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Original Research

Epstein-Barr virus and mismatch repair deficiency status differ between oesophageal and gastric cancer: A large multi-centre study

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Abstract ***Background:*** Oesophageal (OeC) and gastric (GC) cancer patients are treated with similar multimodal therapy and have poor survival. There remains an urgent clinical need to identify biomarkers to individualise patient management and improve outcomes. Therapy with immune checkpoint inhibitors has shown promising results in other cancers. Proposed biomarkers to predict potential response to immune checkpoint inhibitors include DNA mismatch repair (MMR) and/or Epstein–Barr virus (EBV) status. The aim of this study was to establish and compare EBV status and MMR status in large multi-centre series of OeC and GC.

Methods: EBV was assessed by EBV-encoded RNA (EBER) *in situ* hybridisation and MMR protein expression by immunohistochemistry (IHC) in 988 OeC and 1213 GC from

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multiple centres. In a subset of OeC, microsatellite instability (MSI) was tested in parallel with MMR IHC.

Results: Frequency of MMR deficiency (MMRdef) and MSI was low in OeC (0.8% and 0.6%, respectively) compared with GC (10.3%). None of the OeCs were EBER positive in contrast to 4.8% EBER positive GC. EBV positive GC patients were younger ($p = 0.01$), more often male ($p = 0.001$) and had a better overall survival ($p = 0.012$). MMRdef GC patients were older ($p = 0.001$) and showed more often intestinal-type histology ($p = 0.022$).

Conclusions: This is the largest study to date indicating that EBV and MMRdef do not play a role in OeC carcinogenesis in contrast to GC. The potential clinical usefulness of determining MMRdef/EBV status to screen patients for eligibility for immune-targeting therapy differs between OeC and GC patients.

1. Introduction

Oesophageal cancer (OeC) and gastric cancer (GC) are the eighth and fifth most common cancer worldwide, respectively, with an estimated total of 1,407,000 new cases and 1,123,000 deaths in 2012 [1]. The two main histological OeC subtypes are squamous cell carcinoma (SqC) and adenocarcinoma (AdC). The vast majority of GCs are adenocarcinomas.

In Europe, the standard of care for OeC and GC patients with locally advanced resectable disease is chemotherapy or chemoradiotherapy, followed by surgery [2,3]. GC patients receive perioperative platinum/fluorouracil based chemotherapy. For OeC, patients with SqC are treated with preoperative chemoradiotherapy with carboplatin/paclitaxel. Patients with AdC receive perioperative platinum/fluorouracil or preoperative chemoradiotherapy. Nevertheless, survival remains poor, with 5-year overall survival between 36 and 47% [4,5].

To date, few targeted therapy options are available to OeC/GC patients with metastatic disease: trastuzumab for HER2 positive disease [6] and ramucirumab, a VEGFR-2 antagonist without biomarker based patient selection [7,8]. All other trials evaluating receptor tyrosine kinase or downstream signalling inhibitors in OeC/GC were unable to show a survival benefit [9]. There remains an urgent clinical need to identify biomarkers to individualise and improve OeC/GC patient management.

DNA mismatch repair (MMR) has been used as a predictive biomarker for PD1 inhibitor therapy response in multiple different cancer types, including colorectal cancer [10]. Evidence of Epstein-Barr virus (EBV) infection has been proposed as a potential marker for response to PD1/PDL1 inhibitors in GC [11]. Pembrolizumab, an antibody against PD1, was approved by the FDA for the treatment of unresectable or metastatic solid tumours, including OeC and GC, with mismatch repair deficiency (MMRdef) or microsatellite instability (MSI)-High [12].

The potential of immunotherapy in OeC was shown recently in phase 2 trials in non-selected oesophageal SqC and GC patients treated with nivolumab, a monocolonal antibody inhibiting PD1, in second line treatment [13,14] and in a phase 3 trial in heavily pretreated non-selected Asian GC patients [15]. Furthermore, recent results from the phase 1b trials in patients with PD-L1 expressing OeC (KEYNOTE-028) and GC (KEYNOTE-012), showed promising activity of pembrolizumab in the metastatic setting [16,17]. In metastatic colorectal cancer, a phase 2 study demonstrated the clinical benefit of pembrolizumab in patients with MMRdef [18].

In addition to the potential role of MMR proteins in selecting patients for immunotherapy, MMRdef has shown prognostic value [19] and seems to predict a poor response to fluorouracil based chemotherapy in colorectal cancer [20,21]. It has been shown recently in MAGIC trial patients, that gastro-oesophageal cancer patients with MMRdef/MSI tumours treated with surgery alone survived longer compared with those treated with perioperative cytotoxic chemotherapy [22]. In OeC, MLH1 and MSH2 deficiency has been shown to be associated with poor prognosis in small series of SqC [23].

To date, the frequency of MMRdef/MSI in OeC cancer remains unclear because of the small sample size of studies. The reported frequency of MSI-High (MSI-H) ranges from 0 to 27%, but a number of previous studies did not distinguish between MSI-H and MSI-Low (MSI-L) (for an overview of all published studies on MMR and MSI in OeC, see Table 1). The recent study by The Cancer Genome Atlas (TCGA) did not find MSI in any of the 162 OeC [24]. With respect to the frequency of EBV infection in OeC, the majority of previous studies investigated SqC using different methodology, included relatively small number of patients and reported a frequency of EBV positivity from 0 to 36% (for an overview of all published studies on EBV in OeC, see Table 2). Thus, neither MSI/MMRdef nor EBV status has been investigated in large series of OeC