mechanism and identify eligible patients more effectively.

Ethics approving committee
Yale Human Research Protection Program Human Investigation Committee #1201009533. First approved 1/17/2012; last re-approved: 1/17/2015. This study fulfilled the criteria for waiver of informed, written consent. It was performed according to the Declaration of Helsinki.

Conflict of interests
The authors have no conflicts of interests or sources of funding to disclose.

PD-L1 expression in tumour buds of colorectal carcinoma
DOI: 10.1111/his.12915
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Sir: Antiprogrammed death 1 (PD1) and antiprogrammed death ligand 1 (PD-L1) treatment effect tumour responses in a significant number of cases across various types of cancer, and is often substantial.1 This has fostered considerable optimism in the field of cancer immunotherapy. Even at this juncture, surgical pathologists are requested to provide immunohistochemical assessment of PD-L1 expression in biopsy or surgical resection specimens, because for malignant melanoma, bladder cancer and non-small cell lung cancer, at least, the expression of PD-L1 appears to be predictive of therapeutic effects.1 In these types of cancer, PD-L1 immunohistochemistry decorates intra- and peritumoural immune cells as well as the tumour cells themselves to varying degrees, making it amenable to scoring; as would be expected for a cell surface receptor, the immunostaining pattern is membranous. Effective anti-PD-L1 treatment of colorectal cancer patients in a Phase 2 study was shown to be restricted largely to patients with high-degree microsatellite-unstable tumours2 which, by PD-L1 immunohistochemistry, are reported in many cases as ‘peppered’ with large numbers of PD-L1-positive immune cells, macrophages in particular,3 whereas the tumour cells themselves are often negative.

In this communication we wish to draw attention to some details of PD-L1 immunohistochemistry in colorectal carcinoma that seem to have escaped previous investigators’ attention and that, in view of

Table 2. (Continued)

<table>
<thead>
<tr>
<th>CR</th>
<th>Odds ratio</th>
<th>CI</th>
<th>P</th>
<th>CR+PR</th>
<th>Odds ratio</th>
<th>CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classical Hodgkin lymphoma (n = 12)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% CD30 of the cellularity</td>
<td>1.09</td>
<td>(0.82, 1.45)</td>
<td>0.543</td>
<td>1.67</td>
<td>(0.29, 9.54)</td>
<td>0.563</td>
<td></td>
</tr>
<tr>
<td>Stain strength</td>
<td>1.93</td>
<td>(0.14–26.79)</td>
<td>0.625</td>
<td>5.90</td>
<td>(0.46–76.64)</td>
<td>0.174</td>
<td></td>
</tr>
</tbody>
</table>

CR, Complete remission; PR, partial remission; CI, confidence interval.

increasing requests for it by clinicians, may be of interest to surgical pathologists. A small series of colorectal carcinomas was selected from our archives for PD-L1 immunohistochemistry. Eight carcinomas were microsatellite-stable (MSS) and five were high-degree microsatellite-unstable carcinomas of the sporadic type (spMSI-H). Surgical pathologists are familiar with the fact that, at the invasive fronts of many colorectal carcinomas, distinction between activated fibroblasts, stromal immune cells and carcinoma cells can often be difficult, and we also found this to be the case when trying to determine the nature of PD-L1-positive cells. Therefore, sequential immunohistochemistry was applied. This recently described elegant technique allows immunophenotyping of cells in tissue sections with more than one immunohistochemical reaction. Our minor modifications of the original protocol consisted of using diaminobenizidine (DAB) for PD-L1 immunohistochemistry in the first step, thus conserving this immunostaining throughout the next steps (in which visualization was performed by Novared (Vector Laboratories, Peterborough, UK) as in the original protocol); using a non-aqueous mounting medium, thus preventing the rapid (within hours) diffusion of the Novared chromogen; and taking in-frame digital microphotographs (instead of scanning whole slides). PD-L1 immunohistochemistry was performed with the E1L3N antibody (Cell Signaling Technology, Danvers, MA, USA), followed by the CD68 (clone PGM-1) and cytokeratin (CK)18 immunoreactions. Viewing the microphotographs side by side on a double monitor we made the following observations: in eight tumours (five spMSI-H, three MSS), all without or at most low-degree tumour budding, a dense peritumoural rim of macrophages was seen, and in most cases many
Lipid droplets are involved in the process of high-grade transformation of adenoid cystic carcinoma

DOI: 10.1111/his.12916

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Sir: It is well known that the occurrence of high-grade transformation in adenoid cystic carcinoma (HGT–ACC) results in poor prognosis and a higher propensity for metastasis, which sets aside this entity from the main group of ACC. Histologically, the transformed component presents loss of myoepithelial cell differentiation and features of a moderately/poorly differentiated adenocarcinoma or solid carcinoma. The pathogenesis of different neoplasias is related to metabolic changes towards cell proliferation, based on the reprogrammed metabolism providing energy to cell division and growth. Concerning this matter, our group has already shown increased expression of glucose transporter 1 (GLUT1) in the transformed component of HGT–ACC, suggesting that the process of high-grade transformation may change the metabolic state of cancer cells. In addition to increased tumour glycolytic capacity, the activation of lipogenic pathways is another cancer-associated metabolic change which can lead to the accumulation of intracytoplasmic lipid droplets (LDs) in various human carcinomas. This phenomenon has been associated with differentiation, proliferation and aggressiveness of the tumour. Recently, we analysed the accumulation of LDs across a diverse group of salivary gland tumours with the antibody adipophilin (LDs marker). Regarding the ACC group, we found that only few lesions (26%) presented LD accumulation. Furthermore, in these ACC, LD was detected in only a small amount of cells (<50%).

To investigate whether the process of high-grade transformation of ACC could modify the accumulation of LD, we compared the expression of adipophilin between conventional (CA) and transformed areas (TA) in a series of HGT–ACC. It is of interest that adipophilin belongs to the PAT protein family (perilipin, adipophilin and tail-interacting protein of 47 kDa), which has been considered as a potential target for interventional strategies.

The present study was approved by the Institutional Ethics Committee and was performed in six cases of ACC–HGT which were retrieved from the files of the University of Campinas. The median age at tumour presentation was 55.8 years (range 44–65 years). The salivary glands affected were submandibular region (n = 2), palate (n = 1), lip (n = 1), parotid (n = 1) and paranasal sinus (n = 1). With regard to histopathological features, CA presented the morphological features of conventional ACC and TA was identified according to the recommendations of Seethala et al., and at least three of the major criteria were present. Concerning outcome, two patients presented distant metastasis and one patient died due to the disease. Median follow-up was 37.3 months (range 7–140 months).

Friedrich Prall
Maja Hühns
Institute of Pathology, University of Rostock, Rostock, Germany