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Molecular stratification of esophageal adenocarcinoma: implications for prognosis and treatment strategy

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Genome-wide molecular profiling has emerged as a promising approach for advancing the clinical management of esophageal adenocarcinoma (EAC), with the potential to improve prognostic accuracy and enable more personalized treatment strategies. In this review, we summarize current evidence from genomic and epigenomic EAC stratification studies, highlighting the proposed molecular subtypes and evaluating their clinical relevance. We discuss how these subclassifications may inform disease outcomes, refine patient selection for specific therapies and uncover new treatment opportunities aligned with tumor molecular profiles. Additionally, we explore molecular subtypes associated with Barrett's esophagus, a precursor lesion of EAC, and consider how these insights can help elucidate the mechanisms underlying EAC development. Such understanding may inform improved strategies for early tumor detection, risk stratification and prevention, ultimately aiming to reduce the burden of EAC. We also address the current challenges limiting the clinical application of these molecular classifiers, including restricted sample availability, insufficient validation and the difficulty of translating genome-wide findings into practical and clinical useful biomarkers. Integrating molecular subtyping into clinical workflows is a key step toward precision medicine in EAC, with the goal of enhancing treatment response rates and patient outcomes. Future advances will require collaborative efforts and robust clinical validation in large prospective studies to ensure that molecular stratification strategies can be effectively translated into improved management of EAC.

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INTRODUCTION

Esophageal cancer is the eleventh most diagnosed cancer and the seventh leading cause of cancer-related death worldwide [1]. It is classified into two main histological entities—squamous cell carcinoma and adenocarcinoma—with distinct epidemiologic, biologic and clinical characteristics [2]. Esophageal adenocarcinoma (EAC) is the most frequent type in Western countries, where its incidence has been rapidly rising.

A main risk factor for EAC is gastroesophageal reflux disease, with male sex and lifestyle factors such as obesity as contributing factors [3]. EAC usually develops from Barrett's esophagus (BE), a metaplastic condition in which chronic gastroesophageal reflux disease replaces the normal stratified squamous epithelium with columnar-lined epithelium, typically containing intestinal-type goblet cells [4]. The progression from BE to EAC is a multistep process involving increasing grades of dysplasia [5] and accumulation of genetic and epigenetic alterations [6–8]. One of the pathways by which BE can advance to EAC involves complex genomic catastrophes, such as chromothripsis and breakage-fusion-bridge events, observed in around 20% of patients with dysplastic BE [9]. The occurrence of these mechanisms has been confirmed in several studies [10–13] and proposed to accelerate EAC development [14]. Although the annual risk of progression for individual BE patients is low, the overall lifetime risk is substantially elevated compared to the general population [5].

Despite improvements in EAC prognosis due to modern multimodal therapy regimens, the 5-year overall survival rates remain around 20% [15]. Several factors contribute to this dismal prognosis. First, in early phases, EAC is typically asymptomatic, and a large proportion of patients are diagnosed with metastatic or locally inoperable tumors. For these patients, curative therapy may not be available. Furthermore, the demanding surgery and the oncological treatment renders a significant subset of patients unfit for potential curative treatment. Lastly, a substantial percentage of patients undergoing curative treatment experiences tumor relapse with limited treatment options.

Across different therapeutic regimens, up to one fifth of patients with EAC receiving neoadjuvant oncologic treatment may achieve a pathologic complete response, i.e., absence of viable tumor tissue in the resected specimen [16]. Patients with residual disease in the resected specimen following neoadjuvant chemoradiotherapy, along with selected patients with gastro-esophageal metastatic or unresectable disease, may be offered treatment with immune checkpoint inhibitors (ICIs) [17]. Also in the context of immunotherapy, only a subset of patients show a complete or partial response [17].

The unclear drivers of therapeutic resistance and the variation of individual tumor response to same treatment in EAC underscore the need for improved molecular characterization. Although biomarkers such as PD-L1 expression, microsatellite instability

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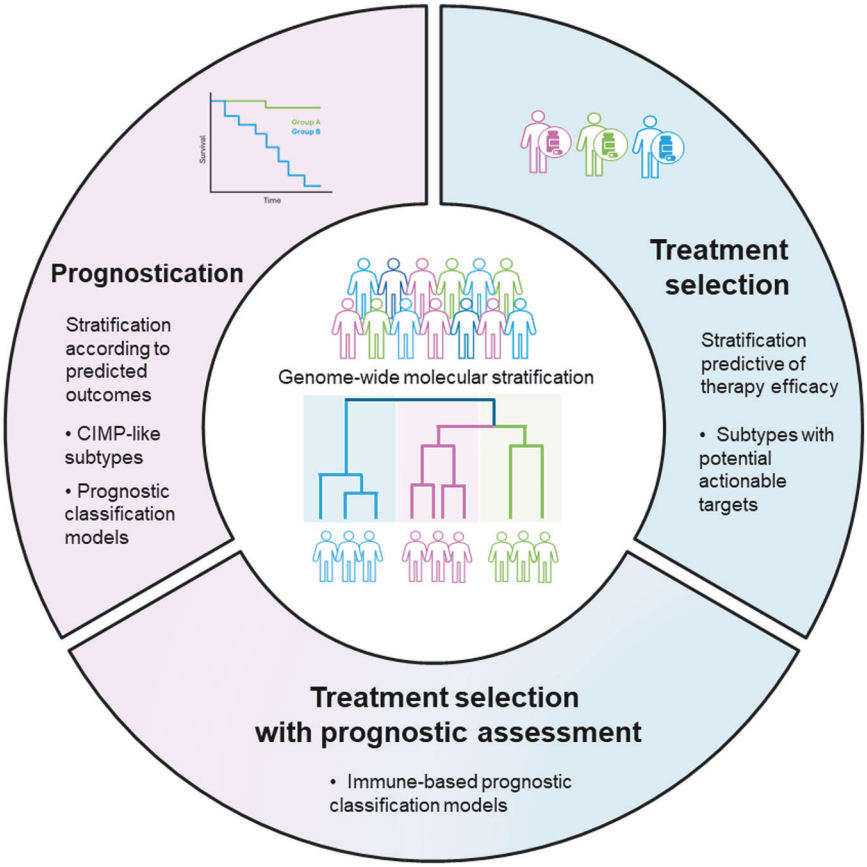

Fig. 1 Clinical utility of genome-wide molecular stratification of patients with esophageal adenocarcinoma (EAC). In this review, studies on EAC were categorized according to the clinical questions they address, either the stratification's value for prognostication or impact on therapy selection. Several studies combined therapeutic decisions with prognostic assessment of the patients. Particularly relevant examples of subtyping are highlighted. CIMP: CpG island methylator phenotype.

(MSI) status and tumor mutation burden have been implemented in clinical practice, molecular profiling on the genome and epigenome level still has a limited role in EAC prognostication and treatment.

Genome-wide approaches have enabled tumor classification into clinically relevant subtypes. Well-established examples include the consensus molecular subtypes (CMS) in colorectal cancer [18] and the molecular subtypes identified in breast cancer [19]. Emerging molecular subgroups have also been identified in EAC and BE, offering important insights into their molecular diversity. This review summarizes genome-wide genomic and epigenomic stratification studies and evaluates EAC subclassifications for clinical utility. A summary of the key aspects discussed for EAC in this review is illustrated in Fig. 1. In addition, we explore reported molecular subtypes associated with BE and how they can contribute to elucidating the mechanisms driving EAC development.

SELECTION CRITERIA

Original research studies were eligible for inclusion in this review if they aimed to identify features that grouped EAC and/or BE cases into distinct subtypes or risk groups using genome-wide genomic or epigenomic approaches. Such criteria resulted in a list of 29 candidate studies. Among these, 5 used stratification strategies in which EAC molecular profiles were analyzed in a pan-cancer context. One of these pan-cancer studies was excluded due to the lack of information regarding cluster assignment of EAC cases.

Studies reporting combined results from EAC and squamous cell carcinoma ($n = 4$) were also excluded, as joint analysis of these two histological subtypes could introduce bias in patient stratification due to their distinct molecular profiles identified across multiple platforms [20]. The included studies were categorized according to the clinical questions they addressed, i.e., (i) prognostic relevance, (ii) influence on therapy selection and (iii) contribution to elucidating the mechanisms underlying EAC development.

Applying these selection criteria resulted in the inclusion of 24 studies (Fig. 2), summarized in Table 1. Among these, 22 studies investigated EAC either alone or in combination with BE, while the remaining 2 studies focused exclusively on BE.

MOLECULAR STRATIFICATION AND IMPROVEMENT OF PROGNOSTIC ACCURACY

Currently, the tumor-node-metastasis (TNM) staging system and histopathological characteristics such as tumor differentiation remain the cornerstones for predicting EAC prognosis [21]. While tumor response to neoadjuvant oncological therapy provides additional prognostic insight [22–24], these tools show limited ability to predict individual survival probabilities. For example, stage-matched patients present considerable variation in clinical outcomes and complete histopathological tumor response in resected specimens may not be an appropriate surrogate marker for survival [25]. This prognostic uncertainty underscores the need for more precise stratification tools. Molecular profiling addresses this gap by identifying biologically distinct tumor subtypes associated with disease outcome. Such stratification can help identify patients who have a lower chance of survival and may benefit from alternative therapeutic

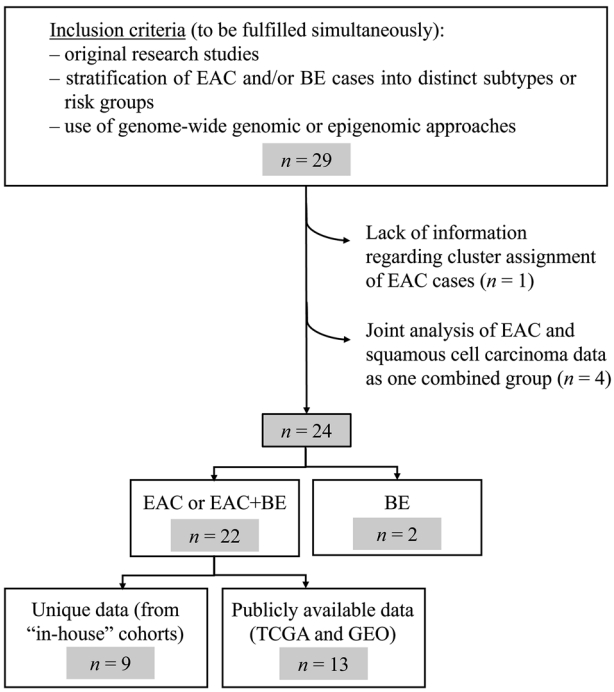

Fig. 2 Flow diagram illustrating the selection process of the studies included in this review. Twenty-nine studies were eligible for inclusion. Among this, one was excluded due to lack of information regarding cluster assignment of esophageal adenocarcinoma (EAC) cases in a pan-cancer stratification, and four others were excluded as they performed a joint evaluation of EAC and squamous cell carcinoma. Application of these selection criteria resulted in the inclusion of 24 studies, 22 of which investigated EAC either alone or in combination with Barrett's esophagus (BE), while the remaining 2 studies focused exclusively on BE. Only around 40% of the studies stratifying EAC relied on unique data from "in-house" cohorts.

interventions, and spare patients with a higher chance of survival from therapies that may have limited benefit and potential adverse effects. Ultimately, this approach may allow for more accurate survival predictions beyond conventional clinical parameters.

Early molecular stratification studies used gene expression profiling to categorize patients with EAC. Already in 2010, Kim and colleagues grouped EAC into three clusters with distinct recurrence-free survival [26]. The cluster with the poorest prognosis showed strong enrichment of NF-κB pathway activity. Importantly, a two-gene signature comprising *SPARC* and *SPP1* was validated as an independent prognostic marker for overall survival, with their combined expression significantly associated with patient outcomes even after adjusting for conventional clinicopathological factors. These results highlight the potential for subtype-specific molecular markers to refine risk assessment.

More recently, analysis of DNA methylation profiles from gastrointestinal (GI) adenocarcinomas grouped patients with EAC across four distinct pan-GI subtypes [27] (Fig. 3). These subtypes – hypermethylated, hypomethylated, intermediate and normal-like – showed a wide range of DNA methylation levels, revealing extensive epigenetic heterogeneity in EAC. Notably, 18% of EAC patients belonged to the normal-like subtype, which presented a low degree of aberrant DNA methylation and the poorest survival. Consistent with the range of DNA methylation levels, the four pan-GI subtypes including EAC cases overlapped with a range of CpG island methylator phenotype (CIMP) statuses.

CIMP-like subtypes

CIMP is characterized by widespread hypermethylation at CpG islands in gene promoter regions, resulting in transcriptional silencing of multiple genes. CIMP was originally described in colorectal cancer by Toyota et al. in 1999 [28], and has since been identified in various other cancer types, including glioma, bladder -, pancreatic -, lung -, hepatocellular -, and gastric cancer [29]. While the prognostic significance of CIMP varies among cancer types [29], it is often associated with more aggressive biology in GI tumors, particularly in colorectal cancer [30–32].

In EAC, genome-wide DNA methylation studies have identified CIMP-like subtypes [33–36], although their clinical significance remains unclear. In 2011, Kaz and colleagues were among the earliest to analyze variations in CpG island methylation in EAC and BE [33]. By using microarrays to analyze global DNA methylation patterns, they identified high- and low-methylation epigenotypes, along with CpG sites potentially involved in disease progression. This groundwork described for the first time methylation subgroups in BE and EAC, and established aberrant DNA methylation as a key feature of EAC pathogenesis.

Building on this, Krause and colleagues [34] also used DNA methylation arrays to identify two EAC subtypes: a CIMP-like hypermethylated group and a non-CIMP group. The CIMP-like group was characterized by extensive CpG island hypermethylation, including high methylation levels of CIMP markers previously proposed in gastric and colorectal cancer [37–39]. This supports the utility of an array-based approach for detecting CIMP in EAC. The hypermethylated tumors also showed significant overlap with regions marked by the repressive histone modification H3K27me3 and binding sites for Polycomb Repressive Complex 2 proteins, indicating coordinated epigenetic gene silencing. Clinically, patients with the most hypermethylated tumors had significantly poorer survival outcomes compared to other groups. Analysis of an independent cohort from The Cancer Genome Atlas (TCGA) in the same study further supported the presence of a CIMP-like phenotype in EAC. However, the prognostic value of this stratification has not been assessed. It is also noteworthy that probe selection was based on CpG islands with highest methylation variation observed across tumors and with low methylation in normal squamous epithelium. While this approach is appropriate for detecting CIMP, it may have missed other potentially clinically relevant methylation patterns, such as CpG sites characterized by frequent hypomethylation.

Further advancing the understanding of DNA methylation in EAC, Sánchez-Vega et al. [36]. classified 87 EAC tumors from TCGA into three categories – CIMP-positive, CIMP-intermediate and CIMP-negative – based on differentially methylated CpG islands (probes) when comparing tumor and healthy adjacent tissue. According to TCGA original study (ref), these data were generated from samples with high tumor purity (≥ 60%; Table 1). Approximately one-third of EAC samples were classified as CIMP-positive, exhibiting pronounced DNA hypermethylation, while CIMP-negative tumors had methylation profiles closer to those observed in normal tissues. Interestingly, unlike colorectal cancer, no significant association between *MLH1* promoter hypermethylation and CIMP categories was found in EAC, suggesting possible mechanistic differences in epigenetic regulation between these cancers. The authors highlighted that subdividing samples according to CIMP status could reduce heterogeneity within cancer subtypes and result in more uniform molecular and phenotypic characteristics. This could help achieve more consistent response rates in clinical trials.

In a broader context, Liu et al. [35] integrated 79 TCGA EAC samples into a pan-GI molecular taxonomy, revealing that GI adenocarcinomas displayed markedly higher frequencies of CpG island hypermethylation compared to non-GI adenocarcinomas, partly attributable to the higher CIMP frequency. Patients with EAC were distributed across four out of the seven pan-GI subtypes

Table 1. Summary of the selected studies discussed in the present review that investigate molecular stratification of esophageal adenocarcinoma, Barrett's esophagus or a combination of both.

Study	Number of samples	Source	Tumor content in the samples	Other samples included in the clustering	Validation dataset	Number of subtypes	Type of data used for stratification	Feature selection for stratification	Method used for sample clustering	Signature	Main findings
Improvement of prognostic accuracy											
Kim et al., 2010, PLoS One [26]	75 EAC	in-house	Not provided	-	-	**3**	Expression microarrays	Genes with an expression ratio that was at least two fold different relatively to reference in at least 8 tissues	Unsupervised hierarchical clustering	-	Among the genes whose expression was significantly associated with prognosis, two (*SPARC* and *SPP1*) were highlighted as a potential prognostic biomarker signature.
Pinto et al., 2024, Mol Oncol [27]	201 EAC	TCGA and GSE72872	≥60% for TCGA samples [20] and ≥50% for GEO samples [34]	STAD, COAD and READ	201 EAC samples from TCGA and GSE72872 (splitted)	**4 (out of 6 pan-GI)**	DNA methylation (450 K)	DVPs between tumor and normal samples	Consensus hierarchical clustering	-	The four subtyes where EAC patients were identified span a range of distinct DNA methylation levels, and agree with the distribution of the different CIMP status enrichment. The normal-like subtype showed the poorest prognosis of all subtypes.
Improvement of prognostic accuracy: CIMP-like subtypes											
Kaz et al., 2011, Epigenetics [33]	29 EAC	in-house	≥75%	-	-	**2 (high and low methylation epigenotypes)**	DNA methylation (Golden Gate)	Probes located in CpG islands and in the neighborhood of the transcription starting site	Unsupervised hierarchical clustering	-	First genome-wide study indicating higher similarity of DNA methylation profiles between BE and EAC than between each one of them and the normal squamous esophagus.
Krause et al., 2016, Carcinogenesis [34]	125 EAC and 19 BE	in-house	≥50%	BE / -	89 EAC from TCGA	**2 (non-CIMP and CIMP-like)**	DNA methylation (450k)	CpG island-located most variable probes in EAC samples, and not highly methylated in normal squamous esophagus	Unsupervised hierarchical clustering	-	The clustering separated EAC and BE from normal squamous esophagus, but not EAC from BE. A group of EAC patients with a CIMP-like methylation pattern was proposed. Patients with the most hypermethylated tumors exhibited significantly poorer survival outcomes compared to all the other tumors.
Liu et al., 2018, Cancer Cell [35]	79 EAC	TCGA	≥60% [20]	STAD, COAD and READ	-	**4 (out of 7 pan-GI) CIMP-high, GEA CIMP-low and two non-CIMP**	DNA methylation (450k)	Gene promoter loci unmethylated in normal tissues and leukocytes (mean $\beta < 0.2$) and methylated ($\beta > 0.3$) in more than 5% samples in at least one of the GI adenocarcinoma types.	Unsupervised hierarchical clustering	-	EAC patients were also included in hypermutated-single-nucleotide variants, genome stable and mostly chromosomal unstable subgroups defined by multiplatform analyses.

Table 1. continued

Study	Number of samples	Source	Tumor content in the samples	Other samples included in the clustering	Validation dataset	Number of subtypes	Type of data used for stratification	Feature selection for stratification	Method used for sample clustering	Signature	Main findings
Sánchez-Vega et al., 2017, World J Gastrointest Oncol [36]	87 EAC	TCGA	≥60% [20]	-	-	**3 (CIMP +, CIMP-intermediate and CIMP-)**	DNA methylation (450k)	Identification of CpG islands-located DMPs between tumor and normal samples, among those with a high variance across samples (standard deviation ≥ 0.1), low methylation in control samples (average $\beta < 0.05$) and increased methylation in tumor samples (average $\beta > 0.25$).	Unsupervised hierarchical clustering	-	No significant association was observed between *MLH1* promoter hypermethylation and CIMP categories.
Improvement of prognostic accuracy: classification models											
Lan et al., 2021, Medicine [40]	75 EAC	TCGA (split into test and training set)	≥60% [20]	-	43 EAC from GEO (GSE72874)	**2 (low-/high-risk)**	Expression (RNA-seq)	Identification of DEGs between tumor and normal samples. Lasso penalized Cox regression was then applied to identify prognostic-related mRNAs.	Median survival risk score (calculated based on the expression levels of the gene signature)	5 genes (*LC26A9*, *SINHCAF*, *MICB*, *KRT19* and *MT1X*)	The 5-mRNA signature was promising as a biomarker for predicting 3-year survival rate of EAC in the internal test set, the entire TCGA set, and the external test set. The sensitivity and specificity of the mRNA signature performed numerically better than the TNM stage for 1-, 2-, and 3-year prognostic evaluation of EAC.
Mao et al., 2024, Transl Cancer Res [41]	80 EAC	GEO (GSE13898 and GSE26886, for DEGs identification) and TCGA (for DEGs identification and model construction)	≥60% for TCGA samples [20]; not provided for GEO samples	-	-	**2 (low-/high-risk)**	Expression (RNA-seq)	Identification of DEGs between tumor and normal or adjacent non-cancerous tissues. Cox analysis together with Akaike information criterion were then performed to find DEGs associated with prognosis.	Median survival risk score (calculated based on the expression levels of the gene signature)	4 genes (*ALAD*, *ABLIM3*, *IL17RB* and *IFI6*)	Multivariate Cox regression analyses suggested that the four-gene signature served as an independent factor in overall survival prediction. Stage stratified analysis showed that the four-gene signature had better predictive performance for patients with advanced tumor stage (III and IV).
Chen et al., 2021, Biomed Res Int [42]	78 EAC	TCGA	≥60% [20]	-	-	**2 (low-/high-risk)**	Expression (RNA-seq) and DNA methylation (450 K)	Identification of methylation-driven genes by comparing DNA methylation status of tumor and normal samples and correlating it with transcriptomic data. Lasso penalized Cox regression was then applied to identify prognostic-related features.	Median survival risk score (calculated based on the expression levels of the gene signature)	4 methylation-driven genes (*GPBAR1*, *OLFM4*, *FOXI2* and *CASP10*)	Multivariate Cox regression analyses showed that the prognostic risk score was an independent prognostic factor.

Table 1. continued

Study	Number of samples	Source	Tumor content in the samples	Other samples included in the clustering	Validation dataset	Number of subtypes	Type of data used for stratification	Feature selection for stratification	Method used for sample clustering	Signature	Main findings
Li et al., 2019, Aging [43]	79 EAC	TCGA (not clear)	≥60% [20]	-	-	**2 (low-/high-risk)**	DNA methylation (450 K)	Lasso-Cox model applied to differentially methylated CpG sites between EAC and normal samples to identify prognostic-related features.	Median survival risk score (calculated based on the DNA methylation levels of the gene signature)	3 CpG sites (cg01192745, cg19801256 and cg18276155)	The 3-CpG prognostic methylation classifier was an independent risk factor by multivariate Cox regression adjusting for clinical risk factors. The classifier improved the predictive ability of the TNM staging system.
Personalized treatment selection: Subtypes with potential actionable targets											
Secrier et al., 2016, Nat Genet [48]	129 EAC	OCCAMS / ICGC	>70%	-	87 EAC samples from EGAS00001000750 and ICGC	**3 (DNA damage repair impaired, mutagenic and C > A/T dominant)**	Mutational signatures obtained from whole-genome sequencing via non-negative matrix factorization	Six mutational signatures: S1 (age), S2 (*APOBEC*), S3 (*BRCA*), S17, S17B and S18-like	Consensus clustering	-	Drug sensitive assays in EAC cell lines showed that the subtypes can be a basis for therapy selection.
Guo et al., 2018, BMC Genomics [49]	215 EAC (from three independent cohorts) and 15 BE	GEO (GSE13898, GSE19417) and TCGA (independently analysed and in a meta-analysis)	≥60% for TCGA samples [20]; not provided for GEO samples	1) BE and normal esophageal tissues; 2) squamous esophageal carcinoma and gastric carcinoma	215 EAC samples from three independent cohorts: GSE13898, GSE19417 and TCGA (independently analysed and in a meta-analysis)	**2 (Subtype I, gastric-like; and Subtype II, squamous-like)**	Expression (microarrays and RNA-seq)	Standard deviation	Consensus hierarchical clustering (performed on each of the three datasets independently and in a meta-analysis)	-	The subtypes showed distinct expression patterns and mutation profiles. The EAC gastric-like subtype II exhibited gene expression patterns closely resembling those found in BE. The study suggested that subtype II EAC patients might be more likely responsive to chemotherapy. However, a limited number of patients had available therapeutic information.
Yu et al., 2019, Gut [50]	23 EAC	in-house	>70%	-	87 EAC from TCGA	**4 (high-, intermediate-, low-and minimal-methylator)**	DNA methylation (450k)	Most variable probes among EAC	Recursively partitioned mixture model clustering	-	Cell lines representative for each subtype responded differently to anti-cancer chemotherapies.
Jammula et al., 2020, Gastroenterology [51]	285 EAC and 150 BE	OCCAMS / ICGC	>70%	BE	19 BE and 125 EAC samples from GEO (GSE72872)	**4**	DNA methylation (EPIC)	Optimal metagenes obtained by non-negative matrix factorization	Non-negative matrix factorization with k-means clustering	-	Subtype 2 was enriched in BE samples. Methylation profiles of BE and EAC were more similar to each other than normal esophagus. Subtype 3 was associated with the shortest time of patient survival.

Table 1. continued

Study	Number of samples	Source	Tumor content in the samples	Other samples included in the clustering	Validation dataset	Number of subtypes	Type of data used for stratification	Feature selection for stratification	Method used for sample clustering	Signature	Main findings
Sundar et al., 2019, Eur J Cancer [54]	229 EAC	in-house (MRC OE02 trial)	≥30%	-	13 in-house EAC samples	**2**	DNA methylation (Illumina GoldenGate Cancer Panel I)	Cox proportional hazard analysis to select probes predictive of survival in the chemotherapy +surgery arm	Non-negative matrix factorization	Non-negative matrix factorization metagene signature involving 11 probes.	In cluster 1, patients in the chemotherapy +surgery arm had significantly better overall survival, appearing to benefit from chemotherapy. In cluster 2, patients in the chemotherapy +surgery arm exhibited worse survival compared with that of patients in the surgery-only arm, suggesting that they may not derive any survival benefit from neoadjuvant chemotherapy. The identified epigenetic signature may serve as a predictive biomarker for neoadjuvant chemotherapy (cisplatin + fluorouracil) benefit in EAC.
Hoadley et al., 2018, Cell [55]	Not provided	TCGA	≥60% [20]	ESCC and 32 other cancer types	-	**7 (out of 28 pan-cancer) C2: BRCA (HER2 amp) C4: Pan-GI (CRC) C10: Pan-SCC C13: Mixed (Chr 8 del) C18: Pan-GI (MSI) C20: Mixed (Stromal/ Immune) C25: Pan-SCC (Chr 11 amp)**	Copy number, DNA methylation, mRNA and miRNA expression	Variable (depending on the type of data)	Multi-platform integrative clustering with iCluster	-	Cell-of-origin influences, but does not fully determine, tumor classification. Mutation frequencies and mutational signatures varied among the clusters, as well as the enriched pathways.
Personalized treatment selection: Immunological profiling											
Ling et al., 2022, Pharmaceuticals [61]	223 EAC	TCGA and GEO (GSE72874, GSE92396 and GSE13898)	≥60% for TCGA samples [20]; not provided for GEO samples	-	44, 45 and 48 EAC samples from GEO (GSE72874, GSE13898 and GSE19417, respectively)	**2**	Gene expression (mRNA and lncRNA) and TME scores	Identification of DEGs (mRNA and lncRNA) with a significant prognostic value and TME scores with median absolute deviation > 0.5	IntNMF (Integrative Clustering of Multiple Genomic Dataset) and consensus clustering	50 subtype-specific DEGs	The group has developed a classifier to stratify samples into two subtypes based on 50 subtype-specific signature genes. Stratified survival analyses based on the age and clinical stage subgroups confirmed the prognostic value of the two EAC subtypes The subtypes showed differences in prognostic and in tumor microenvironment landscape.

Table 1. continued

Study	Number of samples	Source	Tumor content in the samples	Other samples included in the clustering	Validation dataset	Number of subtypes	Type of data used for stratification	Feature selection for stratification	Method used for sample clustering	Signature	Main findings
Naeini et al., 2023, Nat Commun [62]	68 EAC	in-house (mostly DOCTOR clinical trial)	Variable	-	78 EAC from TCGA	**4 immune clusters (immune hot, immune cold, immune suppressed and immune moderate)**	18 immune cell proportions (deconvoluted from RNA-seq data)	18 immune cell proportions (deconvoluted from RNA-seq data)	Unsupervised k-means clustering	-	The four immune clusters associated with both overall survival and progression-free survival.
Thorsson et al., 2018, Immunity [64]	76 ECA	TCGA	≥60%	ESCC and 29 other cancer types	76 EAC samples from TCGA (splitted)	**5 (out of 6 pan-GI) C1: wound healing, C2: IFN-γ dominant, C3: inflammatory, C4: lymphocyte depleted, and C6: TGF-β dominant**	Expression (RNA-seq)	5 cancer immune expression signatures	Consensus clustering of the pairwise correlation of the signature scores	5 cancer immune expression signatures	Immunogenomic features were predictive of outcome, with overall survival and progression-free interval differing between immune subtypes both within and across cancer types.
Personalized treatment selection: Immune-based classification models for improved mortality risk assessment											
Yang et al., 2023, Med Sci Monit [65]	78 EAC	TCGA	≥60% [20]	-	64 EAC samples from GEO (GSE13898)	**2 (low-/high-risk)**	Expression (RNA-seq)	Identification of DEGs between tumor and normal tissue samples and combination with immune-related genes list from ImmPort database. Performance of weighted correlation network analysis on this list, followed by Cox regression model to identify prognostic-related genes.	Median survival risk score (calculated based on the expression levels of the gene signature)	4 genes (*UNC93B1*, *HSPA14*, *AR* and *FGF13*)	Multivariate Cox regression analyses showed that the prognostic risk score was an independent prognostic factor. The results suggested that the high-risk group is more suitable for immunotherapy, which may provide a reference value for the treatment of EAC patients.
Zhang et al., 2021, BMC Bioinformatics [66]	80 EAC	TCGA	≥60% [20]	-	48 EAC samples from GEO (GSE72873)	**2 (low-/high-risk)**	Expression (RNA-seq)	Identification of DEGs between tumor and normal tissue samples and combination with immune-related genes list from ImmPort database. Cox regression analysis was then performed on immune-related DEGs to identify prognostic-related genes.	Median survival risk score (calculated based on the expression levels of the gene signature)	12 immune-related genes (*ADRM1*, *CXCL1*, *SEMG1*, *CCL26*, *CCL24*, *AREG*, IL23A, *UCN2*, *FGFR4*, *IL17RB*, *TNFRSF11A*, and *TNFRSF21*)	Multivariate Cox analysies and nomogram indicated that a combined analysis of the risk score, sex, M stage, and tumor stage can accurately predict survaval prognosis factors. The significance of the survival rate difference between high- and low-risk groups was kept when patients were stratified by age and tumor stage. The signature constructed for EAC patients was proven not suitable for ESCC patients.

Table 1. continued

Study	Number of samples	Source	Tumor content in the samples	Other samples included in the clustering	Validation dataset	Number of subtypes	Type of data used for stratification	Feature selection for stratification	Method used for sample clustering	Signature	Main findings
Elucidation of the mechanisms underlying EAC carcinogenesis											
Krause et al., 2016, Carcinogenesis [34]						BE stratified in combination with EAC, as above.					
Jammula et al., 2020, Gastroenterology [51]						BE stratified in combination with EAC, as above.					
Guo et al., 2018, BMC Genomics [49]						BE stratified in combination with EAC, as above.					
Nones, 2014, Nat Commun [14]	22 EAC	in-house	≥50%	-	-	**Unstable genome, scattered and complex localized**	Whole-genome sequencing and single-nucleotide polymorphism-arrays	Structural variants	-	-	The number of structural variants and their genomic distribution revealed considerable inter-tumor heterogeneity.
Yu et al., 2019, Gut [50]	59 BE	in-house	>70%	-	-	**4 (high-, intermediate-, low-and minimal-methylator)**	DNA methylation (450k)	Most variable probes among EAC	Recursively partitioned mixture model clustering	-	The four subtypes mirrored those identified in EAC in the same study.
Kaz et al., 2011, Epigenetics [33]	29 BE	in-house	≥75%	-	-	**2 (high and low methylation epigenotypes)**	DNA methylation (Golden Gate)	Probes located in CpG islands and in the neighborhood of the transcription starting site	Unsupervised hierarchical clustering	-	The four subtypes mirrored those identified in EAC in the same study, as well as CIMP groups in other cancer types.

BE Barrett's esophagus, *CIMP* CpG island methylator phenotype, *COAD* colon adenocarcinoma, *DEG* differentially expressed gene, *DVP* differentially variable probe, *EAC* esophageal adenocarcinoma, *ESCC* esophageal squamous cell carcinoma, *GEA* gastroesophageal adenocarcinoma, *GEO* Gene Expression Omnibus, *GI* gastrointestinal, *ICGC* International Cancer Genome Consortium, *READ* rectal adenocarcinoma, *STAD* stomach adenocarcinoma, *TCGA* The Cancer Genome Atlas, *TME* tumor microenvironment, *TNM* tumor-node-metastasis.

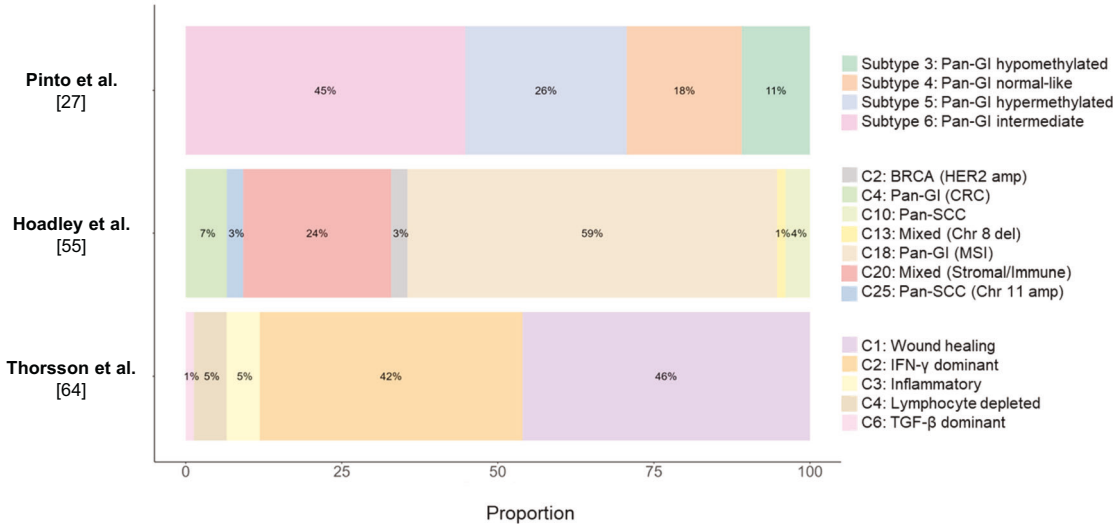

Fig. 3 Distribution of patients with esophageal adenocarcinoma across pan-cancer subtypes identified in three distinct studies.

identified – CIMP-high, gastroesophageal CIMP-low and two non-CIMP subtypes. When present, the CIMP-like methylation patterns were associated predominantly with chromosomal instability rather than MSI or *MLH1* methylation, which are more characteristic of lower GI tumors. This work underscored once again the molecular heterogeneity of EAC and its closer resemblance to chromosomally unstable gastric cancers than to colorectal cancers.

Collectively, these studies provide compelling evidence that CIMP-like methylation patterns exist in EAC and may contribute to tumor heterogeneity and progression. However, their clinical significance remains uncertain due to inconsistent validation across cohorts, and lack of standardized criteria for CIMP classification currently limit its clinical application. Future research should focus on establishing consensus definitions for CIMP in EAC and validating its prognostic and predictive utility in large, well-characterized patient cohorts.

Classification models for improved mortality risk assessment

Ideally, prognostic evaluation in EAC would associate patient outcomes to specific molecular biomarkers involved in tumor progression. Molecular classifiers may facilitate individualized mortality risk assessment, enabling clinicians to more accurately identify patients with a low survival probability for tailored therapeutic interventions. Furthermore, the discovery of prognostically relevant genes may reveal novel biological pathways and potential therapeutic targets, advancing precision medicine and ultimately improving patient survival.

Several molecular signatures developed from genome-wide approaches have been suggested for prognostic assessment of patients with EAC, stratifying them into high- and low-risk groups. While studies differ in the type of data used to build the prognostic models, the methodologies for identifying these signatures are similar: prognostic-related features are derived from differentially altered genes or regions between tumor and normal samples, followed by selection using various modalities of Cox regression analyses. Patients are then categorized into risk groups based on the median survival risk score calculated from the identified signatures, with those in the low-risk group demonstrating improved outcomes compared to those in the high-risk groups.

In 2021, Lan et al. [40] identified a 5-mRNA signature (*SLC26A9*, *SINHCAF*, *MICB*, *KRT19* and *MT1X*) that outperformed the traditional TNM staging system in predicting 3-year survival rates for EAC. This signature maintained predictive accuracy across all tumor stages and was validated in an external dataset, supporting its utility as a robust prognostic biomarker. In the study of Mao et al. [41], a four-gene expression signature (*ALAD*, *ABLIM3*, *IL17RB* and *IFI6*) strongly associated with overall survival was identified. This signature showed particularly strong predictive performance in advanced-stage disease. Expanding beyond gene expression, Chen et al. incorporated DNA methylation data into their analyses and defined a signature comprising four methylation driven genes (*GPBAR1*, *OLFM4*, *FOXI2*, and *CASP10*) [42], while Li et al. [43] used DNA methylation data alone to developed a 3-CpG prognostic classifier (mapped to *ITGA1* and *MCC* genes). Time-dependent ROC curve analysis indicated that the methylation-based classifier outperformed established clinical risk factors – including age, gender, BMI, smoking, alcohol use and tumor stage, – in predicting patient survival. This classifier effectively distinguished low- and high-risk groups across both early and advanced stages. Multivariate analyses showed that the risk scores calculated from all these signatures were independent predictors of overall survival for EAC. While the study of Lan et al. achieved external validation, those of Mao et al., Chen et al. and Li et al. lack such validation, limiting their immediate clinical applicability despite initially promising results.

MOLECULAR STRATIFICATION AND PERSONALIZED TREATMENT SELECTION

The highly heterogeneous molecular landscape of EAC complicates the development of effective targeted therapies. Currently, patients are typically treated with a "one-size-fits-all" approach, including surgery and perioperative chemotherapy and/or radiation. Clinical guidelines suggest the use of trastuzumab for HER2-positive EAC patients with metastatic disease [44], based on its demonstrated survival benefit when combined with chemotherapy in HER2-positive metastatic gastric and gastroesophageal junction cancers [45]. Zolbetuximab has also been recently recommended as first-line therapy in combination with chemotherapy for treatment of claudin 18.2 (CLDN18.2)-positive, HER2-negative, locally advanced, and metastatic gastric and gastroesophageal junction adenocarcinomas [46, 47]. This suggests that CLDN18.2-targeted therapies could be applied to EAC outside the gastroesophageal junction, but clinical validation is still needed. In addition, anti-PD-1 inhibitors are used for EAC treatment but, in the adjuvant setting, this is done regardless of PD-1 or PD-L1 expression levels, likely resulting in suboptimal efficacy.

Molecular classification systems could therefore give insights into new strategies for therapeutic intervention. Advances in molecular profiling – including genomics, epigenomics, transcriptomics and tumor microenvironment (TME) analysis – have revealed distinct EAC subtypes with specific biological characteristics and with the potential to guide more precise therapies and, in some cases, complement prognostic assessment.

Subtypes with potential actionable targets

Whole-genome sequencing of 129 EAC cases by Secrier *et al* [48]. identified three subtypes with suggested therapeutic relevance: (i) DNA damage repair induced, BRCA-like tumors, with homologous recombination defects, likely sensitive to PARP inhibitors; (ii) hypermutated tumors with high neoantigen loads, potential candidates for immunotherapy; and (iii) C > A/T dominant, aging-associated tumors likely benefiting from conventional chemotherapies. Importantly, these treatment suggestions have been derived from in vitro experiments using cell lines representative of each subtype and from findings in other cancer types, hence requiring validation specifically in EAC. Moreover, frequent co-amplification of receptor tyrosine kinases (RTKs) was observed across subtypes, suggesting that combinatorial RTK inhibition might be necessary to overcome resistance mechanisms. Translating subtype classification into clinical benefit requires methodologies that are both practical and accessible. While whole-genome sequencing remains costly, the same subtypes were identified using cost-effective low-coverage sequencing, making this approach more feasible for routine clinical use and expanding opportunities for tailored treatment.

Transcriptomic profiling of 215 EAC samples from three independent cohorts performed by Guo et al. [49] added another dimension, distinguishing gastric-like (Subtype I) and squamous-like (Subtype II) EACs. Subtype I displayed an epithelial and keratinocyte differentiation signature, with molecular features resembling gastric adenocarcinoma. In contrast, subtype II was characterized by cytochrome P450-related metabolism signatures and shared gene expression patterns with esophageal squamous carcinoma. The authors hypothesized that patients in subtype II could be more sensitive to chemotherapy than patients in subgroup I. However, with only three annotated samples analyzed this hypothesis remains speculative. Moreover, distinct mutation signatures were observed in both subgroups, though there was no significant difference in overall mutation burden. Considering the findings from Secrier et al. [48], it can be hypothesized that both transcriptomic subtypes are represented within the hypermutated subgroup. Consequently, ICIs, suggested for hypermutated tumors, could potentially benefit both subtype I and II, depending on neoantigen load. This underscores the molecular complexity of EAC and highlights the importance of multi-omics stratification.

Epigenetic profiling by Yu et al. [50] using an in-house discovery dataset ($n = 23$) further explored EAC stratification with therapeutic relevance. The group identified four methylator subtypes: high, intermediate, low, and minimal. These subtypes resembled previously described CIMP groups [34, 36] and were validated in the TCGA EAC cohort ($n = 87$). By analyzing the genomic alterations in both cohorts and integrating methylome and transcriptome data from all TCGA samples in the validation cohort, the authors further characterized the subtypes. They found that most actionable features were present in the high methylator subtype, specifically characterized by *ERBB2* alterations (mutations or amplifications) and silencing of the tumor suppressor *PTPN13* by aberrant methylation. This subtype also displayed an elevated mutational load, suggesting a possible overlap with Secrier's hypermutated group and theoretically implying shared vulnerabilities to combined epigenetic and immunotherapy strategies. Since only samples with high tumor content were included in the study (Table 1), the presented differences in the number of actionable features are plausible and not likely an effect of variable tumor cell percentages across the four methylation subtypes. Moreover, drug sensitive assays in EAC cell lines representing the methylator subtypes suggested distinct susceptibilities to conventional and targeted therapies, which, if validated clinically, may improve precision medicine for EAC patients. Thus, DNA methylation-based subtyping could provide insights into the functional roles of epigenetic alterations in EAC and serve as predictive markers. However, Yu et al. [50] promising results are based on relatively small cohorts (discovery $n = 23$; validation $n = 87$), necessitating further validation.

Jammula et al. further provided a comprehensive description of epigenetic heterogeneity, stratifying a larger cohort of patients with BE ($n = 150$) and EAC ($n = 285$) collected by the Oesophageal Cancer Clinical and Molecular Stratification (OCCAMS) Consortium [51]. They identified four subtypes associated with patient outcome and potential therapy response. Subtype 1 showed DNA hypermethylation, aligning with a CIMP-like profile, as most hypermethylated probes overlapped with CpG islands and promoter regions. The characteristics of this subtype also matched the high methylator subtype proposed by Yu et al. [50], including high mutation burden and *ERBB2* amplifications. Jammula and colleagues proposed that patients in subtype 1 could be sensitive to DNA methyltransferase and topoisomerase I inhibitors, which have shown efficacy in tumors with high levels of methylation [50, 52]. Interestingly, this subtype had the best overall survival. Subtype 3 did not show significant changes in DNA methylation compared to normal tissues and it was associated with the poorest survival, in line with other studies indicating that the DNA methylation "normal like" subtype in GI adenocarcinomas, including EAC, is linked to worse prognosis [27]. Notably, gene expression data revealed significant infiltration of both innate and adaptive immune cells in this subtype, a feature typically associated with better immunotherapy response. However, frequent *MDM2* amplification – often linked to resistance to ICIs [53] – was also observed in this subtype, creating a clinical contradiction where apparent immunological responsiveness conflicts with molecular resistance mechanisms. The study did not include data on immunotherapy administration or outcomes in this subgroup, leaving it unclear whether *MDM2* amplification ultimately contradict the potential benefits suggested by immune cell infiltration. Additionally, analysis of EAC organoids provided insights into potential subtype-specific targeted therapeutics. For example, CDK2 inhibitors were more effective in organoids representing hypomethylated subtype 4, characterized by *CCNE1* amplification, an alteration reported to be sensitive to CDK2 inhibitors.

Sundar and colleagues identified a DNA methylation signature that stratified patients with EAC into two clusters with distinct survival outcomes based on treatment received [54]. Both clusters included patients who had undergone neoadjuvant chemotherapy followed by surgery or surgery alone. In cluster 1, patients receiving neoadjuvant chemotherapy showed significantly improved median overall survival compared to those treated with surgery alone. Conversely, patients in cluster 2 had no survival benefit from chemotherapy, suggesting that alternative strategies are needed for this group. These observations demonstrate the predictive value of the epigenetic signature, as the survival differences were specifically tied to chemotherapy response rather than general prognosis. The signature was subsequently validated in an independent cohort, strengthening its potential for predicting survival outcome and chemotherapy response in EAC.

Notably, by integrating genetic, epigenetic and expression data from TCGA, Hoadley and colleagues identified 28 pan-cancer clusters [55], seven of which included EAC cases. Cell-of-origin patterns strongly influenced tumor clustering and EAC was highly linked to pan-GI lineages. Yet, EAC cases were also distributed across more heterogeneous clusters characterized by immune-

related features or distinct copy-number alteration patterns (Fig. 3). In addition, a minority of cases exhibited molecular similarities to HER2-amplified breast cancers or squamous cell carcinomas. This framework suggests broad opportunities for targeted therapeutic strategies in EAC. The conserved pathways across GI cancers, along with the resemblance to HER2-amplified tumors, support the potential utility of therapies such as trastuzumab, which has demonstrated clinical benefit in gastric and colorectal cancers with similar molecular features [56, 57]. Furthermore, the significant proportion of EAC cases in the Mixed (Stromal/Immune) cluster (24%), displaying strong immune-related signaling profiles, suggests that a subset of patients with EAC may have increased susceptibility to immunotherapeutic approaches.

Immunological profiling with potential clinical relevance

As research indicates that patient response to immunotherapy is strongly influenced by dynamic tumor-immune interactions [58], comprehensive analysis of the TME may offer new opportunities to identify patient subgroups most likely to benefit from immunotherapy. In other tumor types, classifying patients into immunological "hot" or "cold" subtypes has proven successful in guiding clinicians on the feasibility of immunotherapy [59]. In EAC, however, robust biomarkers predictive of immunotherapy response have been lacking, which may contribute to the generally limited efficacy of these treatments in most patients. Although PD-L1 expression, tumor mutational burden, and MSI have been investigated [17], none have consistently or reliably predicted response to immunotherapy. Stratifying EAC cases based on their immunological states therefore appears to be a promising approach for developing more individualized and effective therapeutic strategies [60].

By integrating RNA expression data and immune infiltrate scores Ling et al. developed a classifier to stratify patients with EAC into two subtypes with significant prognostic differences and distinct immunological profiles [61]. Age- and tumor stage-stratified survival analyses confirmed the prognostic value of these subtypes. The subtype with the best prognosis showed up-regulated immune-related signaling pathways and enriched immune cell infiltration, including CD8 + /CD4 + T cells, B cells, as well as M2 macrophages and cancer-associated fibroblasts. This subtype also presented high enrichment scores for antigen-presenting signatures, suggesting that it could be highly sensitive to immunotherapy. Conversely, the subtype with the poorest prognosis showed significantly decreased macrophage function, indicating that patients in this group may respond better to chemotherapy or combination therapy.

In another study by Naeini et al. [62], the proportion of 18 immune cell types in the TME of pre-treatment samples was used to categorize 68 patients with EAC into four immune clusters associated with overall and progression-free survival. Among these, immune hot, immune cold and immune suppressed clusters were identified. The immune hot cluster was enriched with lymphocytes (i.e., CD4+ and CD8 + T cells), myeloid-derived cells (i.e., macrophages, monocytes and dendritic cells) and demonstrated high levels of immune checkpoint molecules, suggesting a potential response to immune checkpoint blockade immunotherapy. This cluster had the best outcome, consistent with one of Ling's clusters [61], and with previous studies linking immune hot tumors to prolonged survival in other cancer types [63]. On the other hand, the immune cold cluster was characterized by low immune infiltrate and low expression of immune checkpoint molecules, suggesting limited benefit from ICI therapy. However, enrichment of metabolic pathways in tumors within this cluster suggested that alternative therapy strategies could be effective. The immune suppressed cluster, enriched with macrophages and myeloid-derived cells but depleted of lymphocytes, was associated with the worst survival. Tumors in this cluster showed high expression of immune suppression markers, such as *SPP1*, and invasive phenotypes marked by increased angiogenesis, G2M checkpoint activity, and activation of MYC and E2F targets – features mostly reduced in the immune hot cluster. Although not statistically significant, lower pathological responses to cisplatin/5-FU chemotherapy were found in this cluster compared to the others.

The characterization of immunological profiles in EAC has also been explored in broader contexts. Using a collection of immune expression signatures scores, Thorsson et al. classified TCGA tumors – including 76 EAC – into six major immune subtypes spanning multiple cancer tissue types [64]. Each immune subtype was defined by dominant immunogenomic features with potential therapeutic implications and likely impact on prognosis, as the subtypes were associated with both overall survival and progression-free interval. Patients with EAC were distributed across five clusters – C1: wound healing, C2: IFN-γ dominant, C3: inflammatory, C4: lymphocyte depleted and C6: TGF-β dominant (Fig. 3) – demonstrating the immune heterogeneity of EAC. Only the immunologically quiet C5 cluster, characterized by low immune cell infiltration, did not include any EAC patients. The poorest outcomes were observed for C4 and C6, whereas C3 showed the most favorable prognosis. These observations are consistent with above-mentioned studies [61, 62], where immunosuppressed TMEs were associated with poor prognosis, while tumors with high immune cell infiltration had better outcomes. The comprehensive immunogenomic characterization of these clusters allowed the suggestion of subtype-appropriate therapies, spanning from potential response to ICIs in tumors with high lymphocyte infiltration and neoantigen load (as in C2), to the use of TGF-β inhibitors in combination with chemotherapy (for patients in C6).

Immune-based classification models for improved mortality risk assessment

Molecular signatures developed for prognostic assessment of patients with EAC may also inform potential response to immunotherapy or suggest new immunotherapy targets, especially if they are associated with immune-related genes. Patient stratification based on such signatures could enable improved treatment interventions for those with otherwise poor prognoses. The following studies aimed to build prognostic models for EAC based on the differential expression of immune-related genes between EAC and normal tissue samples. Prognosis-associated, differentially expressed features were refined into signatures that allowed categorization of patients into high- and low-risk groups, based on the median survival risk score calculated from the identified signatures. Patients in the low-risk groups consistently showed improved outcomes compared to those in the high-risk groups, and the two groups frequently display distinct immune landscapes.

From gene expression data, Yang and colleagues [65] constructed a four-gene immune-related signature associated with prognosis. The survival risk score calculated from this signature was an independent risk factor for overall survival in EAC patients. Differences in immune cell infiltration, tumor immune escape (TIDE) scores and expression of immune checkpoint-related genes suggested that patients in the high-risk group might be more suitable for immunotherapy. Another immune-based prognostic signature based on gene expression data was proposed by Zhang et al. [66], and the survival rate difference between high- and low-risk groups remained significant when patients were stratified by age or tumor stage. Furthermore, multivariate analyses and a nomogram indicated that combining the survival risk score with sex, M stage and tumor stage could accurately predict survival in patients with EAC. The proportions of M0, M1, and plasma cells differed between the high- and low-risk groups, although the potential for targeted therapy response has not been explored.

MOLECULAR STRATIFICATION AND ELUCIDATION OF THE MECHANISMS UNDERLYING EAC CARCINOGENESIS

Understanding the molecular changes occurring during transition from BE to EAC may provide essential mechanistic insights into early EAC carcinogenesis, and may help improve early detection, risk stratification and prevention strategies [67]. A significant number of publications have characterized the genomic events occurring in BE, as reviewed by Killcoyne and Fitzgerald [68]. While such studies are important for understanding the mechanisms underlying EAC development, they do not use these events for direct patient stratification and were therefore not included in this review.

Genome-wide molecular profiles of BE samples have been compared to those of EAC [34, 49, 51]. Characterizing inter-lesional molecular heterogeneity may allow identification of novel tumor suppressors involved in esophageal carcinogenesis and potential biomarkers for malignant progression of BE. Krause et al. investigated genome-wide methylation profiles of EAC, BE and normal squamous esophagus [34]. The most variable probes across all samples separated EAC and BE from normal squamous esophagus, but not EAC from BE, highlighting the molecular similarity between tumors and their precursor lesion. As a result, EAC and BE were grouped together in two distinct clusters, one of them characterized by a CIMP-like methylation pattern. The distribution of BE samples across clusters was independent of whether they were collected from patients with EAC. Jammula et al. reported similar findings, showing that methylation profiles of BE more closely overlap with EAC than with normal tissues [51]. These observations confirm that aberrant methylation is an early event in EAC progression [8, 69], and that BE and EAC samples share genome-wide methylation features [7, 34, 51], distinct from normal squamous esophagus. Interestingly, one of the four subtypes identified by Jammula et al. was dominated by BE cases (83% against 17% EAC). The few EAC cases in this subtype had adjacent BE, moderate differentiation, and the best prognosis compared to EAC cases in the other subtypes. These observations are in agreement with another study showing that EAC with adjacent BE has a better prognosis [70].

Molecular similarities between BE and EAC are also supported by transcriptomics and genomics data. In a meta-analysis of gene expression data from three independent EAC cohorts, Guo et al. identified two subtypes with specific expression and mutation profiles [49]. The gastric-like subtype II exhibited gene expression patterns closely resembling those found in BE, supporting the concept of a progression pathway from BE to EAC. Additionally, whole-genome sequencing has shown that BE exhibits a high mutational burden [11, 71], present already in non-dysplastic BE samples, underscoring the presence of extensive early molecular changes in this premalignant condition. Complex genomic catastrophes found in dysplastic BE [9] have been suggested as a potential driver of malignant transformation [14, 68]. Structural rearrangement patterns derived from such complex genomic events were used by Nones and colleagues [14] to subtype 22 EAC cases. The number of structural variants and their genomic distribution revealed considerable inter-tumor heterogeneity and enabled categorizing EAC cases into unstable genomes, scattered and complex localized.

These studies, in agreement with others, indicate that molecular alterations occur early in EAC development and that they can be valuable for tumor stratification. However, some studies have highlighted molecular differences that distinguish BE from EAC [33, 51]. In addition to similarities in hypermethylation patterns, Jammula et al. also identified a set of unmethylated probes highly specific to the BE-dominated subtype, suggesting that these may maintain tissue specificity in BE and become methylated in EAC [51]. This subtype lacked DNA methylation at binding sites for key transcription factor motifs (including HNF4A/G, FOXA1/2/3, GATA6 and CDX2), consistent with their role in EAC progression. Similarly, Kaz et al. found distinct methylation signatures between EAC, BE, normal squamous esophagus and high-grade dysplasia [33]. By identifying molecular signatures that may separate EAC from BE, the authors provided insights into EAC progression. This approach also offers the potential for developing biomarkers for early detection and risk stratification among patients with BE.

To identify specific markers for these histological groups, Kaz et al. conducted differential methylation analysis, revealing the highest number of differentially methylated CpG sites between EAC and squamous epithelium (SQ; 442 sites), followed by BE *vs.* SQ (225 sites), and only a few between EAC and BE (17 sites) [33]. The low number of sites differentiating EAC and BE supports the substantial overlap in methylation profiles, but the identified sites may be involved in progression and may serve as diagnostic or prognostic markers.

Although BE is recognized as a pre-malignant condition, clinical heterogeneity exists among individuals with BE. Clustering analyses of BE samples independently from EAC have revealed methylation subtypes in BE [33, 50]. Yu et al. [50] identified four distinct non-dysplastic BE methylation subtypes that mirrored those identified in EAC, without significant differences in gene alteration frequency between them. As for EAC, Kaz et al. [33] found two BE clusters with distinct methylation profiles, described as high and low methylation epigenotypes, analogous to CIMP groups in other cancer types. The presence of similar clusters in BE and EAC suggests that molecular heterogeneity is established at the BE stage and highlights the importance of molecular profiling for identifying patients at higher risk of progression.

COMMON CHALLENGES LIMITING THE CLINICAL APPLICATION OF EAC MOLECULAR STRATIFICATION STUDIES

Despite research efforts in molecular subtyping of EAC, several limitations currently restrict the clinical utility of published stratification studies and prognostic signatures. First, EAC is a relatively rare cancer type, and most studies have therefore relied on modest sized cohorts (Table 1). Limited sample availability is also reflected by the fact that approximately 60% of the studies included here used data from the same patient cohorts, publicly available from TCGA or Gene Expression Omnibus (GEO), rather than producing novel datasets from independent, population-representative patient cohorts (Fig. 2 and Table 1). Moreover, validation of findings in separate, external cohorts is often lacking (Table 1).

Furthermore, the subgroup stratification and the relative frequency of various molecular alterations risk being influenced by underlying confounding factors such as bias in clinical representativeness or variable tumor cell fraction within samples. Although several studies have included samples with a high tumor content only (Table 1), few of them have systematically addressed the distribution of tumor percentages across and within the subtypes [36] to evaluate its potential impact on subtype assignment.

Most studies reviewed here used surgical specimen samples from locally advanced, resectable tumors, while a large proportion of patients with EAC are diagnosed with metastatic or locally inoperable tumors. Although understanding molecular characteristics of localized disease can provide valuable information applicable to the metastatic context, – for example by informing therapeutic strategies or identifying biomarkers relevant throughout disease progression, – relying solely on data from locally advanced tumors may limit the applicability of findings in advanced disease.

Finally, methodological differences, such as the choice of genome-wide platform, feature selection strategies, clustering methods and study design, may also influence the molecular subtyping are identified and whether these can be validated.

CLINICAL IMPLICATIONS AND FUTURE DIRECTIONS

Despite challenges, molecular profiling may advance clinical management of EAC by providing more precise prognostic assessments and tailored therapeutic interventions. Stratification efforts have identified genomic and epigenomic subgroups with the potential to inform disease outcomes, refine patient selection for existing therapies, and uncover new treatment opportunities.

To enable clinical translation, future research should focus on larger, well-characterized, prospective patient cohorts and rigorous external validation. Collaboration across centers serve as an effective approach to overcome current sample limitations and to ensure consistent methodologies and standardization throughout studies. Such initiatives may also facilitate the use of patient cohorts that are representative with respect to demographic characteristics and clinically relevant features, including tumor stage.

Transparent reporting of tumor cell fraction within samples, clinical variables and stratification methods is also necessary to enhance reproducibility and facilitate fair comparison between studies. Controlling for these factors is important to ensure the quality of data analyses and assess the clinical relevance of proposed subtypes.

Moreover, stratification of BE, either independently or combined with EAC, may provide valuable insights into the early mechanisms driving tumor development. Such findings may facilitate the identification of biomarkers for malignant progression in BE, which could improve early detection, refine risk stratification, and inform effective prevention strategies to reduce the burden of EAC. Prioritizing the development and validation of molecular markers for patient subgroups at higher risk of malignant progression, as well as for early detection of EAC, remains essential for improving survival outcomes.

Collectively, these strategies may facilitate the implementation of promising molecular classifiers in routine clinical practice, enable the design of clinical trials with more homogeneous populations, and ultimately advance precision medicine in EAC.

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AUTHOR CONTRIBUTIONS

RP and GEL conceived and planned the paper; RP carried out the literature search, article collection and organization; RP, IVS, HMV and HP drafted the manuscript; RP and IVS designed the figures; GEL supervised the writing process, revised the first draft and made critical contributions; TM critically revised the manuscript. All the authors approved the final version of the manuscript.

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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