Editorial for IJC-23-1164.R1:

Understanding the molecular biology of Anal Squamous Cell Carcinoma

Anal squamous cell carcinomas (ASCC) are strongly associated with prior infection with high-risk Human Papilloma Viruses (HPV). Although relatively sensitive to chemoradiotherapy, local recurrence requires salvage surgery with significant morbidity and is associated with poor outcomes. A better understanding of anal cancer biology is a recognised unmet research need [1] that Hamza and colleagues set out to address [2]. Surgical samples from 158 anal cancer patients with abdominoperineal resections following first line chemoradiotherapy (or radiotherapy alone) were assessed for pathogenic variants in 571 cancer-related genes, focusing on prognostic and targetable mutations. Mutations in PIK3CA and KMT2C were found to be associated with worse outcomes (overall survival) in HPV+ve patients, with 40% of all patients having targetable mutations. We congratulate the authors on a comprehensive analysis of a larger number of patients with a rare disease that supports including these patients in trials of precision medicine.

As with other mucosal squamous cell carcinomas, the HPV status of SCCA is associated with improved outcomes after initial radical treatment [3] but does not yet alter treatment approaches or regimens. Given that 90-95% of SCCA patients are HPV+ve, there is a higher absolute number of HPV+ve SCCA patients that relapse after first-line treatment. The differences in mutational profiles between these groups in this paper demonstrate the need for a better understanding of SCCA biology to inform future treatment. As discussed in the paper, all but one HPV+ve tumour in this cohort harboured mutations in the PI3K/AKT/mTOR pathway which is different to findings in HPV+ve head and neck [4] and penile cancers [5],

supporting the case for specific SCCA biology research rather than always extrapolating from the other HPV-associated cancers.

Although the samples in this study are from persistent or recurrent disease, similar results have been reported in primary tissue. Zhu et al profiled 116 SCCA cases [6], with 30% of HPV+ve cases having demonstrable mutations in or amplification of PI3KCA, 53% of HPV-ve cases containing p53 mutations and 21% CDKN2A. Comparisons between these studies are limited, given that there was no differentiation between treatment failure and treatment response in primary tissue, but the results are remarkably consistent. Characterisation of paired tissue from primary and recurrence tissue is a potential avenue for future work in this space.

This biology is also consistent with a panel of recently developed anal cancer cell lines [7]. PIK3CA mutations were found in three HPV+ve SCCA primary lines, with 2 showing some sensitivity to the PI3Kα-specific inhibitor BYL719 (Alpelisib), demonstrating the potential therapeutic utility of targeting this pathway. Two HPV+ve cell lines (one of primary origin the other derived from locally recurrent disease) also harboured KMT2C mutations. Other drugs could be tested using this model to further the case for inclusion of these patients in future precision medicine trials.

All patients in this study were not only the most advanced cases of SCCA but were those who had already failed first-line treatment. Some of these patients had radiotherapy only rather than standard CRT as first-line treatment before their surgery, suggesting some of these patients may have had poor performance status or contraindications to chemotherapy at

diagnosis. Therefore, although some of these tumours may have had targetable mutations, the patients may be more suitable for best supportive care rather than precision medicine. Assessment of potential differences in mutational profile between those treated with CRT and radiotherapy in this study would have been useful.

It is also important to consider the tumour microenvironment and see the mutational profiles identified here within the context of tumour-host interaction, where local immune response (e.g. tumour infiltrating lymphocytes (TILs)) [8] or relative hypoxia are seen to play a role. Loss of function KMT2C variants have been associated with response to immune checkpoint blockade in colorectal cancer and were consistent with TILs [9]. It would be interesting to assess TILs in these surgical samples and their association with both outcome and KMT2C status. Interestingly, in colorectal cancer KMT2C was also associated with microsatellite instability (MSI), whereas no patients in this study were MSI high. Assessment of KMT2C variants in published and ongoing immunotherapy trials for recurrent anal cancer may allow us to target patients most likely to benefit.

Less invasive techniques will play a role in our understanding of ASCC, whether circulating markers of disease (cell free DNA / circulating tumour cells) or imaging-based analyses. MRI is used routinely as part of the diagnostic work-up for SCCA and texture analysis has been used to predict p53 status in head and neck cancers [10]. Given high-quality imaging and outcome data available from previous anal cancer trials, if this approach could be assessed in SCCA images it would facilitate assessment of these mutations in a much larger cohort of patients and potentially support the development of biomarker-driven anal cancer trials.

In bringing all available technologies to bear on ASCC the future is bright for learning as much as possible about this disease, and ultimately improving outcomes for our patients.

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