Biomarker evaluation in radically resectable locally advanced gastric cancer treated with neoadjuvant chemotherapy: an evidence reappraisal

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***Abstract*:** Neoadjuvant chemotherapy (NAC) significantly improved the prognosis of patients with locally advanced resectable gastric cancer but, despite important progresses, relapse-related death remains a major challenge. Therefore, it appears crucial to understand which patients will benefit from peri-operative treatment. Biomarkers such as human epidermal growth factor receptor-2 (HER2), microsatellite instability (MSI), and Epstein-Barr Virus (EBV) have been widely studied; however, they do not yet guide the choice of perioperative treatment in clinical practice. We performed a narrative review, including 23 studies, addressing the value of tissue- or blood-based biomarkers in the neoadjuvant setting. Ten studies (43.5%) were prospective, and more than half were conducted in East-Asia. Biomarkers were evaluated only post-NAC (on surgical samples or blood) in seven studies (30.4%), only pre-NAC (on endoscopic specimens or blood) in 10 studies (43.5%), and both pre- and post-NAC (26.1%) in six studies. Among the high variety of investigated biomarkers, some of these including MSI-H or enzymatic profile (as TS, UGT1A1, MTHFR, ERCC or XRCC) showed promising results and deserve to be assessed in methodologically sound clinical trials. The identification of molecular biomarkers in patients treated with NAC for locally advanced resectable gastric or EGJ cancer remains crucial.

***Keywords*:** biomarkers, gastric cancer, neoadjuvant chemotherapy, predictive factors, prognostic factors

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Introduction

Patients with gastric cancer, including those with adenocarcinoma of the esophagogastric junction (EGJ) treated with curative intent, have a poor prognosis with 5-year survival rates varying between 30% and 40%. Relapse-related death remains a major challenge for curative treatment. Several strategies have evolved to improve survival, such as adjuvant systemic chemotherapy, typically used in Asian countries, peri-operative chemotherapy, mainly used in European countries and, adjuvant chemoradiation, historically preferred in North America. In particular, throughout the last decade, perioperative chemotherapy significantly improved the prognosis of patients with resectable gastric cancer, becoming the standard of care in Caucasian patients with resectable locally advanced disease. Perioperative (neo-adjuvant + adjuvant) rather than preoperative (just neo-adjuvant) therapy represents the standard treatment for locally advanced gastric cancer (LAGC) based on the results of the MAGIC and FFCD trials.[1,2] Recently, the taxane-containing FLOT [docetaxel, oxaliplatin, leucovorin and 5-fluorouracil (5-FU) regimen] showed superiority over ECF (epirubicin, cisplatin, and 5-FU, as applied in the MAGIC study) in terms of histological response, relapse-free survival, and overall survival.[3] The

greatest benefit from perioperative chemotherapy seems to come from the pre-operative part [neoadjuvant chemotherapy (NAC)] since, even in the AIO-FLOT4 trial, less than half of the study population completed the post-operative treatment as planned by protocol. Similar results come from the SAKK 43/99 trial,[4] which compared pre- and post-operative taxane-containing chemotherapy for resectable gastric cancer. This trial also found a higher proportion of patients able to complete the chemotherapy treatment in the pre-operative arm (94% *versus* 66%).

In any case, despite this important progress, relapse of the disease is still observed in a significant proportion of patients, often with a fatal outcome due to metastatic spread. Therefore, it appears crucial to understand which patients will benefit from NAC, based on reliable predictive factors, in order to personalize the therapeutic approach. Currently, no molecular marker has been shown to guide systemic treatment in the peri-operative setting. Data correlating the clinical outcome with molecular characteristics in patients receiving chemotherapy are scarce and are mainly based on The Cancer Genome Atlas (TCGA) four molecular-defined subtypes [Epstein–Barr virus (EBV)-positive subtype, microsatellite unstable subtype (MSI), genomically stable (GS) subtype, and chromosomal instability subtype (CIN)].[5–7]

Prognostic and predictive factors are essential for personalized medicine; several clinical and tumor characteristics may identify patients with a poor prognosis, irrespective of the received treatment. Prognostic factors can be identified from logistic regression analyses and can be used to stratify patients for treatment allocation and, on the long run, create risk assessment models or nomograms. Predictive factors indicate patient subgroups which could benefit from a specific treatment over the other.[8] However, besides well-known histological parameters, such as pathological tumor-node-metastasis (pTNM) stage, no further molecular analyses are used thus far to stratify the role for chemotherapy in the perioperative setting of gastric and EGJ cancer. MSI and EBV status have been widely studied,[9,10] but evidence is still heterogeneous and hardly applicable to clinical practice.[11] In addition, the majority of data derived from surgical specimens, often pre-treated with NAC. Ideally, to assess the predictive value of a biomarker to a specific NAC, it should be determined from material obtained before the treatment. Moreover, even for the prognostic value, post-operative samples may not accurately reflect the original biology of the tumor, due to the impact of treatment itself. Therefore, due to these potential biases, we performed a literature reappraisal about biomarkers evaluation in radically resectable gastric and EGJ cancers. The aim of our critical review was to verify the lines of investigation on this topic, with aspects of consistency and controversy, and to discuss the most promising ones according to their future clinical application.

Methods

Leaving aside the rigorous criteria of a systematic review, we searched Pubmed, Embase, and Cochrane Library Databases updated to July 2020 for all the potentially relevant publications. The key search terms were 'biomarkers' AND 'gastric cancer' OR 'stomach cancer' OR 'gastroesophageal cancer' OR 'esophagogastric junction cancer' AND 'neoadjuvant chemotherapy'. Narrowing the selection to English language papers, 143 articles were identified. Among these, we selected studies which included patients with radically resected locally advanced gastric cancer receiving NAC and that analyzed tumor tissue or circulating molecular biomarkers. In addition, studies including some patients with potentially resectable stage IV gastric cancer were also included, if they reported subgroups analysis based on stage (e.g. stage I–III *versus* IV). Studies assessing exclusively post-operative treatment or metastatic gastric cancer were excluded. According to this strategy, we selected 42 articles, that have been carefully evaluated and, lastly, 23 of them fulfilled our criteria. We arbitrarily included results with the highest potential clinical implications and/or the easiest reproducibility along with clinical practice. High quality data from established international research groups and results consistent with known literature background have been primarily selected. We extracted information from each eligible study, including first author, publication year, country, type of study, source of the biomarker (tissue or blood), timing of evaluation (pre-NAC EGDS or post-NAC surgery), clinical features including stage, histotype and tumor location, chemotherapy regimens, HER2 status, and clinical outcomes. Due to the descriptive intent of the paper, we did not perform any direct correlation with clinical outcomes.

Results

Eligible studies and their characteristics

Twenty-three articles were selected and included in our review.[12–33] These studies were published between 2006 and 2020, properly reflecting the current clinical scenario. Ten studies (43.5%) were prospective, whereas 13 were retrospective. Concerning geographical area, more than half of the studies (13/23, 56.5%) were conducted in East Asian countries. Patient characteristics were balanced between studies in term of age, gender, and clinical tumor characteristics. Only five studies (21.7%) reported information on the HER2 status of the tumor. Chemotherapy regimens were various, although all studies used fluoropyrimidines as a kind of backbone, combined with other drugs such as oxaliplatin, cisplatin, or taxanes. Patients were treated with tegafur/gimeracil/oteracil (S-1) in eight studies (34.8%) reflecting the current Asian standard of care in this setting. One study included a combined approach with chemoradiation and another one with intra-arterial chemotherapy. All the main characteristics of the population enrolled in the selected studies are reported in Table 1.[12–33]

Biomarkers

All publications included in the final selection reported the determination of a molecular biomarkers in locally advanced gastric and EGJ cancer patients undergoing NAC. In detail, biomarkers analyzed, samples type, and outcomes are shown in Table 2.[12–33]

For what concern the timing of the determination, biomarker analyses were performed exclusively post-NAC (on surgical specimens or blood) in seven studies (30.4%), only pre-NAC (on endoscopic biopsies or blood) in 10 studies (43.5%), and both pre- and post-NAC (26.1%) in six studies. Twelve studies of the total (52.2%) analyzed tissue biomarkers[12,8,16,18–20,26,28–31,33] four studies performed the determination on the endoscopic biopsy and the surgical sample, other four only on the endoscopic biopsy and the last four just on surgical specimen. Ten studies (43.5%) looked at circulating biomarkers,[13–15,17,21,22,24,25,27,32] in the vast majority assessed pre-NAC (80%, 8/10). Only one examined both circulating and tissue biomarkers, but exclusively on samples obtained post-NAC.[23] Among circulating biomarkers, lymphocyte ratio or neutrophil/platelet to lymphocyte ratio were the most frequently analyzed parameters (3/10).[15,17,21] On tissue sample, MMR/MSI status was the most examined one (3/12):[12,18,33] two studies, assessed MSI by polymerase chain reaction (PCR) analyzing five nucleotide repeats (mono nucleotide BAT25, BAT26 and dinucleotide D2S123, D5S346, D17S250) and cases with at least two markers of instability were defined as MSI-H, (while cases with only one markers of instability were classified as MSI-L and no markers as MSS). The third study performed MSI analysis using a panel of three mononucleotide (BAT25, BAT26, and CAT25) and cases were divided in MSI-H (instability in two or three of the markers) and MSS if one only or no markers of instability. In addition, this study also performed the immunohistochemical staining of MMR proteins (MLH1, PSM2, MSH2, MSH6).

Outcomes

Clinical outcomes analyzed in the studies were heterogenous: 17 studies performed analyses on overall survival (OS), relapse-free survival (RFS), and disease-free survival (DFS), seven studies assessed rates of response to treatment, including radiological or pathological tumor responses (in four cases by RECIST criteria, the others using Becker, Mandard, and Japanese criteria of response).[8,14,20,24,26,28,29] Three studies created a nomogram, stratifying patients according to biomarker results. Looking specifically at 17 studies which analyzed survival outcomes, half determined only OS and half both OS and DFS. In detail, the correlation between biomarker and survival has been performed on both pre- and post-NAC specimens in two studies (12.5%), on surgical samples post-NAC in four studies (4/16, 25%) while the majority of studies (n = 10) analyzed pre-NAC samples only (10/16, 62.5%). However, among these latter, only three studies, representing one sixth of the total selection, investigated tissue molecular biomarkers, whereas the vast majority looked at circulating biomarkers. Conversely, studies conducted on surgical samples analyzed mainly tissue biomarkers.

Specifically, retrospective studies conducted on MSI showed similar results with higher rate of RFS and OS for MSI-H subpopulation (RFS 21.4 months for MSS *versus* not reached for MSI-H patients, OS 38.6 months *versus* not reached in the MSI-H group).[18] However, one study analyzed MSI on endoscopic pre-NAC samples and in this case, the presence of an

Table 1. Patient characteristics of studies included in the review.[11–33]

Author	Study (Retro/Prosp)	Country	Age (median)	Male (%)	Intestinal histotype (%)	G3 (%)	Clinical stage (cN+)	Pathological stage (pN+)	Her2 positive (%)	Chemotherapy
Grosser et al.[12]	R	Europe	60.9	70.2	56.4	69.0	NA	69.2	NA	Cisplatin + 5-FU
Li et al.[8]	P	Asia	56.6	77.1	31.4	NA	68.6	42.9	NA	SOX/XELOX
Catenacci et al.[13]	P	USA	66	78	33.3	72.2	75	47.0	16.7	FOLFIRINOX
Qin et al.[14]	P	Asia	57.6	69	22	NA	NA	NA	NA	DOS/XELOX
van Hootegem et al.[15]	R	Europe	62	88.4	NA	71.9	37.5	65.4	NA	ECF/Cisplatin + 5-FU
Yeh et al.[16]	P	Asia	66	65.5	NA	29.3	100	67.2	NA	FOLFOX
Chen et al.[17]	R	Asia	NA	77	NA	59.3	73.6	73.6	NA	SOX/XELOX
Haag et al.[18]	R	Europe	61	69.3	53.3	NA	89.1	62.6	4.3	FLOT/FLO/ECX/ECF
Kohlruss et al.[33]	R	Europe	61	76.6	56.4	63.9	NA	68.4	NA	Cisplatin + 5-FU
Smyth et al.[19]	P	Europe	64	81	86.9	NA	60	NA	NA	ECF
Liu et al.[20]	P	Asia	61	80	NA	NA	100	53.3	NA	SOX and RT+S-1
Li et al.[21]	R	Asia	59	80	NA	61.6	100	60.7	NA	FLEEOX
Bozkaya et al.[22]	P	Asia	55	72	NA	70	NA	84	NA	DCF
Stahl et al.[23]	P	Europe	61	72	NA	NA	80	63	8.2	ECX +/– panitumumab
Tan et al.[24]	P	Asia	55.3	67.5	NA	NA	NA	NA	NA	SOX
He et al.[25]	R	Asia	62	67.5	NA	NA	100	7 (CR)	NA	FLEEOX
Li et al.[26]	R	Asia	66	87	NA	48,3	34.4	66.7	NA	SOX
Li et al.[27]	R	Asia	58.3	71.8	NA	NA	68	31.2	NA	FOLFOX/SOX/XELOX
Qu and Qu[28]	R	Asia	59	75.4	NA	71.7	64.2	47.2	22.6	FOLFOX
Jia et al.[29]	R	Asia	60	71.6	NA	64.7	81.2	77.5	NA	FOLFOX
Hirakawa et al.[30]	P	Asia	65	74.4	39.5	60.5	90.7	73.8	NA	DCS
Mutze et al.[31]	R	Europe	NA	74	41.2	84.3	NA	59	NA	Cisplatin + 5-FU
Ott et al.[32]	R	Europe	56	72	44.4	83	NA	62	NA	Cisplatin + 5-FU

5-FU, 5-fluorouracil; DCF, docetaxel, cisplatin and 5-FU; ECF, epirubicin, cisplatin, 5-FU; ECX, epirubicin cisplatin and capecitabine; FLO, calcium leucovorin, citrovorum factor and folinic acid; FLOT, docetaxel, oxaliplatin, leucovorin and 5-FU; FOLFIRINOX, folinic acid, 5-FU, irinotecan, oxaliplatin; NA, not available; P, prospective; R, retrospective; SOX, S-1 and oxaliplatin; XELOX, oxaliplatin and capecitabine.

Table 2. Biomarkers and outcomes of studies included in the review.[11–33]

Author	Biomarker	Endoscopic (E)/ surgical (S) samples	N° cases (E/S)	Sample	Method	Outcome	Results S	Results NS
Grosser et al.[12]	p53 and correlation with MSI and EBV	Y/Y	138/294	Tissue	IHC, NGS	OS	p53 (surgical)	p53 (endoscopic), p53 correlation with MSI/EBV
Li et al.[8]	WES	Y/Y	35/35	Tissue	NGS	ORR	C10orf71mut, MDM2 amplification	MYC amplification
Catenacci et al.[13]	UGT1A1	N/Y	–/36	Blood	Liquid chromatography	OS, DFS	/	UGT1A1
Qin et al.[14]	IgG glycomics profiling	Y/Y	49/49	Blood	Liquid chromatography	ORR	Glycans G4, G6, G18	Others GP
van Hootegem et al.[15]	NLR	Y/N	139/–	Blood	Flow cytometry	OS, DFS	/	NLR
Yeh et al.[16]	ERCC1, ERCC2, XRCC	N/Y	–/58	Tissue	IHC	ORR, OS, DFS	ERCC1, ERCC2	XRCC1
Chen et al.[17]	PLr	Y/N	91/–	Blood	Flow cytometry	OS, DFS	PLR (high plt count)	PLR (low plt count)
Haag et al.[18]	MSI	N/Y	–/101	Tissue	IHC	OS, DFS	MSI-H	/
Kohlruss et al.[33]	MSI, EBV	Y/Y	143/326	Tissue	PCR	ORR, OS	MSI-H, EBV+ for OS in resected non-CTx cohort	MSI-H, EBV+ for response to CT and OS in biopsy cohort
Smyth et al.[19]	CDH1, ELOVL5, EGFR, PIP5K1B, FGF1, CD44v8.10, TBCEL (7-gene Signature)	N/Y	–/84	Tissue	PCR	OS	High Risk (HR) versus Low Risk (LR)	/
Liu et al.[20]	microRNA	Y/N	15/–	Tissue	PCR	ORR	/	/
Li et al.[21]	CLR	Y/Y	112/112	Blood	Flow cytometry	ORR, OS	High CLR versus Low CLR	/
Bozkaya et al.[22]	Survinin	Y/N	50/–	Blood	PCR	ORR, OS, DFS	/	Survinin
Stahl et al.[23]	EGFR, HER2, MET	N/Y	–/160	Tissue and blood	IHC, ISH/FISH	OS, DFS	HER2+, MET	EGFR

(continued)

Table 2. (continued)

Author	Biomarker	Endoscopic (E)/ surgical (S) samples	N° cases (E/S)	Sample	Method	Outcome	Results S	Results NS
Tan et al.[24]	microRNA	Y/N	120/–	Blood	PCR	ORR	miR-145, miR-185	miR-381, miR-195
He et al.[25]	T cell subpopulations	Y/N	105/–	Blood	Flow cytometry	OS	High CD3+ CD8+ Tcell longer OS	CD3+CD4+ T cell
Li et al.[26]	P-gp, GST, topo II, MRP, LRP, Ki-67, p53	Y/N	93/N	Tissue	IHC	ORR	Ki-67, p53	P-gp, GST, Topo II, MRP, LRP
Li et al.[27]	MTHFR, DPYD UMPS, ABCB1, ABCC2, ERCC1, XRCC1, and GSTP1 polymorphism	Y/N	103/N	Blood	PCR	OS	ABBC2-24C >T	Others polymorphisms
Qu and Qu[28]	C-met, EGFR, HER2, Ki-67, MMP7, p53, Topo II	Y/Y	53/53	Tissue	IHC, FISH	ORR	HER2, p53	c-MET, EGFR, Ki-67, MMP7, topoII
Jia et al.[29]	DAP-3	N/Y	–/85	Tissue	IHC, PCR	ORR	DAP-3	/
Hirakawa et al.[30]	ERCC1, DDB2	Y/N	43/–	Tissue	IHC	OS, DFS	ERCC1, DDB2	/
Mutze et al.[31]	DNMT1, DNMT3b	Y/N	127/–	Tissue	IHC	OS	DNMT1	DNMT3b
Ott et al.[32]	TS, MTHFR	N/Y	–/135	Blood	PCR	OS	TS 2rpt/2rpt, TS 2rpt/3rpt	TS 3rpt/rpt

CDH1, cadherin-1; CLR, circulating lymphocyte ratio; DAP3, death-associated protein 3; DDB2, DNA damage-binding protein 2; DPYD UMPS, dihydropyridine dehydrogenase; DNMT1/3b, DNA cytosine-5-methyltransferase 1/3b; EBV, Epstein-Barr Virus; EGFR, epidermal growth factor receptor; ERCC1/2, DNA excision repair protein ERCC-1/2; ELOVL5, elongation of very long chain fatty acids protein 5; FGF1, fibroblast growth factor 1; GST, glutathione–S-transferase; HER2, human epidermal growth factor receptor 2; IgG, immunoglobulin G; LRP, low density lipoprotein receptor related protein 1; MMP7, matrix metalloproteinase 7; MRP, MARCKS-related protein; MSI, microsatellite instability; MTHFR, methylenetetrahydrofolate reductase; NLR, neutrophil-lymphocyte ratio; P-gp; P-glycoprotein; PIP5K1B, phosphatidylinositol 4-phosphate 5-kinase type-1; PLR, platelet-lymphocyte ratio; TBCEL, tubulin-specific chaperone cofactor E-like; UGT1A1, UDP glucuronosyltransferase 1 family, polypeptide A1; WES, whole exome sequencing.

aberrant p53 expression was associated with a worse OS in the MSI-H subgroup even compared to MSS/EBV negative (median MSI-H 23.4 months, MSS/EBV− 36.6 months), potentially losing the positive prognostic effect of MSI-H.[8] Thymidylate synthase (TS) tandem repeat polymorphism analyzed in blood samples has been identified as independent prognostic factors in the NAC group, with a significant survival benefit for the 2rpt/2rpt ($p = 0.002$) and 2rpt/3rpt genotypes ($p = 0.004$).[32] Regarding response to NAC, multi-omics characterization and RNA sequencing on tumor tissues allowed to identify C10orf71 mutations that were associated with treatment resistance ($p = 0.00011$) as well as *MDM2* ($p = 0.033$),[8] while *MYC* amplification correlated with treatment sensitivity. Similarly, polymorphisms in genes involved in drug metabolism has been associated with NAC response: for example, patients with TT and TC genotypes of *ABCC2-24C > T (rs717620)* responded to NAC 3.80 times more often than those with the CC genotype.[27] Outcomes of studies included in the review are summarized in Table 2.

Discussion

The identification of predictive biomarkers for NAC in radically resectable locally advanced gastric and EGJ cancer is an unmet clinical need. Tissue-derived or circulating biomarkers have been widely studied, but these data are hardly applicable to the curative setting at the time being.[11] Starting from that, we searched the literature for articles investigating biomarkers in LAGC and 23 articles were finally included in our review.[12–33] The population evaluated in the analysis was homogeneous in terms of clinical setting, since all patients had a gastric or EGJ cancer, received NAC, and underwent radical surgery. The main characteristics of the population were well balanced among the different studies, with a slight predominance of Asiatic patients, since 13 studies out of 23 were conducted in Eastern countries. Similarly, NAC was consistent across the selection: fluoropyrimidines were the common denominator of the various regimens, with limited differences related to standard of care. In this setting, clinical practice varies between geographical areas, mainly due to differences in tumor characteristics and local preferences. While perioperative chemotherapy is the preferred strategy in Europe, adjuvant chemotherapy is preferred in Asia and adjuvant chemo(radio)therapy in US.[34–36] Conversely, we

observed a high heterogeneity in the evaluated biological samples (blood, biopsy specimen, surgical specimen), in the types of biomarkers tested, utilized methods and analyzed clinical outcomes. Circulating biomarkers derived from liquid biopsy (LB) have potentially a great role in gastric and EGJ cancer where molecular characterization usually relies on a single or a few endoscopic biopsies in the pre-operative setting, sometimes even inadequate for complete molecular characterization. Research conducted in solid tumors showed promising results about feasibility and relevance of LB to detect predictive biomarkers in colorectal and lung cancer.[37,38] In gastric and EGJ cancer, LB research is in its beginnings, and only few studies looked at the correlation between HER2 amplification in plasma and in histological samples.[39,40] Our review included 11 papers analyzing circulating biomarkers looking prevalently at blood count ratio or gene polymorphisms involved in chemotherapy metabolism. Some results are encouraging, but prospective trials conducted in larger populations are needed.

Microsatellite instability (MSI) is one of the most studied biomarkers in solid tumors, including gastric cancer. In early-stage colorectal cancer, it is associated with a lack of benefit from adjuvant chemotherapy,[41] and its determination is now clinical practice to tailor adjuvant treatment decision in stage II. Similarly, MSI impact on outcomes was tested in resected gastric cancer. Pietrantonio and colleagues[8] performed an individual patient data meta-analysis from the MAGIC, CLASSIC, ARTIST, and ITACA-S trials showing statistically significant longer 5-year OS rate for MSI-H group compared to MSI-low and MSS (77.5% *versus* 59.3%). Moreover, the addition of chemotherapy was beneficial for MSI-low/MSS GC (5-year DFS of 57% *versus* 41% with surgery alone), in contrast with the MSI-H subgroup (70% *versus* 77%). These results support the use of MSI as prognostic marker for resectable gastric cancer. However, the meta-analysis included trials assessing mainly adjuvant chemotherapy, as well as patients who received chemoradiation[1,42–44] with only less than 10% of the whole dataset treated with NAC and analyzed for MSI status. Furthermore, no pre-NAC biomarkers evaluation was reported. As a result, it appears premature to translate these findings into clinical decision making about NAC. Three studies included in our review looked at MSI status confirming the positive prognostic value of this marker. Two of them checked the

MSI both pre- and post-NAC, essential and mandatory to get solid information on prediction to NAC response and survival benefit.

Programmed death ligand-1 (PD-L1) status is emerging as a predictive factor of response to immunotherapy. After first FDA approval for pembrolizumab and nivolumab in advanced lines,[45,46] negative results of subsequent studies[47,48] highlighted the necessity of identify which patient will benefit from these agents. Currently, a combined positive score (CPS) greater than or equal to one identifies PD-L1 positive tumors, even though first line CHECKMATE-649 just presented at ESMO 2020 looked specifically to CPS $\geqslant$ 5 population, showing an increase OS of 3.3 month (median) with the addition of nivolumab to chemotherapy.[49] However, in our review, no study did analyze PD1/PD-L1 status, likely due to the lack of immunotherapy in the peri-operative setting at the time being.

Our study presents several limitations: first, the heterogeneity of biomarkers analyzed and utilized methods, which prevents from performing statistical analyses. Secondly, patients enrolled in the chosen studies display important epidemiologic differences (with regard to ethnicity, age, etc) resulting in biological diversity, as well as great dissimilarities in the administered chemotherapy regimens. Thirdly, different clinical outcomes, different time points of the biomarkers evaluation, and different correlations increased the risk of biases in interpreting general results from this analysis Lastly, we arbitrarily chose to focus only on biological biomarkers, excluding imaging. For instance, 18-fluorodeoxyglucose-PET (PET) has shown promising results in response prediction in the neo-adjuvant setting of EGJ cancers.[50] On the other side, a potential strength of our review is the focus on only locally advanced radically resectable gastric and EGJ adenocarcinoma treated with NAC. Moreover, a considerable number of studies included in our review assessed biomarkers on pre-NAC samples, which is the best approach for the implementation of predictive markers, and closest to the setting needed for clinical practice.

In conclusion, our review showed a high heterogeneity in investigating prognostic and predictive biomarkers for NAC in gastric and EGJ cancer, particularly due to the type of biomarker, type of sample used, methods of detection, timing of evaluation and, lastly, clinical endpoints correlated.

Therefore, for all these aspects, our results cannot be considered conclusive but just descriptive. However, some insights could be drawn as hypothesis-generating. Even though MSI is, among all the investigated biomarkers, the one with the potentially highest clinical impact, any study of our analysis reported a solid and strict correlation with NAC. In fact, there are reports about the potential detrimental effect of chemotherapy for MSI-H gastric cancer,[51] but data are deduced comparing MSI-H with MSS tumors and a specific study on solely MSI-H gastric cancer treated or not with chemotherapy has not been performed. Moreover, one study presented a challenging negative interaction of aberrant p53 with MSI-H, although number of patients is limited.[12] This data deserves to be specifically investigated in future well-designed clinical trial addressing NAC in radically resectable locally advanced EGJ and gastric cancer. Furthermore, some enzymatic biomarkers, as TS, UGT1A1, MTHFR, ERCC, or XRCC, raised as promising predictive factors of NAC in several studies of our analysis, suggesting that it could be useful their determination for tailoring the therapeutic algorithm and, lastly, to include them in future studies on NAC in EGJ and gastric cancer, always with uniform techniques and a consistent timing.

Conflict of interest statement

services MSD Advisory services Sanofi-Aventis Speaking engagements ACCMED Speaking engagements Wren Laboratories Europe Advisory services EANM Advisory services Springer Writing engagements Pensiero Scientifico EditoreWriting engagements All other authors declare no COIs.

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