



Esophageal carcinoma: Towards targeted therapies

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Abstract

Background

Patients with esophageal cancer are confronted with high mortality rates. Whether it is esophageal squamous cell carcinoma (ESCC) or esophageal adenocarcinoma (EAC), patients usually present at advanced stages, with treatment options traditionally involving chemotherapy in metastatic settings. With the comprehensive genomic characterization of esophageal cancers, targeted therapies are gaining interest and agents such as ramucirumab, trastuzumab and pembrolizumab are already being used for the treatment of EAC.

Conclusions

Pembrolizumab has recently been FDA-approved for PD-L1 positive, locally advanced or metastatic ESCC. Despite comprehensive molecular characterization, however, available targeted therapies for ESCC are still lagging behind. Herein, we discuss current trends towards more targeted therapies in esophageal cancers, taking into consideration unique features of ESCCs and EACs. Patients progressing on standard therapies should be subjected to genomic profiling and considered for clinical trials aimed at testing targeted therapies. Future targeted therapies may include CDK4/6 inhibitors, PARP inhibitors and inhibitors targeting the NRF2 and Wnt signaling pathways. Ultimately, optimized biomarker assays and next generation sequencing platforms may allow for the identification of subcategories of ESCC and EAC patients that will benefit from selective targeted therapies and/or combinations thereof.

Keywords Esophageal carcinoma · Targeted therapies · Molecular pathways · Precision medicine · Clinical outcomes

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1 Introduction

The esophageal cancer incidence has been estimated to be ~500,000 cases worldwide in 2018. Most patients present in advanced stages and, therefore, the overall survival rate remains dismal: 20% at five years [1–3]. It is a heterogeneous disease that can broadly be categorized into esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC). To date, treatment options have been largely similar for the different types of esophageal cancer [1]. More recent cellular and molecular data suggest, however, that these types of cancer represent different entities [2]. ESCCs closely resemble head and neck cancers, whereas EACs mimic gastric cancers [2–4]. Herein, we review the latest findings on the molecular pathways driving esophageal cancer and highlight recent advances in targeted therapies. We also present a summary of clinical outcomes with respect to currently available targeted therapies. Finally, we focus on ongoing research in the field and discuss prospective options for translation into clinical use.

2 The esophagus

2.1 Anatomy and histology

Anatomically, the esophagus comprises three distinct sections of clinical relevance: cervical, thoracic and abdominal [1, 5, 6]. The cervical portion extends from the pharyngo-esophageal junction to the suprasternal notch. Esophageal cancers that arise here are mostly of squamous histology. The thoracic segment extends from the suprasternal notch to the diaphragmatic hiatus. The abdominal portion extends from the diaphragmatic hiatus to the esophago-gastric junction. Histologically, adenocarcinomas typically arise in this latter segment. For gastroesophageal adenocarcinoma, the Siewert-Stein classification uses anatomical location to help guide surgical intervention and treatment [7]. Siewert Type 1 cancers constitute adenocarcinoma of the distal esophagus and are treated as esophageal adenocarcinoma [7]. Siewert Type 2 cancers constitute adenocarcinoma of the cardia and Siewert Type III cancers constitute subcardial adenocarcinoma, both of which are treated in a similar fashion to gastric cancers [7].

Histologically, the esophageal wall consists of innermost mucosa, submucosa, muscularis propria and outermost adventitia. The mucosa is further subdivided into stratified squamous epithelium, lamina propria and muscularis mucosa. The muscularis propria consists of purely skeletal muscle (upper third of the esophagus), purely smooth muscle (lower third) and a transition mix of skeletal and smooth muscle in between. The adventitia includes loose connective tissue and connects the esophagus with its surrounding tissue [1, 5, 6].

2.2 Esophageal cancers

The majority of esophageal cancers are ESCC and EAC. Additionally, there exist small cell/neuroendocrine and rare basaloid esophageal cancers. ESCC occurs in the upper and mid-esophagus and originates from squamous epithelial lining of the esophagus [1, 5]. It is associated with smoking, alcohol consumption, diet (hot beverages, red meat and nutritional deficiencies), caustic injury and poor oral hygiene. ESCC has a relatively higher incidence among Eastern Asian and Eastern and Southern African populations [1, 5]. The mechanism of carcinogenesis involves direct mechanical injury secondary to exposure of the esophageal mucosa to carcinogens [1, 5]. This leads to epithelial changes from normal epithelium to basal cell hyperplasia, low-grade and high grade intra-epithelial neoplasia and, finally, invasive carcinoma [1, 5]. EAC occurs in the lower third of the esophagus and is thought to initiate around glandular cells near the stomach [1, 8]. It is associated with male gender, gastroesophageal reflux disease (GERD), obesity, reduced *H. pylori* infection, and has an increased incidence among European, North American and Australian populations [6, 8]. In this case, the mechanism

involves a chronic process of GERD causing metaplastic changes in the esophageal epithelium from normal squamous cell epithelium to columnar epithelium, known as Barrett's esophagus, to low grade dysplasia, high grade dysplasia and ultimately invasive adenocarcinoma [6]. According to a population-based national registry, the Surveillance Epidemiology and End Results (SEER) database for outcomes on ESCC versus EAC, there are no differences in overall survival between ESCC and EAC [9]. Chinese ESCC patients, however, tend to have a better overall survival compared to Caucasian ESCC patients [9].

Thus far, treatment options have been largely similar for both ESCC and EAC, and generally depend on the stage of esophageal cancer (Tables 1 and 2) [10–13]. Endoscopic resection for mucosal lesions versus esophagectomy for submucosal lesions is the main approach for early stage esophageal cancer [10–12]. For locally advanced cancers, based on the patient's performance status, neoadjuvant chemo-radiation followed by esophagectomy versus definitive chemo-radiation is offered in the case of ESCC [14–17]. For locally advanced EAC, neoadjuvant chemotherapy or chemo-radiation is offered with surgery, or surgery alone in low risk patients with well-differentiated tumors that are less than 2 cm in size. In the case of unresectable tumors, definitive chemo-radiation has been found to be associated with a better overall survival compared to radiation therapy alone [10–12, 18]. Management of metastatic disease includes palliative systemic therapy, radiation therapy or supportive care [10–12]. Endoscopic therapy of metastatic disease is offered for palliative purposes and includes balloon dilation to relieve dysphagia, resection of advanced lesions and placement of endoscopic stents [10–12]. Esophagectomy is associated with high morbidity and mortality rates [19]. Chemotherapy options include platinum-based regimens with 5-fluorouracil or taxanes and sometimes anthracyclines, all of which exhibit considerable cytotoxic effects. To mitigate these limited therapeutic options, there is an increasing interest in the molecular characterization of esophageal cancers allowing the design of personalized targeted therapies that can improve overall patient survival and quality of life.

3 Molecular characterization of esophageal cancers

A number of studies has used molecular analyses to characterize esophageal cancers, to identify driver mutations and to uncover therapeutic targets (Tables 3 and 4). Here we review several seminal studies that highlight the molecular characteristics of ESCCs and EACs, respectively.

Table 1 TNM staging system

Category	Criteria
<i>T Category</i>	
TX	Tumor cannot be assessed
T0	No evidence of primary tumor
Tis	High grade dysplasia
T1	Tumor is in the lamina propria, muscularis mucosa or submucosa
T1a	Tumor is in the lamina propria or muscularis mucosa. (M1 epithelium, M2 lamina propria, M3 muscularis mucosa)
T1b	Tumor is in the submucosa (SM1 upper third of submucosal layer, SM2 middle third of the submucosal layer, SM3 lower third of submucosal layer)
T2	Tumor is in the muscularis propria
T3	Tumor is in the adventitia
T4	Tumor is in the adjacent structures
T4a	Tumor is in the pleura, pericardium, azygos vein, diaphragm or peritoneum
T4b	Tumor is in the adjacent structures, such as aorta, vertebral body or trachea
<i>N Category</i>	
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	1–2 regional lymph nodes involved
N2	3–6 regional lymph nodes involved
N3	7 or more regional lymph nodes involved
<i>M Category</i>	
M0	No distant metastasis
M1	Distant metastasis

Adapted from AJCC [13]

T refers to the depth of cancer invasion into the esophageal wall in cm, which can be determined through imaging or endoscopic resection; N refers to the number of lymph nodes involved and M refers to the presence of metastasis. The Japanese esophageal society (JES) further classifies T1a and T1b tumors into (M1, M2, M3) and (SM1, SM2, SM3), respectively, depending on the esophageal wall layer involved as highlighted in brackets [10, 90]

3.1 ESCC

A number of studies from China and Japan has provided comprehensive analyses of driver mutations in ESCCs as listed in Table 3 [3, 4, 20–27]. One of the earlier studies considered 158 Chinese patients with ESCC and identified 8 significantly mutated genes and highlighted alterations in key pathways including cell cycle, TP53, NOTCH, Wnt and RTK/PI3K pathways [25]. *ZNF750* is a known tumour suppressor gene, with mutations leading to impaired differentiation causing ESCC [28]. Genetic alterations in *CDKN2A*, *PIK3CA*, *TGFBR2* and, less commonly in *ERBB*, *EGFR* and *BRCA* have been noted and may provide opportunities to improve clinical outcomes through targeted therapies.

The Cancer Genome Atlas (TCGA) integrated genomic analyses of 164 esophageal cancers from eastern and western hemispheres, including ESCCs and EACs [2]. Similar to previous studies, common mutations were found in the *TP53* gene, which occur in the majority of dysplastic lesions and malignant ESCC lesions [2]. In addition, mutations were found in *NOTCH1*, *NFE2L2*, *ZNF750*, *PIK3CA* and

CDKN2A. Notable amplifications of *SOX2*, *TERT*, *FGFR1*, *MDM2*, *NKX2-1* and *CCND1*, and deletions of *RB1*, *VGLL4* and the negative regulator of the Hippo pathway and autophagy factor *ATG7* were highlighted. Genomic alterations affecting the histone-modifying factors *KDM6A*, *KMT2D* and *KMT2C* were also found to be more common in ESCCs. The TCGA also integrated clustering of somatic copy number alterations, DNA methylation, mRNA and microRNA expression levels in ESCCs to further classify these cancers into three subtypes [2]. Type I ESCCs tend to be more common among Vietnamese, exhibit alterations in the NRF2 pathway, are associated with a poor prognosis and are resistant to chemo-radiotherapy [2]. This subtype has a higher frequency of *SOX2* and/or *TP63* amplifications and resembles lung SCC and head and neck SCC [2]. Type II ESCCs tend to be more common among Eastern Europeans and South Americans and are associated with higher mutations rates in the *NOTCH1* and *ZNF750* genes, more frequent inactivating alterations of *KDM6A* and *KDM2D*, *CDK6* amplifications and inactivation of *PTEN* or *PIK3R1* [2]. This type of ESCC also exhibits a greater leukocyte infiltration. Finally, type III ESCCs, which represent the lowest number of cases, occur more commonly

Table 2 Staging of esophageal cancer including ESCC and EAC (Adapted from AJCC) [13]

Stage	T	N	M
<i>ESCC</i>			
0	Tis	N0	M0
I	T1	N0–1	M0
II	T2	N0–1	M0
	T3	N0	N0
III	T3	N1	M0
	T1–3	N2	M0
IVA	T4	N0–2	M0
	T1–4	N3	M0
IVB	T1–4	N0–3	M1
<i>EAC</i>			
0	Tis	N0	M0
I	T1	N0	M0
IIA	T1	N1	M0
IIIB	T2	N0	M0
III	T2	N1	M0
	T3–4a		
IVA	T1–4a	N2	M0
	T4b	N0–2	M0
	T1–4	N3	M0
IVB	T1–4	N0–3	M1

among North Americans and exhibit alterations predicted to activate the PI3K pathway [2].

3.2 EAC

Based on TCGA data, gastric cancers are classified into four subtypes including EBV-positive cancers, genomic stable cancers, microsatellite unstable cancers and cancers with chromosomal instability (CIN) [2]. EACs tend to more closely resemble gastric cancers with CIN, characterized by structural DNA variations and copy number changes. These cancers exhibit oncogene amplifications, including *ERBB2*, *KRAS*, *EGFR*, *IGF1R* and *VEGFA*. With respect to cell cycle dysregulation, EACs exhibit mutations in *CDKN2A* and amplifications of *CCNE1*. Regarding epigenetic modifications, alterations affecting the SWI/SNF-encoding genes *ARID1A*, *SMARCA4* and *PBRM1* have been found to be more common in EAC [2].

Using a cohort of 551 genomically characterized EACs with matched RNA sequencing data, a recent study identified 77 EAC driver genes, 21 noncoding driver elements, and highlighted potential therapeutic targets [29]. Of note, *GATA 4* amplification and *SMAD4* mutation or homozygous deletion were found to be associated with significantly poorer prognoses. Novel EAC drivers included *B2M*, which encodes a core component of the MHC class I complex and is a marker of acquired resistance to immunotherapy and *ABCBI*, which

encodes a channel pump protein that is associated with drug resistance [29]. Chromatin-modifying genes belonging to the SWI/SNF complex were also found to be selectively mutated. In terms of sub-classification of EACs, mutation analysis of 129 EACs identified 3 dominant subtypes with potential personalized therapeutic targets. The DNA damage response (DDR)-impaired subtype was found to be genomically unstable and to be deficient in the homologous recombination repair (HR) pathway, suggesting a potential role for PARP inhibitors in addition to DNA-damaging agents. The C > A/T dominant mutation subtype exhibited age as a risk factor and a higher frequency of *ERBB2/MET* co-amplifications that might benefit from combined receptor tyrosine kinase (RTK) inhibitors. The mutagenic subtype showed higher mutation rates as well as higher levels of immune-mediated signaling with neo-antigen presentation, which could benefit from PD1 blockade therapy [29].

4 Targeted therapies

Despite recent advances in identifying genomic drivers of esophageal cancer, only a handful of targeted therapies are clinically available. Trastuzumab, ramucirumab and pembrolizumab are three agents that have been approved by the US FDA for the treatment of advanced and/or metastatic gastroesophageal cancers [30–34]. Below, we review completed and ongoing clinical trials of targeted therapies and suggest potential options for future targets (Fig. 1).

4.1 Anti-angiogenesis agents

EACs tend to over-express pro-angiogenesis factors, such as vascular endothelial growth factor (VEGF), which is secreted by both tumor and stromal cells to form blood vessels allowing tumor progression [35]. Targeting angiogenesis is used as a second line treatment in advanced EACs. Various clinical trials have been conducted with bevacizumab as a VEGF inhibitor, but without clear clinical benefit [35, 36]. As yet, ramucirumab, an anti-VEGFR2 monoclonal antibody, is the only anti-angiogenesis agent that is approved as a single agent or in combination with paclitaxel to treat patients with advanced esophageal and gastro-esophageal junction (GEJ) adenocarcinomas who have progressed on fluoropyrimidine/platinum chemotherapy [32, 34]. In a ramucirumab monotherapy trial for previously treated advanced gastric or GEJ adenocarcinomas (REGARD), a total of 355 patients was randomized to ramucirumab versus placebo [32]. The results showed that patients who received ramucirumab had a higher median progression-free survival (PFS) (2.1 vs 1.3 months) and a higher overall survival (OS) (5.2 vs 3.8 months, $p = 0.047$) compared to those who received conservative

Table 3 Summary of molecular features in ESCCs

Study	ESCC
Lin et al., 2014 [4] N = 139 samples, Chinese patients	The study identified 31 genes with alterations in ESCC including <i>ERBB4</i> , <i>PTEN</i> , <i>PIK3CA</i> , <i>MAP3K15</i> , <i>JAK1</i> , <i>TP53</i> , <i>ZNF750</i> , <i>FAT1</i> , <i>FAT2</i> and <i>FAT3</i> . The study noted common <i>CCND1</i> amplification and <i>CDKN2A</i> deletion. Altered pathways included the mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K) pathways, cell cycle progression, ERBB, HDAC and PI3K family, XPO1, FGFR1, TP53, JAK-STAT3 and MTOR-RPS6K signaling.
Goa et al., 2014 [3] N = 113 samples, Chinese patients	The study identified a total of 70 genes altered in at least 50% of the cases, including <i>TP53</i> , <i>CCND1</i> , <i>CDKN2A</i> , <i>NFE2L2</i> , and <i>RB1</i> , <i>KMT2D</i> , <i>KMT2C</i> , <i>KDM6</i> , <i>EP300</i> , <i>CREBBP</i> , <i>FAT1</i> , <i>FAT2</i> , <i>FAT3</i> or <i>FAT4</i> or <i>AJUBA</i> , <i>NOTCH1</i> , <i>NOTCH2</i> <i>NOTCH3</i> and <i>FBXW7</i> .
Song et al., 2014 [25] N = 158 samples, Chinese patients	Common genetic mutations included <i>TP53</i> , <i>RB1</i> , <i>CDKN2A</i> , <i>PIK3CA</i> , <i>NOTCH1</i> , <i>NFE2L2</i> , <i>ADAM29</i> , <i>FAM135B</i> , <i>KMT2D</i> , <i>ASH1L</i> , <i>KMT2C</i> , <i>SETD1B</i> , <i>CREBBP</i> , <i>EP300</i> . Gene amplifications included <i>CCND1</i> , <i>CDK4</i> , <i>CDK6</i> , <i>E2F1</i> and <i>MDM2</i> . Gene deletions included <i>RB1</i> , <i>CDKN2A</i> , <i>CHEK1</i> , <i>CHEK2</i> and <i>TP53</i> . Altered pathways include Wnt pathway, cell cycle, NOTCH pathways, RT/RAS/AKT pathways.
Zang et al., 2015 [27] N = 104 samples, Chinese patients combined with 88 previously reported samples	The study identified three mutational signatures: signature A was characterized by C > G, C > T, and C > A mutations associated with <i>ADOBEC</i> mutations; signature B was characterized by C > T mutations at XpCpG trinucleotides; signature C was represented by types which are yet to be determined. Common gene mutations included <i>APOBEC</i> , <i>PIK3CA</i> , <i>AJUBA</i> , <i>ZNF750</i> , <i>PTCH1</i> , <i>CREBBP</i> and <i>BAP1</i> , <i>ZNF750</i> , <i>FAT1</i> , <i>FAT2</i> , and <i>FAM135B</i> , <i>PLCE</i> , <i>XPF</i> , <i>ALDH2</i> , and <i>MTHFR</i> . Amplifications included <i>CBX4</i> , <i>CBX8</i> , and <i>SOX2</i> . Altered pathways included hedgehog signaling, PI3K pathway, cell cycle, NOTCH signaling, PI3K signaling, cell adhesion, MAPK and chromatin-remodeling genes.
Cheng et al., 2016 [21] N = 31 samples, Chinese patients	Common gene alterations included <i>FGFR1</i> , <i>CDKN2A</i> , <i>LETM2</i> , <i>CDCA7</i> , <i>TRAPPC9-CLVS1</i> , <i>EIF3E-RAD51B</i> , <i>CCND1</i> , <i>MYC</i> , <i>MMPs</i> , <i>EGFR</i> and <i>ERBB2</i> . Higher frequency of C > G transversions occurred in ESCCs than EACs, whereas A > C transitions were more frequent in EACs than ESCCs.
Sawada et al., 2016 [66] N = 144 samples, Japanese patients	The study identified 15 common gene mutations, <i>TP53</i> , <i>NOTCH1</i> , <i>MLL2</i> , <i>NFE2L2</i> , <i>ZNF750</i> , <i>FAT1</i> , <i>PIK3CA</i> , <i>EP300</i> , <i>CDKN2A</i> , <i>CREBBP</i> , <i>NOTCH3</i> , <i>TET2</i> , <i>FBXW7</i> , <i>TGFBR2</i> and <i>AJUBA</i> . Amplifications included 3q, 8q, 5p, 7p and 20q. Deletions included 3p, 9p, 4p, 5q, and 13p and loss of heterozygosity in 9p, 3p, 9q, 13q, 13p, and 17p.
Qin et al., 2016 [24] N = 67 samples, Chinese patients combined with survival data of a cohort of 321 samples	Common gene mutations included <i>TP53</i> , <i>TTN</i> , <i>NOTCH1</i> , <i>NFE2L2</i> and <i>CDKN2A</i> . ESCCs with deleterious <i>NOTCH1</i> mutations or down-regulated gene expression had a better prognosis. Gene amplifications included <i>MYBL2</i> , <i>TP73</i> on 1p36, <i>SOX2</i> on 3p24.3, <i>FGFs</i> (<i>FGF19</i> , <i>FGF4</i> and <i>FGF3</i>), <i>SHANK2</i> on 11q13.2–11q13.4, <i>FOXA1</i> on 14q21.1 and <i>MYC</i> on 8p23.3. Gene deletions included <i>TP53</i> , <i>CDKN2A</i> , <i>NFE2L2</i> , <i>LRP1B</i> , <i>FHIT</i> , <i>TGFBR2</i> and <i>FOXP1</i> . Altered pathways included the TP53, cell-cycle, NOTCH, Wnt and the cadherin signaling pathways.
Xiong et al., 2017 [26] N = 360 samples, East Asian patients	The study identified four distinct subtypes with prognostic significance and potential for personalized medicine: ESCC1 tumors were mostly well-differentiated and had a better survival than other subtypes. ESCC2 tumors were mostly metastatic, associated with poor differentiation, epithelial to mesenchymal transition (EMT), higher stroma, higher immune content, and a poor prognosis. The ESCC3 subtype was much less well-characterized and needs further investigation. The ESCC4 subtype was associated with CIN, a poor prognosis, over-expression of <i>MYC</i> target genes, and a high frequency of loss of heterozygosity (LOH).
Chang et al., 2017 [20] N = 94 samples, Chinese patients combined with 704 reported samples	The study identified 20 putative driver genes, including: <i>MLL2</i> , <i>FAT1</i> , <i>PIK3CA</i> , <i>EP300</i> , <i>ZNF750</i> , <i>CREBBP</i> , <i>NOTCH1</i> , <i>NOTCH3</i> , <i>PTCH1</i> , <i>RB1</i> , <i>KDM6A</i> , <i>TGFBR2</i> , <i>PTEN</i> , <i>CUL3</i> , <i>RBPJ</i> , <i>CDKN2A</i> , <i>FBXW7</i> , <i>NFE2L2</i> , <i>AJUBA</i> and <i>TP53</i> . Among the mutations, those in <i>TP53</i> , <i>CDKN2A</i> and <i>PIJ3CA</i> were common in EACs. The study identified six signatures: Signature E1 associated with APOBEC alterations, Signature E2 associated with smoking, Signature E3/5 associated with aging, Signature E4 associated with T > C mutations and Signature E6 associated with T > A, as well as T > G mutations. Genomic amplifications included 3q, 5p, 7p, 8q, 12p, 16p, 20p and 20q.

Table 3 (continued)

Study	ESCC
	Genomic deletions included 3p, 4p, 4q, 5q, 9p, 13q, 18q and 21p. Altered pathways included the K/RAS/ PI3K, cell cycle regulation, Wnt, NOTCH and TP53 pathways.
TCGA, 2017 [2] <i>N</i> = 164 samples, including eastern and western patients	Common gene mutations included <i>TP53</i> , <i>NFE2L2</i> , <i>MLL2</i> , <i>ZNF750</i> , <i>NOTCH1</i> , <i>TGFBR2</i> , <i>KDM6A</i> , <i>KMT2D</i> and <i>KMT2C</i> and <i>EGFR</i> . Gene amplifications included <i>SOX2</i> , <i>TERT</i> , <i>FGFR1</i> , <i>MDM2</i> and <i>NKX2-1</i> . Gene deletions included 3p25.2, the negative regulator of the Hippo pathway <i>VGLL4</i> and the autophagy factor <i>ATG</i> , as well as <i>RB1</i> .
Deng et al., 2017 [22] <i>N</i> = 158 samples, Chinese (<i>N</i> = 78), Caucasian (<i>N</i> = 39) and Vietnamese (<i>N</i> = 41) patients	<i>CSMD3</i> mutations were prognostic in Asians. <i>TP53</i> , <i>NFE2L2</i> and <i>EP300</i> had a significantly higher mutation rates in Asian populations, while <i>KRTAP9-1</i> , <i>LRFN5</i> and <i>MAP2</i> were more significantly mutated in Caucasians.
Deng et al., 2019 [23] <i>N</i> = 77 samples, Chinese Patients	The study identified 84 pathogenic/likely pathogenic mutations and 51 rare variants of uncertain significance (VUS). Gene mutations included <i>TP53</i> , <i>CYP21A2</i> , <i>SPINK1</i> , <i>OTOA</i> , <i>PRAMEF7/8</i> and <i>PRAMEF10/33</i> . Altered pathways included the galactose metabolism, MAPK signalling and central carbon metabolism pathways.
Yan et al., 2019 [75] <i>N</i> = 39 samples, Chinese patients	Driver gene mutations included <i>TP53</i> , <i>PIK3CA</i> , <i>ZNF750</i> and <i>BRCA2</i> . Mutations in <i>NOTCH1</i> , <i>CREBBP</i> , <i>PTCH1</i> , <i>MET</i> , <i>mTOR</i> and <i>AXIN2</i> occurred later during tumorigenesis. The study identified five dominant signatures: aging, activation of APOBEC family members, deficient homologous recombination repair and <i>BRCA1/2</i> mutations, mismatch repair deficiency and aristolochic acid exposure.

treatment [32]. In a phase III ramucirumab plus paclitaxel versus placebo plus paclitaxel trial in patients with previously treated advanced gastric or GEJ adenocarcinomas (RAINBOW), a total of 665 patients who progressed on fluoropyrimidine/platinum-containing chemotherapy was randomized to receive ramucirumab combined with weekly

paclitaxel versus placebo plus weekly paclitaxel. Patients who received the treatment combination showed an improved OS (9.6 vs 7.4 months) compared to those who received paclitaxel alone [34]. The side effect profile of ramucirumab includes hypertension, thromboembolic events, rash, diarrhea and myelosuppression [31, 34].

Table 4 Summary of molecular features in EACs

Study	EAC
Dulak et al., 2013 [91] <i>N</i> = 149 samples, Caucasian patients	The study identified 26 altered genes including <i>TP53</i> and <i>CDKN2A</i> , being mostly affected, and <i>ARID1A</i> , <i>PIK3CA</i> , <i>SMAD4</i> , <i>ELMO1</i> , <i>DOCK2SMARCA4</i> , <i>ARID2</i> , <i>SPG20</i> , <i>TLR4</i> , <i>AKAP6</i> , <i>HECW1</i> , <i>AJAP1</i> , <i>KRAS</i> , <i>CTNBN1</i> and <i>ERBB2</i> . Altered pathways included the PI3K, TGFβ/SMAD, cell cycle and Wnt pathways.
Sercier et al., 2016 [29] <i>N</i> = 129 samples, Caucasian patients	Common gene mutations included <i>TP53</i> (81%), <i>ARID1A</i> (17%), <i>SMAD4</i> (16%), <i>CDKN2A</i> (15%), <i>KCNQ3</i> (12%), <i>CCDC102B</i> (9%), <i>CYP7B1</i> (7%) and <i>SYNE1</i> (23%). Three dominant signatures included C > A/T dominant (age), DNA Damage Repair (DDR) impaired/ <i>BRCA</i> mutated and mutagenic.
TCGA, 2017 [2] <i>N</i> = 164 samples, ESCC (<i>N</i> = 90), EAC (<i>N</i> = 72) including eastern and western patients	Common gene mutations included <i>TP53</i> , <i>CDKN2A</i> , <i>ARID1A</i> , <i>SMAD4</i> and <i>ERBB2</i> , <i>ARID1A</i> , <i>SMARCA4</i> and <i>PBRM1</i> . Gene amplifications included <i>VEGFA</i> , <i>ERBB2</i> , <i>GATA6</i> and <i>CCNE1</i> , <i>KRAS</i> , <i>EGFR</i> , <i>IGF1R</i> or <i>VEGFA</i> , <i>GATA4</i> and <i>GATA6</i> .
Dai et al., 2018 [92] <i>N</i> = 10 samples, Chinese patients	Common gene mutations included <i>NBPF20</i> , <i>NOTCH1</i> , <i>TP53</i> , <i>MUC16</i> , <i>F8</i> , <i>HARS</i> , and <i>TTN</i> .
Frankell et al., 2019 [86] <i>N</i> = 551 samples, Caucasian patients	The study identified 76 EAC driver genes and 21 noncoding driver elements. Common gene mutations included <i>ARID2</i> , <i>RNF43</i> , <i>ARID1B</i> , <i>ERBB2</i> , <i>B2M</i> , <i>MUC6</i> , <i>ABCBI</i> , <i>SMAD4</i> and <i>TP53</i> . The study noted <i>CDKN2A</i> over-expression, <i>MYC</i> over-expression and <i>GATA4</i> amplification. Alterations included activation of the Wnt pathway and mutations in the SWI-SNF complex.

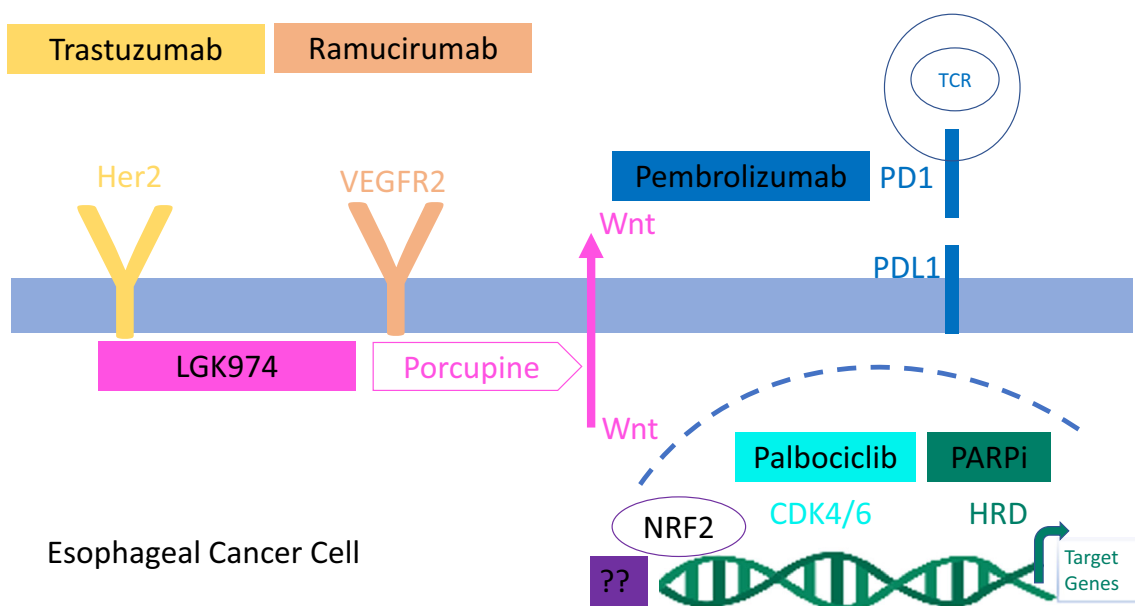


Fig. 1 Schematic representation of targeted pathways in esophageal cancer. Current FDA-approved therapies include trastuzumab for advanced HER2-positive esophageal/GEJ adenocarcinomas, ramucirumab for advanced esophageal/GEJ adenocarcinomas and pembrolizumab for advanced esophageal cancers, whether ESCC or EAC, expressing PD-

L1. Potential targets include CDK4/6 inhibitors, such as palbociclib in esophageal cancers that over-express cell cycle genes, PARP inhibitors for esophageal cancers with HR deficiency and/or *BRCA 1* mutations, LGK974 for esophageal cancers with an activated Wnt signaling pathway, and a possibility for NRF2 targeting in ESCC

4.2 Anti-proliferation agents

As previously noted, EACs may exhibit activating mutations in proliferation-related pathways, including those in the epidermal growth factor receptor (EGFR) and the human epidermal growth factor receptor 2 (HER2) [30, 35, 37, 38]. Targeting EGFR has been investigated in various clinical trials without clinical benefit [39]. In contrast, targeting HER2 has become standard of care in HER2-positive (HER2+) metastatic EACs. Tumors are considered to be HER2-positive when they exhibit a 3+ HER2 expression score by immunohistochemistry (IHC) or when they exhibit HER2 amplification as detected by FISH [30].

Trastuzumab is a monoclonal antibody that selectively targets the extracellular domain of HER2 and causes antibody-mediated toxicity in HER2 over-expressing cells [30]. In the ToGa trial, assessing the addition of trastuzumab to chemotherapy for HER2-positive advanced gastric or GEJ cancers, 3665 patients were screened for HER2 by IHC/FISH and 22% were scored HER2-positive; 584 patients were randomly assigned to receive trastuzumab plus chemotherapy versus chemotherapy alone (cisplatin/fluoropyrimidine). Patients meeting the current standards for HER2-positive disease that received trastuzumab showed a higher median OS (mOS) (16.0 vs 11.8 months) [30]. The side effect profile of trastuzumab includes cardio-toxicity, which is largely reversible, nausea, vomiting, diarrhea and musculoskeletal pain [30].

Pertuzumab is a monoclonal antibody that inhibits dimerization of HER2 with other HER family members [36]. It can

result in cell-mediated toxicity as well as inhibition of downstream MAP kinase and PI3K signaling, leading to growth arrest and apoptosis [36]. Unlike breast cancer [40], adding pertuzumab to trastuzumab did not significantly improve the OS in EACs [37]. In the phase III JACOB trial, 780 patients with metastatic gastric and GEJ cancers were randomized to receive trastuzumab/chemotherapy \pm pertuzumab. After a median follow-up of approximately 2 years, the addition of pertuzumab provided a non-significant improvement in mOS (17.5 months vs 14.2 months) [37]. Currently, a trial is ongoing assessing the addition of trastuzumab, with or without pertuzumab, to chemotherapy versus chemotherapy alone in the neoadjuvant treatment of gastric/GEJ adenocarcinomas [41].

Lapatinib, a small molecule inhibitor of both EGFR type 1 and HER2, was not found to improve the OS when combined with chemotherapy as a first line treatment in HER2-positive esophageal cancers. This was noted in the phase III TRIO-013/LoGiC trial, where 545 previously untreated patients with HER2-positive gastric, esophageal and GEJ adenocarcinomas received capecitabine/oxalipatin with or without lapatinib. No statistically significant differences were observed in the mOS and/or PFS between the two treatment arms [42].

Trastuzumab emtansine (T-DM1), an antibody-drug conjugate of trastuzumab and the cytotoxic agent emtansine (DM1), a microtubule inhibitor, did not yield any clinical benefit in patients with advanced gastroesophageal cancers who were previously treated with chemotherapy plus HER2-targeted therapy. T-DM1 was compared to chemotherapy (docetaxel

or paclitaxel) as a second line treatment for HER2-positive advanced gastric and GEJ cancers in the phase 2/3 GATSBY trial with no effect on mOS [38].

4.3 Immunotherapy

Tumor cells evade the immune system by modulating inhibitory signals on T cell receptors resulting in immune tolerance [43, 44]. In particular, tumor cells express programmed death ligand 1 (PD-L1), which binds programmed death (PD) receptors on T cells and inhibit their function. They can modulate CTLA4, which is known to inhibit T cell function. The overall aim of immunotherapy in cancer management is to release the inhibition on T cells such that they can attack the tumor cells. The risk is activation of the immune system elsewhere in the body, which can cause immune-mediated adverse effects including, but not limited to, colitis, transaminitis, pneumonitis, thyroiditis and dermatitis [43].

As indicated above, molecular characterization of esophageal cancers has led to the identification of subsets of ESCCs and EACs that could benefit from immunotherapy [26]. Checkpoint inhibitors include monoclonal antibodies directed against PD1/PD-L1/CTLA4, which are used in various solid tumors such as melanoma, renal cell carcinoma and non-small cell lung carcinoma [43]. Key clinical trials have shown significantly improved OS rates when using immunotherapy compared to conventional chemotherapies.

In the phase II KEYNOTE-059 trial, patients with previously treated advanced gastric and GEJ cancers who had progressed on at least two lines of therapy received pembrolizumab [31]. The objective response rate (ORR) was 11.6% with a median response duration of 8.4 months. Interestingly, the ORR was 15.5% for patients with a tumor PD-L1 expression $\geq 1\%$ and 6.4% for patients with a tumor PD-L1 expression $< 1\%$ [31]. PD-L1 expression status was determined by IHC using a PD-L1 IHC 22C3 pharmDx assay. PD-L1 positive tumors exhibited combined positive scores (CPS) ≥ 1 calculated as the number of PD-L1-positive tumor cells, lymphocytes and macrophages divided by the total number of tumor cells [31]. Based on these results, pembrolizumab is currently approved by the FDA as a third-line treatment modality for advanced gastric and GEJ adenocarcinomas with a PD-L1 expression score $\geq 1\%$ [45].

In the phase II KEYNOTE-180 study, 121 previously treated esophageal cancer patients with ESCC, EAC or GEJ who progressed after two or more lines of therapy received the single agent pembrolizumab. The overall response rate was 10% with a mOS of 5.8 months [33]. The ORR was 14.3% among patients with ESCC, 5.2% among patients with EAC, 13.8% among patients with PD-L1 positive tumors and 6.3% among patients with PD-L1 negative tumors [33]. In this study, PD-L1 positive tumors had CPS scores ≥ 10 . The data suggest that immunotherapy may be most beneficial in PD-L1

positive ESCCs. As a result of this study and the KEYNOTE-181 trial [46], pembrolizumab has recently been FDA approved as a second-line option for recurrent, locally advanced or metastatic PD-L1 positive ESCCs with CPS scores $\geq 10\%$ [47].

In the phase III KEYNOTE-061 trial, 395 patients requiring second-line treatment for gastric or GEJ adenocarcinomas with a PD-L1 CPS score of $\geq 1\%$ received either pembrolizumab or paclitaxel. The mOS was not found to be significantly longer with pembrolizumab (9.1 months) versus paclitaxel (8.3 months), HR = 0.82, 95% CI 0.66–1.02 [48]. This study suggested that a PD-L1 CPS score of $\geq 1\%$ may not be enough to identify cancers that will preferentially benefit from immunotherapy. Considerations of higher scores or other biomarkers are warranted.

In the phase III ATTRACTION-2 trial, which was conducted in Asia, patients with advanced gastric or GEJ cancers who were refractory to at least two previous lines of therapy received nivolumab versus placebo [49]. Nivolumab was found to improve the mOS (5.3 months) compared to placebo (4.1 months) and the ORR of 11% was found to be independent of PD-L1 positivity [49]. In the ATTRACTION-4 trial, the same research group evaluated combinations of nivolumab with different chemotherapy regimens, including S-1 plus oxaliplatin (SOX) or capecitabine plus oxaliplatin (CAPOX) as first-line therapies for unresectable advanced HER2-negative gastric and GEJ cancers. The study included 40 patients and the results were promising, showing that the combination of nivolumab with SOX resulted in an ORR of 57.1% and a PFS of 9.7 months, while the combination of nivolumab with CAPOX resulted in an ORR of 76.5% and a PFS of 10.6 months. A phase III clinical trial is currently underway [50]. In the JAVELIN Gastric 300 trial avelumab did not improve the OS compared to third-line chemotherapy or best supportive care (BSC) [51]. Ipilimumab did not improve the PFS compared to BSC in a phase II study of 143 patients with pretreated gastric or GEJ adenocarcinomas [52]. In the CheckMate-032 phase I/II trial 160 patients with advanced or metastatic chemotherapy-refractory gastric, esophageal and GEJ cancers received nivolumab or nivolumab/ipilimumab in different dosing schedules [53]. The ORRs ranged from 8 to 24% with a median duration of response > 7 months [53]. A phase III trial is underway.

In the phase III KEYNOTE-062 randomized clinical trial 763 patients with advanced gastric or GEJ adenocarcinomas were randomly assigned to receive first line pembrolizumab, pembrolizumab plus chemotherapy or chemotherapy alone [54]. All patients had PD-L1 CPS scores ≥ 1 , and 37% had a score ≥ 10 [54]. This was the first study showing that pembrolizumab monotherapy was non-inferior to chemotherapy as a first line treatment. The greatest benefit was seen in patients with CPS scores ≥ 10 where the 2-year OS was 39% for pembrolizumab vs 22% for standard chemotherapy, and

the mOS was 17.4 months vs 10.8 months [54]. Moving forward, pembrolizumab monotherapy will likely be offered as a first line treatment option in advanced gastric and GEJ cancers that express PD-L1 with CPS scores ≥ 10 . Ongoing phase III clinical trials include CheckMate-577, which is assessing stage II/III esophageal/GEJ cancer status post chemoradiation therapy, followed by surgery randomized to receive nivolumab or placebo in the adjuvant setting [55]. CheckMate-649 is assessing the efficacy of nivolumab plus ipilimumab or nivolumab with chemotherapy compared to chemotherapy in previously untreated advanced gastric/GEJ cancers [56].

Of note, there are various assays to test for PD-L1 expression depending on which epitope was designed for the antibody used in IHC. There are different antibodies for different checkpoint inhibitors, as well as different cut-offs for PD-L1 positive versus negative scores in different cancers [57]. The variance in PD-L1 testing creates confusion and affects patient outcome as it dictates who is included/excluded from immunotherapy. There is a pressing need for a uniform assay [58].

Tumors with a high microsatellite instability (MSI) and mismatch repair (MMR) deficiency exhibit high mutational burdens and generate neo-antigens that can be used as response predictors to immunotherapy [59]. Identifying EACs and ESCCs with MMR deficiency provides opportunities for immunotherapy. MSI-high tumors can be identified via immunohistochemistry for the mismatch repair proteins MLH1, MSH2, MSH6 and PMS2 [60] or via PCR-based techniques to assess the mononucleotide repeat markers NR1, NR24, BAT25, BAT26 and MONO27 [30]. Future tests will be aimed at combining next generation sequencing, MSI testing and tumor mutational burden to identify cancers eligible for immunotherapy [60]. Targeted immunotherapy refinement in esophageal cancer will likely include uniform assays to accurately measure PD-L1 expression levels as well as assays to select for MSI-high tumors.

5 Emerging and future therapies

5.1 CDK4/6 inhibitors

The CyclinD/CDK4/6 pathway is known to play a central role in the regulation of cell cycle progression and to be activated in various cancers [61]. CDK4/6 inhibitors prevent cell cycle progression from the G1 to the S phase and are currently indicated as first line treatment options for metastatic ER-positive breast cancers [61]. Since both ESCCs and EACs exhibit alterations that activate cell cycle regulatory genes, CDK4/6 inhibitors may serve as attractive agents to be explored in clinical esophageal cancer trials. EACs that overexpress cyclin E are more likely to be CDK4/6 resistant, as they are able to bypass CDK4/6 and activate downstream

signals in the cell cycle pathway [2]. Early preclinical studies have shown that a CDK4/6 inhibitor, PD-0332991, can inhibit ESCC cell growth, induce ESCC cell apoptosis and suppress ESCC cell migration and invasion [62]. Additional in vivo xenograft experiments have shown that PD-0332991 can potentially inhibit ESCC tumor growth and lung metastasis [62]. Currently, CDK4/6 inhibitors are being tested in SCC biomarker-driven trials, including patients with ESCC [63, 64].

5.2 Targeting the NRF2 pathway

Genomic data suggest that NRF2 is hyperactive in human ESCC [65] and that mutations in the *NRF2* gene range from 7.3% to 20%, depending on the studies reported [3, 20, 24, 25, 66]. Also *KEAP1*, the inhibitor of *NRF2*, has been found to exhibit inactivating mutations in ESCC albeit at a lower frequency [20, 66]. *Keap1* knockout mice show constitutive *Nrf2* activation and exhibit esophageal hyper-proliferation and hyperkeratosis [67]. It has been found that ESCCs with a high NRF2 protein expression are more resistant to chemoradiotherapy and exhibit a poorer survival compared to ESCCs with a low expression [68–70]. This is likely secondary to antioxidant effects of NRF2 signaling that reduce the accumulation of reactive oxygen species (ROS) and allow tumors cells to survive in highly oxidative environments [71–73]. In preclinical studies it has been found that NRF2 inhibition may lead to a repressed migration and invasion of ESCC cells in hypoxic microenvironments [74]. Furthermore, it has been found that inhibition of the NRF2 downstream target NQO1 may enhance the antitumor effects of curcumin in ESCC patient-derived xenograft tumors [74].

5.3 Poly (ADP-ribose) polymerase (PARP) inhibitors

Both EACs and ESCCs encompass subtypes with alterations in DNA damage response (DDR) genes and, as such, they may benefit from PARP inhibitors [2, 26, 29]. In EACs, a DDR impaired subgroup comprising 22% of the cancers has been found to be enriched for a BRCA mutation signature and a deficient HR pathway [29]. A BRCA mutation signature was also identified in 7 out of 39 ESCCs [75]. Among the DNA damage response pathways, both EACs and ESCCs exhibit mutations in the TP53 pathway. EACs also exhibit mutations in ARID1A (8%), which is recruited to double strand breaks to process it to single strand breaks. PARP inhibitors are FDA approved for platinum-sensitive relapsed BRCA1/2-associated high-grade ovarian, fallopian tube and primary peritoneal cancers [76], and have shown efficacy in BRCA-associated breast and pancreatic cancers [77–79]. Using the concept of synthetic lethality, PARP inhibitors impair the repair of single strand breaks in cells that are already deficient in the repair of double strand breaks by HR. The

resulting genomic instability leads to apoptosis [76]. In a pre-clinical study using the ESCC-derived cell lines KYSE70 and KYSE140, olaparib, a PARP inhibitor, has been found to enhance the cytotoxicity of chemotherapeutic agents [80]. Ultimately, identifying ESCCs and EACs exhibiting DDR deficiency through molecular profiling or immunohistochemistry will allow the selection of cancers that may be sensitive to PARP inhibitors. This will be an important step forward in facilitating personalized medicine in these specific cancer cases. Currently, there are trials ongoing testing PARP inhibitors in combination with other therapies for the treatment of patients with metastatic or locally recurrent esophageal cancers [81, 82].

5.4 Targeting Wnt signaling

Wnt signaling has been found to be activated in the majority ESCCs through up-regulation of Wnt activators and down-regulation of Wnt inhibitors [25, 66, 83, 84]. Wnt signaling controls stemness and is thought to drive carcinogenesis by promoting the growth of pluripotent stem cells [83]. In vitro studies have shown that WNT10a over-expression can promote the migration and invasion and enhance the proliferation of transformed esophageal cells, and increase the proportion of cells with a stem cell-like phenotype [83]. Other studies have shown that Wnt inhibitory factor-1 (WIF1), a secreted antagonist of the Wnt pathway, may be inactivated through epigenetic mechanisms. Over-expression of WIF1 in ESCC-derived cells was found to result in a significant inhibition of tumor cell colony formation [84]. A phase I clinical trial is currently underway using LGK974, a drug that targets Porcupine, a Wnt-specific acyltransferase that is important for the processing of Wnt ligand secretion in various cancers, including ESCC [85]. Although to a lesser extent, activation of the Wnt pathway has been found to occur in ~19% of EAC cases [86] through a different mechanism: β -catenin mutation preventing the degradation of APC, which in turn reduces Wnt destruction [87–89]. A putative role of Wnt signaling in the esophageal cancer precursor Barrett's esophagus is emerging [87–89], but further research is needed.

6 Conclusions and perspectives

Trastuzumab is used in combination with chemotherapy as first line treatment in metastatic EAC. Ramucirumab is available as monotherapy or in combination with chemotherapy in the second line setting in locally advanced or metastatic EAC. Pembrolizumab is currently available in the second line setting for locally advanced, metastatic or recurrent PDL-1 positive ESCCs and EACs with CPS \geq 10 or MSI-high/deficient MMR cancers and as a third line agent in EACs with CPS \geq 1. Patients with GEJ cancers progressing on standard

chemotherapy, either 5FU + platinum \pm anthracycline or taxane + ramucirumab, should be subjected to PD-L1 testing (if not already done) and next generation sequencing (NGS), ideally on biopsies of progressive lesions, to assess for targeted therapy and clinical trial inclusion eligibility. With recent data showing that pembrolizumab is non-inferior to chemotherapy as first line agent in advanced gastric and GEJ cancers expressing PD-L1, immunotherapy is an attractive option. Moving forward, identifying gene expression/mutation signatures or biochemical markers (other than PD-L1 staining) including MSI and MMR deficiency will help to identify ESCC and EAC subtypes that will benefit most from immunotherapy.

Targeted therapies in ESCCs are limited and immunotherapy is an option only in the second line setting. Molecular classification of esophageal cancers has facilitated the design of new potential therapeutic options, which are precise and targeted. These emerging alternatives are awaiting clinical trials: CDK4/6 inhibitors, PARP inhibitors and Wnt signaling modulators. Targeting NRF2 in ESCCs is a possibility that remains to be fully explored. Ultimately, personalized medicine in esophageal cancer will likely rely on NGS analysis of ESCCs and EACs to pinpoint subtypes that will most likely benefit from targeted therapies. The costs of targeted therapies in conjunction with drug access variability in various countries still pose challenges. Moreover, incorporating diagnostic tests, such as genomic sequencing to identify gene signatures, assays to test for PD-L1 staining, MSI and MMR deficiency, go along with healthcare costs that will need to be balanced with any improvement in survival outcome and/or quality of life, particularly for patients with advanced esophageal cancer.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Ethics No cell lines or human tissues were used for this manuscript.

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