a favourable prognosis (Rakha *et al*, 2010).

Methodological heterogeneity exists between sampling methods used to assess TSR. Two key issues stand out: (1) lack of standardisation in TSR measurement, (2) area of tissue selected for analysis. Our in-house computer algorithm method selects a 9 mm^2 area of a digitally scanned H&E image (Downey *et al*, 2014). Recent related work assessed TSR manually in a single section from the most invasive tumour area (Gujam *et al*, 2014). Mesker *et al* favour assessment of the whole slide, even suggesting an evaluation of all available microscope slides. Although rigorous assessment is to be commended, this technique may have practical implications for histopathologists should TSR evaluation ever become routine. Alternative approaches should be considered, compared and validated.

We believe that there is much more to the stroma in dictating outcome, than simply its proportion in relation to tumour. There is a need to examine the cell types that coexist within tumour stroma, for example, fibroblasts and immune cells (Hanahan and Coussens, 2012); a recent issue of this journal showed that patients with a high TSR had significantly reduced inflammatory cell infiltrate within their stroma (Gujam *et al*, 2014). It remains possible that discrepancies observed between studies of TSR in breast cancer may be due in part to components of the stromal microenvironment.

Consistent with all emerging techniques it takes time for the ideal methodology to become accepted in the field. We respectfully suggest the best way to achieve this for TSR is through collaboration, comparing different techniques, using carefully selected sub groups of breast cancer and working towards reaching a consensus, taking account not only of the stroma but the cells within.

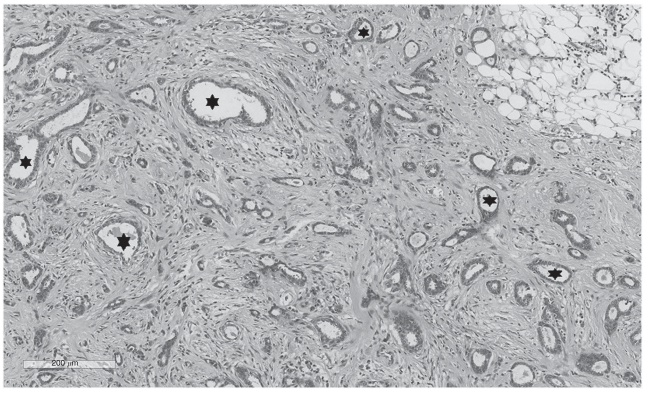

Figure 1. Tubular carcinoma showing arrangement of tumour cells in characteristic tubes (stars) embedded within an abundant multicellular stroma. Scale bar = 200 μm.

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