A cautionary note regarding detection of PD-L1 expression by tumour-associated macrophages

We read with interest the recent paper by Gottlieb et al. (2017) in which the authors evaluated Programmed Death Ligand-1 (PD-L1) expression by tumour cells and tumour-associated macrophages (TAMs) in primary high grade serous ovarian cancer (HGSOC) specimens and in matched metastases. The expression of immune checkpoint molecules in the local tumour environment, particularly of PD-L1, is currently a topic of much research focus. However, there are some technical issues surrounding the immunohistochemical detection of PD-L1 that other researchers who are considering performing this assay should be aware of. These are especially important in the context of TAM PD-L1 expression.

As the authors mention in their discussion, there are now several commercially available anti-human PD-L1 antibodies, and there has been much debate over the reproducibility of staining using different clones. This is particularly true with regard to the staining of tumour-associated immune cells (Hirsch et al., 2017). We recently performed an in-house evaluation of four antibodies (E1L3N, Cell Signaling Technology; 28-8, Abcam; 22C3, Dako and SP263, Roche) for detection of PD-L1 in formalin-fixed paraffin-embedded colorectal tissue, and found significant variation in staining intensity and in sensitivity to alterations in the staining protocol, such as choice of antigen retrieval solution and/or secondary detection reagents (Anyaegbu et al., in press).

During our evaluation, we noticed considerable non-specific staining of macrophages, present when an isotype control was used in place of the primary antibody. Background staining of macrophages is a common and widely recognised phenomenon due to their high level of Fc receptor expression. However, we found it to be particularly pronounced with the protocols required to obtain optimal PD-L1 staining, despite the use of blocking reagents, since signal amplification using polymer detection methods is usually required. We have found this to be the case with HGSOC samples as well as colorectal cancer. Cellular localisation of ‘true’ PD-L1 staining is important to differentiate from non-specific or background staining. The non-specific staining of macrophages have a granular cytoplasmic appearance, lacking the membra- nous staining we judge to be PD-L1-specific. This finding is consistent with other publications and guidelines released for interpretation (PD-L1 IHC 22C3 pharmDx Interpretation Manual - US Version, 2015; Kerr et al., 2015; Roach et al., 2016; Webb et al., 2016). In our experience, non-specific granular cytoplasmic staining can also co-exist with true membranous staining and PD-L1 expression should be recognised in these. While Gottlieb et al. do specify that they assessed membranous PD-L1 staining of tumour cells and TAM, it is difficult to see the staining pattern in the relatively low magnification image provided. Also, these figures cannot demonstrate direct evidence of PD-L1 expression by CD68+ TAM as there are not of serial sections.

We do not mean to suggest that the results presented by Gottlieb et al are not valid. The extent of TAM PD-L1 expression observed in this cohort (74% of patients) and the concordance between primary and metastatic lesions are interesting findings. There is increasing evidence that TAM do express PD-L1 in HGSOC and that this has potential clinical implications (Webb et al., 2016; Curiel et al., 2003; Qu et al., 2016). However, we wish to emphasise that those looking to assess TAM PD-L1 expression should do so with some caution. Inclusion of appropriate staining controls and careful assessment of expression pattern are vital to the meaningful interpretation of these assays.

Conflict of interest statement

The authors have no conflicts of interest directly relevant to this publication.

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