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Reply to: Comment on 'Tumour antigen expression in hepatocellular carcinoma in a low-endemic western area'

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Sir,

We would like to thank Grizzi *et al* for their comments to our paper on the expression of tumour antigens in hepatocellular carcinoma (HCC) in a Western European cohort (Sideras *et al*, 2015). Grizzi *et al* discuss the international collaborative efforts organised by the 'Society for Immunotherapy of Cancer' with a goal to 'identify hurdles that impede effective translation of cancer immunotherapy' (Fox *et al*, 2011). One of the hurdles the panel identified was that of cancer complexity. The question arises if studies using tissue microarrays (TMAs) are capable of capturing the complexity of cancer. To answer the question it is important to first consider not only the disadvantages but also the advantages of TMA-based research, and to understand which research question is being asked by the investigators using TMAs.

Tissue microarray technology was developed as an alternative to laborious and costly histological studies utilising full tissue slides (Camp *et al*, 2008). The advantages are several. Tissue microarrays use small amounts of tissue that markedly increase the number of assays that can be performed per tissue block. In fact, the tissue blocks we used for our study are largely undisturbed after cutting the cores and can be further used to study other questions. Tissue microarrays substantially reduce the amount of antibodies and workload when studying large cohorts of patients. The presence of tissues from multiple patients on a single slide allows for identical experimental conditions.

As pointed out by Grizzi *et al*, a potential disadvantage of using TMAs is that, in case of tumour heterogeneity, antigens of interest, which are focally expressed, may be missed. However, multiple studies have demonstrated strong correlations between TMAs and whole sections (Camp *et al*, 2000; Torhorst *et al*, 2001; O'Grady *et al*, 2003; Zhang *et al*, 2003; Bhargava *et al*, 2004; Schmidt *et al*, 2009), and it is known that sampling error is reduced inversely to the size of the cohort. Although the correlation also depends on the level of heterogeneity, even heterogeneously expressed antigens have shown reasonable correlations between TMAs and full sections (Camp *et al*, 2008).

To come back to our study, several cancer testis antigens (CTAs) showed lower prevalence of expression than studies originating in Eastern Asia, where most HCC patients are HBV positive. We provided several explanations to account for these discrepancies. One of them is the discrepancy in aetiology (in our study only 23% of patients were HBV positive), which has been previously shown to influence the expression of CTAs. For example, in the study by Xia *et al*, which is quoted by Grizzi *et al*, the prevalence of MAGE-C1 expression, as examined in full-tumour sections of 46 patients, was 36% (Xia *et al*, 2013). However, in HBV-negative patients only 13% expression was seen, in agreement with our study. We further discussed how our results are in agreement with other similar studies from European cohorts with low HBV prevalence (Riener *et al*, 2009). On the contrary, the Liang *et al* study, also quoted, had an HBV prevalence of 88% (Liang *et al*, 2013). Incidentally, Liang *et al* also used TMAs to study CTA expression of the 362 HCC patients in their study. The study by Wang *et al*, which was not published at the time of submission of our paper, examined tissue from 142 patients using RT–PCR (Wang *et al*, 2015). However, as we discussed in our paper, mRNA is not always translated to protein, and in fact direct comparisons have shown much lower CTA protein expression than mRNA expression in HCC (Nakamura *et al*, 2006).

A final point raised by Grizzi *et al* is regarding the use of the H-score. We agree that this method provides equivalent mathematical products that 'may', biologically, represent different things. We used the H-score only to investigate the possible biologic role of Glypican-3 and SALL-4 co-expression, and we were quite cautious as to the drawn conclusions, indicating that more research is needed to establish such an association. On the other hand, in Figure 2 of our manuscript, we show three-dimentional graphs of the intensities and percentages of expression of several tumour antigens, providing thus a transparent view of our data.

In conclusion, although we agree with Grizzi *et al* that TMAs are not suitable for answering questions of complex spatial relationships in the tumour microenvironment, or selection of tumour antigens for immunotherapeutic targeting in individual patients, our aim, to compare expression prevalence of a large panel (15) of tumour antigens in HCC, using a relatively large patient cohort (131), justifies the use of TMAs.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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