Anti-tumour Treatment

MSI as a predictive factor for treatment outcome of gastroesophageal adenocarcinoma

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ABSTRACT

Gastroesophageal cancers are a major cause of death worldwide and treatment outcomes remain poor. Adequate predictive biomarkers have not been identified.

Microsatellite instability (MSI) as a result of mismatch repair deficiency is present in four to twenty percent of gastroesophageal cancers and has been associated with favorable survival outcomes compared to microsatellite stable tumors. This prognostic advantage may be related to immunosurveillance, which may also explain the favorable response to immune checkpoint inhibition observed in MSI high (MSI-H) tumors. The value of conventional cytotoxic treatment in MSI-H tumors is unclear and results on its efficacy range from detrimental to beneficial effects.

Here the recent data on MSI as a predictive factor for outcome of gastroesophageal cancer treatment is reviewed.

Introduction

Globally, gastroesophageal adenocarcinomas are among the most common causes of cancer related deaths [1]. Treatment still largely relies on conventional cytotoxic agents which are only limitedly effective in the majority of patients [2,3]. According to a comprehensive molecular analysis by The Cancer Genome Atlas (TCGA) Network, gastric cancer (including gastroesophageal junction cancer) can be classified into four subgroups based on its molecular characteristics: Epstein-Barr virus (EBV)-infected tumors, microsatellite instable (MSI) tumors, genomically stable (GS) tumors and chromosomally instable (CIN) tumors [4]. EBV-infected tumors have shown best prognosis, whereas GS tumors have shown worse prognosis and worse response to neoadjuvant chemotherapy [5]. CIN tumors and MSI tumors have shown an intermediate prognosis and CIN tumors benefit most from neoadjuvant chemotherapy, whereas MSI tumors only benefit modestly from neoadjuvant chemotherapy.

Even though many additional prognostic and predictive factors, including both patient and tumor characteristics have been investigated

[6], unfortunately none of these have led to treatment strategies based on specific biomarkers, except for trastuzumab, which has been proven efficacious in patients with HER2 overexpressing gastric or gastroesophageal cancer [7]. Even ramucirumab, which supposedly specifically targets VEGF-R2, is given to an all-comers population and most other studies with targeted agents have not improved outcome [8,9]. Due to this lack of predictive biomarkers, a selection of patients is over treated with chemotherapy they will not benefit from and identification of biomarkers that would allow treatment to be tailored to specific individual molecular characteristics is of great importance.

Recently, immune checkpoint blockade has emerged as a relevant new therapeutic option for seemingly unbeatable metastatic cancers such as melanoma and lung cancer, and has also shown promising results for at least certain subgroups of gastroesophageal cancer [10].

Among others, important immune checkpoint molecules are programmed cell death protein 1 (PD-1) and cytotoxic T lymphocyte protein-4 (CTLA-4), that respectively inhibit the activation of various immune cells and subsequently lead to resistance to immunosurveillance [11]. PD1 is a co-inhibitory receptor that is expressed on the surface of

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activated T lymphocytes and regulatory T cells (Tregs) and its ligand (PD-L1) is expressed by both immune and tumor cells. By activating the PD1 pathway, T cell activity is suppressed in peripheral tissues, thus protecting these tissues during inflammation. By upregulating PD-L1 expression, tumor cells can evade antitumor immune response. Blockade of PD-1 leads to stimulation of the local immune response and shows to be a promising treatment, although effectiveness depends on the expression of PD-1 of the tumor infiltrating lymphocytes [12].

CTLA-4 is expressed on Tregs and also inhibits immune response, although it seems to act in lymphatic tissues rather than within the tumor [13]. In the draining lymph node, dendritic cells present an antigen and express the surface protein CD28, that stimulates tumor specific T cells through binding to the stimulatory receptors CD80 and CD 86. CTLA-4 however binds CD80 and CD86 with a much higher affinity and reduces their ability to induce an antitumor immune response [14].

Due to a defective mismatch repair systems and subsequently an excessive number of somatic mutations, MSI tumors have a high likelihood of presenting neoantigens, which initiates an antitumor T cell response. Ongoing antigen exposure can lead to T cell exhaustion which is associated with elevated expression of PD-1 by tumor infiltrating lymphocytes and subsequently with a decreased antitumor immune response in PD-L1 expressing tumors [15]. Although this is an effective mechanisms to suppress anti-tumor immunity, drugs that block the PD-1-PD-L1 axis such a pembrolizumab can reverse this defense mechanism and induce unprecedented often durable responses in MSI cancers [16–18]. Data on the predictive value of MSI for efficacy of conventional cytotoxic agents however are not unequivocal. Here we review the recent data on MSI and its role in gastroesophageal cancer treatment.

Microsatellite instability in gastroesophageal cancer

Four to twenty four percent of gastroesophageal cancers are characterized by MSI [18–20]. Microsatellites are tracts of repetitive DNA motifs of 1–6 base pairs and are abundant in the genomes of higher organisms, mainly occurring in non-coding DNA [21].

The mismatch repair (MMR) system detects and corrects replication errors. Protein complexes hMSH2/hMSH6 and hMSH2/hMSH3 detect the replication errors made by DNA polymerase and hMLH1/hPMS2 (hMutLα) subsequently removes the mismatched base or bases and allows DNA resynthesis [22] (Fig. 1).

Germline or somatic mutation and DNA promoter methylation of one of the MMR genes leads to deficient mismatch repair and subsequently an accumulating number of mutations throughout the genome [18]. MSI as a result of germline mutations in the MMR system is strongly associated with the Lynch syndrome [23]. Alternatively in sporadic gastric cancer cases MSI may result from MutL Homolog (*MLH1)* promoter hypermethylation which occurs in 80% of the cases [4,24]. Finally a role for infection by *H. Pylori* should be acknowledged. *H. Pylori* has also been correlated with MMR deficiency (MMR-D) and MSI in clinical data [25,26]. However, even though a reduction of MMR protein levels in H. *Pylori* infected cells was observed, it could not be directly translated into MSI-H in mouse models [27].

Testing for MSI

Microsatellites are exceptionally prone to replication errors and therefore are particularly useful as markers for MMR-D [28].

For long, MSI/MMR-D has been determined by polymerase chain reaction (PCR)–based analysis of microsatellites or immunohistochemical (IHC) analysis for MMR protein expression [29]. PCR-based methods compare the allelic position of the microsatellite locus in tumor and normal tissue. Commonly employed panels are the Bethesda Panel [30], consisting of two mononucleotide loci (BAT-25 and BAT-26) and three dinucleotide loci (D2S123, D5S346 and D17S250), and the Promega Panel [31], which replaces the three dinucleotide loci with three additional mononucleotide loci (NR-21, NR-24 and MONO-27), because the dinucleotide markers have shown to be less sensitive and less specific than mononucleotide markers for the detection of MMR-D. Most commonly, when ≥2 out of the 5 markers show a different size compared to the normal tissue control the genotype is called MSI-high (MSI-H). When one out of five markers shows a different size the genotype is called MSI-low (MSI-L) and when there is no difference in any of the markers the genotype is called microsatellite stable (MSS). IHC staining allows detection of expression or absence of MMR proteins and semiquantative scoring is possible. Loss of expression of one of the single proteins or protein complexes involved in MMR provides indirect evidence of MSI.

Recent work shows that MSI can also be identified via next-generation sequencing (MSI-NGS) which may be even more sensitive than MSI PCR, due to a broader range of inferred microsatellite loci [32].
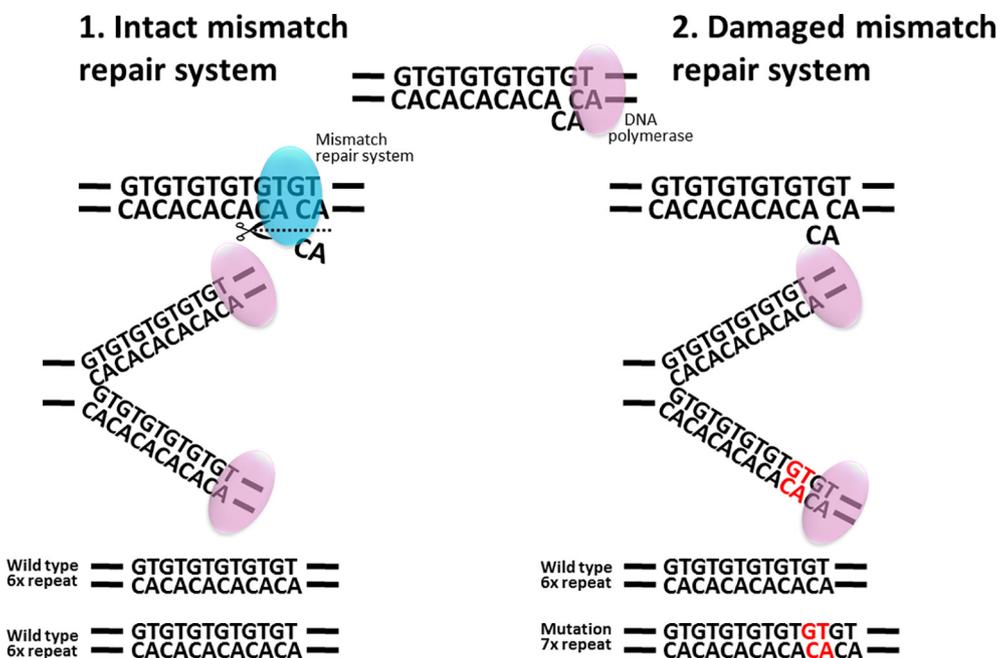

Fig. 1. The microsatellites existing in normal DNA are prone to replication errors (CA). In cells with an intact MMR (1) the wrongly inserted base is recognized and deleted and no mutation is acquired. In cells with a damaged MMR (2) the wrongly inserted base is incorporated in the DNA, thus leading to a frameshift mutation in the microsatellite. MMR deficient cells show highly elevated rates of mutation throughout the genome, which can be objectified by assessing the mutations in the microsatellite, i.e. microsatellite instability.

Table 1
Summary of the number of MSI-H patients and the techniques used to infer MSI in the mentioned publications.

Publication	Cancertype	Number of patients	MSI-H (%)	Techniques used	Inferred loci
TCGA [4]	Gastric adenocarcinoma	295	64 (22%)	PCR	BAT-25; BAT-26; BAT-40; TGFR II; D2S123; D5S346; D17S250
Fuchs et al. 2018 [19]	Metastatic gastric or GEJ (siewert II and III) carcinoma	174	7 (4%)	PCR	BAT-25; BAT-26; NR-21; NR-24; NR-27
Marrelli et al. 2016 [12]	Gastric cancer	472	111 (24%)	PCR	BAT-25; BAT-26; NR-24; NR-21 and NR-27
Choi et al. 2019 [34]	Resectable gastric adenocarcinoma	592	40 (6%)	PCR	BAT-25; BAT-26; NR-21; NR-24; MONO-27
Haag et al. 2019 [35]	Resectable gastric and GEJ carcinoma	101	9 (9%)	PCR	BAT-25; BAT-26; NR-21; NR-24; MONO-27
Hashimoto et al. 2019 [36]	Resectable gastric adenocarcinoma	285	24 (8%)	PCR	BAT-25; BAT-26; NR-21; NR-24; MONO-27
Kim et al. 2015 [37]	Stage II and III gastric cancer	1276	105 (8%)	PCR	BAT-25; BAT-26; D5S346; D2S123; D17S250
Smyth et al. 2017 [38]	Resectable gastric cancer	303	20 (7%)	PCR	BAT-25; BAT-26; NR-21; NR-24; NR-27
Kohlruss et al. 2019 [39]	Resectable gastric adenocarcinoma	617	59 (10%)	PCR	BAT-25; BAT-26; D5S346; D2S123; D17S250
Polom et al. 2017 [40]	Gastric cancer (population of Marrelli et al. 2016)	472	111 (24%)	PCR	BAT-25; BAT-26; NR-21; NR-24; NR-27
Polom et al. 2018 [41]	Stage IV gastric cancer	176	14 (8%)	PCR	BAT-25; BAT-26; NR-21; NR-24; NR-27
Beghelli et al. 2006 [47]	Stage I–IV gastric cancer	510	83 (16%)	PCR	BAT-25; BAT-26
Shitara et al. 2018 [49]	Metastatic gastric or GEJ carcinoma	592	50 (8%)	PCR	BAT-25; BAT-26; NR-21; NR-24; NR-27
Shitara et al. 2019 [50]	Advanced gastric or GEJ carcinoma	763	50 (7%)		Not published
An et al. 2009 [68]	Resectable gastric cancer	1990	170 (9%)	PCR	BAT-25; BAT-26; D5S346; D2S123; D17S250
Pietrantonio 2019 [72]	Resectable gastric cancer (meta-analysis)	1556	121 (8%)	PCR	Different sets of 5 quasi monomorphic mononucleotides
Oki et al. 2009 [73]	Stage I–IV gastric cancer	240	22 (9%)	PCR	D2S123; D5S107; D10S197; D11S904; D13S174
Biesma et al. 2019 [75]	Resectable gastric or GEJ carinoma	168	13 (8%)	PCR	BAT-25; BAT-26; MONO-27; NR-21; NR-24
Miceli et al. 2019 [76]	Resectable gastric cancer	393	35 (9%)	IHC and PCR	BAT-25; BAT-26; NR-21; NR-22; NR-24

Prevalence of MSI in gastroesophageal adenocarcinoma

Reports on the incidence of MSI-H in gastric cancer vary (Table 1), likely because MSI-H status is associated with specific clinical and pathological parameters. In a recent systematic review MSI-H was found to be associated with female sex, older age, intestinal Laurén histological type, mid/lower gastric location, lack of lymph node metastases and TNM stage I–II, but not with ethnicity [33]. Between 6 and 24% of resected gastroesophageal adenocarcinomas are reported to be MSI-H [20,34–39], and this may even mount up to 48% in patients of 85 years and older [40]. However, the percentage sharply drops to less than 10% in patients with synchronous metastatic disease [41], and less than 5% in a beyond-second line treatment setting [19]. MSI is only rarely observed in non-junctional esophageal cancers [42–44].

Prognostic value of MSI in gastroesophageal adenocarcinoma Generally speaking, MSI-H has been associated with favorable survival outcomes compared to MSS and MSI-L [45,46], although the prognostic value may be dependent on the histological subtype. For example, a series of 111 MSI-H primary gastric cancers revealed a notable prognostic difference between MSI-H and MSS in the intestinal subtype but no prognostic value was observed for MSI-H in the diffuse-mixed type and signet-ring cell/mucinous histotypes [20]. It should be noted that only a relatively small number of diffuse cancers were included in the analysis and the study may be underpowered to show any significant differences within this subgroup.

A subgroup analysis in the same cohort showed that the prognostic advantage of MSI-H is present within the same disease stage in resectable gastric cancer and even holds up for stage IV disease [41]. However, this prognostic value of MSI in stage IV gastric cancer was not seen in a different series of 510 stage I–IV gastric cancer patients that underwent gastrectomy including 83 MSI-H tumors [47]. The authors found a positive prognostic value of MSI-H, but in subgroup analysis this only held up for stage II cancers. In contrast to the cohort of Polom et al, none of these patients received any systemic treatment, which might explain the difference in findings.

MSI and immune checkpoint inhibition

The generally good prognostic outcome may be related to

immunosurveillance; MSI-H gastroesophageal tumors are characterized by high levels of CD8 positive T-cells infiltrate [48]. This, in turn, can also explain the favorable response of MSI-H gastroesophageal cancer patients to checkpoint blockade.

The KEYNOTE-059 study evaluated the efficacy and safety of pembrolizumab monotherapy in patients who had progressive gastroesophageal cancer on at least two chemotherapy regimens. Out of the 174 patients available for MSI assessment, 4/7 (57.1%) of the MSI-H patients had objective response to pembrolizumab, whereas 15/167 (9.0%) of the MSS/MSI-L patients responded [19]. The KEYNOTE 061 study randomized 592 patients with advanced gastric or gastroesophageal junction cancer that progressed on first-line chemotherapy with a platinum and fluoropyrimidine to pembrolizumab monotherapy or paclitaxel. In the MSI-H group median overall survival was not reached for the fifteen MSI-H patients treated with pembrolizumab (95% CI 5.6 months–not reached), while it was 8.1 months for the twelve MSI-H patients treated with paclitaxel (95% CI 2.0–16.7) [49]. Very recently the data of patients with MSI-H (n = 50/763) that were included in the KEYNOTE 062 study were presented [50]. In this first line study patients were randomized to pembrolizumab alone, to chemotherapy (cisplatin and fluoropyrimidine) plus pembrolizumab or to chemotherapy plus placebo. Median overall survival of MSI-H patients treated with pembrolizumab, either alone, or in combination with chemotherapy, was significantly better than treatment with chemotherapy alone. Interestingly however, the objective response rate was numerically higher in the group of MSI-H patients receiving both chemotherapy and pembrolizumab compared to pembrolizumab alone (64.7% vs 57.1%), while overall survival was better with pembrolizumab alone. It should be noted that the study was not powered to compare pembrolizumab alone with pembrolizumab plus chemotherapy, but these numbers might suggest that at least some chemotherapy may be beneficial for MSI-H patients, to induce a first response, while prolonged administration of chemotherapy may confer no additional benefit. Further prospective data are needed to test this hypothesis.

Ideally, potential predictive factors for response to checkpoint inhibition had been further tested in a randomized controlled trial, including patients from the predictive subgroup only. Nevertheless, the data from the KEYNOTE studies have been very compelling across

tumor types. In a future immunotherapy trial it may therefore be difficult to randomize MSI-H gastroesophageal patients to chemotherapy only. Thus, new ways for further scientific backing of the data have to be developed. Prospective real world data collection may aid in this respect [51].

In summary, patients with MSI-H gastroesophageal tumors can substantially benefit from immune checkpoint inhibition. It is uncertain whether MSI-H tumors should be treated with immunotherapy alone or if addition of chemotherapy may be beneficial in this subgroup as well.

MSI and effect of cytotoxic agents: preclinical mechanistic data

While MSI is a strong indicator for response to immune checkpoint blockade, the role that cytotoxic agents should have in the treatment of MSI-H gastric cancer remains unclear [52]. For example, resistance in MMR deficient cells has been shown for 6-thioguanine, *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (MNNG), temozolomide, cisplatin and carboplatin and the fluoropyrimidines 5-fluorouracil (FU) and 5-fluoro-2-deoxyuridine (FdUrd) [53–62]. It has been hypothesized that resistance to drugs such as 6-thioguanine, MNNG, temozolomide, cisplatin and carboplatin results from failure of the cell to bind DNA adducts formed by these drugs and to subsequently induce apoptotic cell death [63]. Similarly, differential FdUrd cytotoxicity between MMR-competent and MMR-D cells is mediated at the level of DNA incorporation [57].

Resistance of MSI-H cells has also been described for the topoisomerase inhibitors etoposide and doxorubicin in some, but not all studies [64,65]. In MMR-proficient cells the cleavable complex produced by the binding of these drugs to topoisomerase II may be recognized by the MMR pathway [52]. Also, treatment with etoposide may lead to higher mutation rates producing clones with mutated topoisomerase II, which is unable to bind the inhibitor, thus leading to resistance [66]. Besides binding the topoisomerase II, doxorubicin may participate in redox reactions, generating free radicals and even alkylating agents which may then form DNA adducts recognized by the MMR pathway.

In general, the genomic instability due to MMR-D can increase mutations in the coding or regulatory sequences of other genes that may play a central role in determining drug sensitivity [63]. Thus, in addition to DNA-damage pathways, other mechanisms may be involved in the emergence of resistance to cytotoxic drugs in MMR-D tumor cells.

Interestingly, in contrast to other widely used platinum compounds loss of mismatch repair does not lead to resistance to oxaliplatin. DNA adducts formed by oxaliplatin are, in fact, not recognized by the MMR system, suggesting that for cell lethality induced by oxaliplatin other mechanisms are in place [60,61]. Also for other drugs that produce adducts that are not recognized by the mismatch repair detector, such as the alkylating agents melphalan and perfosfamide, the chloroethylating agent 1,3-bis(2-chloroethyl)-nitrosourea and agents such as paclitaxel and tamoxifen that do not interact with DNA, no resistance is conferred by loss of MMR [62,63]. Furthermore, MSI-H cells may be treated with the hypomethylating agent 5-fluorodeoxycytidine (FdCyd), which through re-expression of hMLH1 evades the resistance to fluoropyrimidine therapy caused by deficiencies in the MMR pathway [67].

In summary, depending on the specific cytotoxic drug used and its interaction with DNA synthesis, treatment resistance may emerge in MSI-H tumors or not (Table 2). Thus, not all cytotoxic drugs should necessarily be avoided in MSI-H tumors, and there may even be approaches to resensitize MSI-H cells to drugs that require the MMR system for tumor cell lethality. Further mechanistic as well as clinical evaluation of the effect of cytotoxic drugs in MSI-H tumors is warranted to identify effective cytotoxic drugs in MSI-H patients.

MSI and effect of cytotoxic agents: clinical data

A recent large scale study in stage II and III gastric cancer patients,

Table 2
Cytotoxic agents and MMR related resistance in vitro (based on Irving et al.) [51].

		MMR related resistance	No MMR related resistance
Methylating agents	Temozolamide	+	
	Procarbazine	+	
	MNNG	+	
	MNU	+	
Other alkylating agents	Busulphan	+	
	Melphalan		+
	Cyclophosphamide		+
	Perfosfamide		+
	BCNU		+
Antimetabolites	6-Thioguanine	+	
	6-Mercaptopurine	+	
	5-Fluorouracil	+	
Platinum compounds	Cisplatin	+	
	Carboplatin	+	
	Oxaliplatin		+
Topoisomerase inhibitors	Etoposide	+	
	Doxorubicin	+	
	Epirubicin	+	
Taxanes	Paclitaxel		+

revealed that 5-FU-based adjuvant chemotherapy improved disease-free survival in the MSS/MSI-low group, but showed no benefit in the MSI-high group [68]. In another study 28 of 285 patients (9.8%) exhibited negative MLH1 and most MLH1-negative tumors (85.7%) showed high MSI [36]. MLH1-negative patients were significantly less likely to respond to preoperative chemotherapy than MLH1-positive patients. Relapse free survival in the MLH1-negative group was significantly longer than in the MLH1-positive group when no preoperative chemotherapy was given, whereas in patients with preoperative chemotherapy there was no significant difference in relapse free survival between the two groups.

Similar results were obtained in the prospective capecitabine and oxaliplatin adjuvant study in stomach cancer (CLASSIC) trial, which demonstrated the benefit of adjuvant chemotherapy (capecitabine with oxaliplatin) after D2 gastrectomy over D2 gastrectomy alone for stage II/III gastric cancer [69,70]. This lack of survival benefit for the MSI-H group in the CLASSIC trial is remarkable because in vitro no resistance to oxaliplatin has been observed. However, oxaliplatin monotherapy has been shown to be significantly inferior to the combination of oxaliplatin and a fluoropyrimidine [71]. It could be hypothesized that when MMRD cells are resistant to 5FU therapy, this also negatively impacts the synergistic effect of this regimen.

In a secondary post hoc analysis of the MAGIC study, which randomized patients with resectable gastric cancer to surgery alone or perioperative chemotherapy (epirubicin with 5-fluorouracil and cisplatin) plus surgery, the association between MMR-D, MSI and survival was assessed [38]. Patients treated with surgery alone who had high MSI or MMRD had a median OS that was not reached (95% CI, 11.5 months - not reached) compared with a median OS among those who had neither high MSI nor MMR-D of 20.5 months (95% CI, 16.7–27.8 months; hazard ratio, 0.42; 95% CI, 0.15–1.15; P = 0.09). In contrast, in the chemotherapy plus surgery group, patients who had either high MSI or MMRD had a median OS of 9.6 months (95% CI, 0.1–22.5 months) compared with a median OS among those who had neither high MSI nor MMR-D of 19.5 months (95% CI, 15.4–35.2 months; hazard ratio, 2.18; 95% CI, 1.08–4.42; P = 0.03). A recent individual patient data meta-analysis pooling data from the CLASSIC and MAGIC trial together with the ARTIST and ITACA-S trial, which both compared different multimodal treatment strategies in curative gastric cancer, emphasize these results [72]. The authors found no benefit of perioperative or adjuvant chemotherapy in MSI-H

Table 3

Design of cited studies. [a]Genomically stable, [b]Chromosomal Instability, [c]Overall Survival, [d]Recurrence Free Survival, [e]Microsatellite instability-Low, [f]Microsatellite instability-High, [g]Individual Patient Data, [h]Chemoradiotherapy. Retrospective cohort analyses that reported to have used prospectively gathered cohorts for their retrospective analyses are marked with an *.

Publication	Study design	Prognostic/predictive factor	Results
Sohn et al. 2017 [5]	Retrospective cohort analysis	Prognostic and predictive	Positive prognostic value over GSa and CINb Negative predictive value for response to chemotherapy
Fuchs et al. 2018 [19]	Post hoc analysis in single arm trial	Predictive	Positive predictive value for response to pembrolizumab
Marrelli et al. 2016 [20]	Retrospective cohort analysis*	Prognostic	Positive prognostic value in non-cardia intestinal type gastric cancer
Polom et al. 2018 [33]	Meta-analysis	Prognostic	Positive prognostic value
Choi et al. 2019 [34]	Post hoc analysis of randomized trial	Prognostic and predictive	Positive prognostic value in stage II/III gastric cancer Negative predictive value for response to adjuvant capecitabine and oxaliplatin
Haag et al. 2019 [35]	Retrospective cohort analysis	Prognostic and predictive	Positive prognostic value for OSc, no significant prognostic value for RFSd No significant predictive value for histological response to platinum-based chemotherapy
Hashimoto et al. 2019 [36]	Retrospective cohort analysis	Predictive	Negative predictive value for response to preoperative chemotherapy
Kim et al. 2015 [37]	Retrospective cohort analysis	Prognostic and predictive	Positive prognostic value when not treated with chemotherapy Negative predictive value for efficacy of chemotherapy in stage II/III gastric cancer
Smyth et al. 2017 [38]	Post hoc analysis in randomized trial	Predictive	Negative predictive value for response to preoperative chemotherapy
Kohlruss et al. 2019 [39]	Retrospective cohort analysis	Prognostic and predictive	Positive prognostic value MSI-Le is a positive predictive value for response to neoadjuvant platinum/5FU containing chemotherapy. No significant predictive value of MSI-Hf
Polom et al. 2018 [40]	Retrospective cohort analysis*	Prognostic	Positive prognostic value in stage IV gastric cancer
Zhu et al. 2015 [45]	Meta-analysis	Prognostic	Positive prognostic value
Choi et al. 2014 [46]	Meta-analysis	Prognostic	Positive prognostic value
Beghelli et al. 2006 [47]	Retrospective cohort analysis	Prognostic	Positive prognostic value, only in stage II gastric cancer
Shitara et al. 2018 [49]	Post hoc analysis in randomized trial	Predictive	Positive predictive value for response to pembrolizumab
Shitara et al. 2019 [50]	Post hoc analysis in randomized study	Predictive	Positive predictive value for response to pembrolizumab with or without chemotherapy.
An et al. 2012 [68]	Retrospective cohort analysis*	Prognostic and predictive	No significant prognostic value Negative predictive value for response to 5FU based chemotherapy
Pietrantonio 2019 [72]	IPDg meta-analysis	Prognostic and predictive	No significant predictive value for the efficacy of chemotherapy. Positive prognostic value.
Oki et al. 2009 [73]	Prospective cohort analysis	Prognostic	No significant prognostic value
Biesma et al. 2019 [75]	Post hoc analysis in randomized trial	Prognostic	No significant prognostic value.
Miceli et al. 2019 [76]	Post hoc analysis in randomized trial	Prognostic and predictive	No significant prognostic value. No significant predictive value for CRTh over chemotherapy.

Table 3 (*continued*)

Publication	Study design	Prognostic/predictive factor	Results
Oki et al. 2009 [73]	Prospective cohort analysis	Prognostic	No significant prognostic value
Biesma et al. 2019 [75]	Post hoc analysis in randomized trial	Prognostic	No significant prognostic value.
Miceli et al. 2019 [76]	Post hoc analysis in randomized trial	Prognostic and predictive	No significant prognostic value. No significant predictive value for CRT[h] over chemotherapy.

patients. It should be noted however that aside from those included in the CLASSIC trial, many patients received only cytotoxic agents that have shown in vitro MMR related resistance. As mentioned earlier, the oxaliplatin and fluoropyrimidine combination might lose its synergistic effect because of resistance to the latter, possibly explaining the lack of benefit in the CLASSIC trial and thus contributing to negative results in the individual patient data meta-analysis

Similar results were obtained in more advanced disease stages. Data from prospective databases at two large European centers on patients who had undergone surgery and were diagnosed with synchronous metastatic gastric cancer at the time point of surgery showed that patients who were MSI-H had superior OS compared to MSS patients, although this did not remain significant in multivariable analysis [41]. The overall survival in the group who did not receive chemotherapy after surgery was 33.3% for MSI-H and 14.3% for MSS, while in the group which received chemotherapy after surgery, overall survival was 20% for MSI-H and 22.6% for MSS.

In contrast, in a cohort study of the MD Anderson of patients with stage II, III, or IV disease without distant metastasis treated with a fluorouracil-based regimen, a detrimental effect of chemotherapy in patients with gastric cancer of the MSI subtype could not be confirmed [5]. In fact, MSI-H patients seemed to benefit from chemotherapy, albeit modestly: the hazard ratio for recurrence among those who received adjuvant chemotherapy was 0.55 (95% confidence interval (CI) 0.22–1.3, P = 0.18). Patients with the CIN subtype exhibited the greatest benefit from adjuvant chemotherapy; the hazard ratio for recurrence among those who received adjuvant chemotherapy was 0.39 (95% CI 0.16–0.94, P = 0.03). No benefit from adjuvant chemotherapy was observed among patients with the genomically stable subtype; hazard ratio for recurrence was 0.83 (95% CI, 0.36–1.89, P = 0.65). Another report showed that the survival of gastric cancer patients after the administration of 5-FU did not correlate with MSI status [73]. In a preliminary analysis of the CRITICS study [74], that randomized gastric cancer patients to perioperative chemotherapy or preoperative chemotherapy followed by postoperative chemoradiotherapy, patients with MSI-H tumors showed favorable overall survival outcomes compared to MSS tumors [75]. However, it remains difficult to determine whether the survival benefit is a result of cytotoxic treatment or due to an inherent prognostic advantage. Furthermore no significant interaction between MSI status and radiotherapy was seen when patients were treated with adjuvant chemotherapy (capecitabine and cisplatin) or adjuvant chemoradiotherapy in the ARTIST trial [76].

In summary, given the conflicting evidence, the low number of MSI-H patients in each individual study and the retrospective character of the analyses in these studies it is hard to draw definite conclusions about the relation between MSI and efficacy of cytotoxic treatment, although commonly used regimens seem to confer little benefit to MSI-H patients in clinical practice. Table 3 summarizes the methodology of cited studies and shows identified prognostic and predictive value of MSI in gastric cancer.

Implications for clinical practice and research

Based on the currently available data, the most optimal approach to treatment of patients with MSI-H gastroesophageal cancer is within the context of immunotherapy trials. Treatment with immune checkpoint inhibition significantly improves outcome in a relatively large proportion of MSI-H tumors compared with MSI-L/MSS tumors, leading to remarkable and often long lasting responses and survival benefits. Nevertheless some cytotoxic agents may still be beneficial for MSI-H patients. Unfortunately, due to the use of combination regimens in most clinical studies it is unclear what agents could have optimal effect in MSI-H tumors and what regimen might facilitate the immunologic antitumor effects of checkpoint blockade best [77]. Future studies could allocate MSI-H patients to immunotherapy only or immunotherapy combined with cytotoxic agents, to investigate whether chemotherapy may be of additional benefit in MSI-H patients and whether specific subgroups within MSI gastric cancer may benefit from cytotoxic therapy. Making use of the available preclinical and mechanistic data, cytotoxic agents used in such trials should preferentially include drug combinations that do not (fully) depend on the MMR system to confer tumor cell lethality (Cf. Table 2). To better understand the underlying mechanisms of resistance and sensitivity of MSI-H tumors to cytotoxic treatment, a large translational component investigating the tumor's immune microenvironment is warranted. By inclusion of MSI-H patients in such studies, patients may benefit from the presumed efficacy of checkpoint inhibition in MSI-H tumors, while at the same time the research community, and ultimately society, will benefit from the accumulation of the body of evidence for both immunotherapy and cytotoxic treatment in this patient population.

Declaration of Competing Interest

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