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## Acquired microsatellite instability status and loss of HER2 positivity during treatment of gastro-esophageal junction adenocarcinoma ☆

*Dear Editor,*

Gastro-oesophageal adenocarcinomas (GOAs) are a common cause of cancer mortality worldwide. Nowadays, beyond histological classification, the management of GOAs depends on molecular classification. The Cancer Genome Atlas consortium identified four different molecular subtypes of GOAs potentially allowing to tailor the treatment strategies [1]. About 20% of patients show an overexpression or amplification of the Human Epidermal Growth Factor Receptor-2 (HER2). These tumors are generally microsatellite stable (MSS) and present chromosomal instability [1]. The anti-HER2 monoclonal antibody trastuzumab combined with platinum and fluoropyrimidine chemotherapy is the standard first line regimen for these patients [2]. Microsatellite instable (MSI) and DNA mismatch repair deficient (dMMR) cancers are a distinct subtype of GOAs with a prevalence of up to ~20% and being more frequent in early stage [3]. These tumors are often associated with a hypermutated phenotype resulting in extensive formation of cancer-specific neoantigens triggering robust anti-cancer immune responses. They benefit extensively from anti-PD-1 immune checkpoint blockade [2,4]. Here, we describe a rare case of acquisition of MSI and the concomitant loss of HER2 positivity potentially due to selective therapeutic pressure in a metastatic MSS GOA treated with platinum-based chemotherapy and trastuzumab.

A 61-year-old man, without family history of cancer, was diagnosed in January 2018 with a moderately (G2) to poorly (G3) differentiated HER2-positive (IHC 2+, mean HER2 copy number of 5.75 and HER2/CEP17 ratio of 2.09 as assessed by in situ hybridization) and MSS gastro-esophageal junction adenocarcinoma with peritumoral and mediastinal lymph nodes and multiple liver metastases. The histology appeared homogeneous across the 2 biopsies (samples) performed in the primary tumor. Combined treatment with trastuzumab and platinum-based chemotherapy was initiated: capecitabine (1000 mg/m² BID) for 14 days, oxaliplatin (130 mg/m²) and trastuzumab (8 mg/kg loading dose followed by 6 mg/kg) every three weeks. Partial response of all metastases was observed on imaging three months after treatment start. Due to poor tolerance and significant neuropathy, chemotherapy was stopped and trastuzumab monotherapy continued for additional 4 months. Seven months after treatment start (August 2018), the patient experienced symptomatic dysphagia. Imagery described a progression of the primary tumor and metastatic lymph nodes without changes in liver metastases. Local progression of the tumor was confirmed by endoscopy. Rebiopsy of the same primary tumor site demonstrated the persistence of a (G2)-G3 adenocarcinoma, however HER2-negative (IHC 1+, mean HER2 copy number of 5.05 and HER2/CEP17 ratio of 1.38) and MisMatch Repair deficiency (dMMR, characterized by loss of expression of *MLH1* and *PMS2* proteins at immunohistochemistry staining) (Fig. 1). Like the diagnostic biopsy, no mixed tumor histology was observed. The MSI status was confirmed by the polymerase chain reaction (PCR) amplification and analysis of seven MSI biomarkers (Idylla™ MSI assay: *ACVR2A, BTBD7, DIDO1, MRE11, RYR3, SEC31A, SULF2*). *MLH1* promoter was not hypermethylated (SALSA-MLPA ME011-D1 method). Considering the MSI status, a treatment with anti-PD1 immunotherapy (nivolumab, 3 mg/kg every two weeks) was initiated leading to partial response for 12 months. At progression (September 2019), the patient was sequentially treated with other types of immunotherapies (association of anti-PD-L1 and anti-TIM3 antibodies for 3 months, anti-CD25 antibody during two months into early-phase clinical studies) and anti-angiogenic treatment (ramucirumab) with and without chemotherapy (paclitaxel). Rebiopsy of the same primary tumor site was performed at each disease progression ($n = 4$) before each treatment modification and revealing the same histology pattern, as well as the persistence of HER2 negativity and MSI/dMMR status. Molecular characteristics of biopsies from the diagnosis (Jan 2018), the first (Aug 2018) and the last (Aug 2020) disease progression before treatment modification are summarized in the Fig. 1. Best supportive care was initiated in December 2020 and the patient eventually died in January 2021.

Comprehensive DNA sequencing (FoundationOne CDx assay) was performed on the initial diagnostic biopsy specimen of 2018 and the latest biopsy performed at progression in August 2020 (Figs. 1 and 2). Sequencing of the initial biopsy specimen confirmed the MSS status with a tumor mutational burden (TMB) of 5.0 Muts/Mb and found a clonal *TP53* and a sub-clonal *ERBB3* alteration (Table 1). Sequencing of the progressive lesion showed a genomic signature of MSI with a TMB of 23.9 Muts/Mb. Accordingly, several new variants were detected, mainly consisting of frameshift mutations (Table 1). Interestingly, the same *TP53, ERBB3, and STK11* alterations were still present, and among others two *MSH6* frameshift mutations had appeared. We observed no base substitution, insertion, deletion or copy number alteration in *MLH1* or *PMS2*.

HER2 and MSI molecular alterations are usually not coexisting or overlapping in time. Each distinguished profile predicts efficacy of a specific treatment. The addition of trastuzumab to platinum-based chemotherapy provided a significant survival benefit for patients with HER2-positive GOAs [2]. Loss of HER2 positivity after trastuzumab-based chemotherapy has been reported in ~30% of HER2 IHC3+ score and/or FISH amplified GOAs [5]. Several

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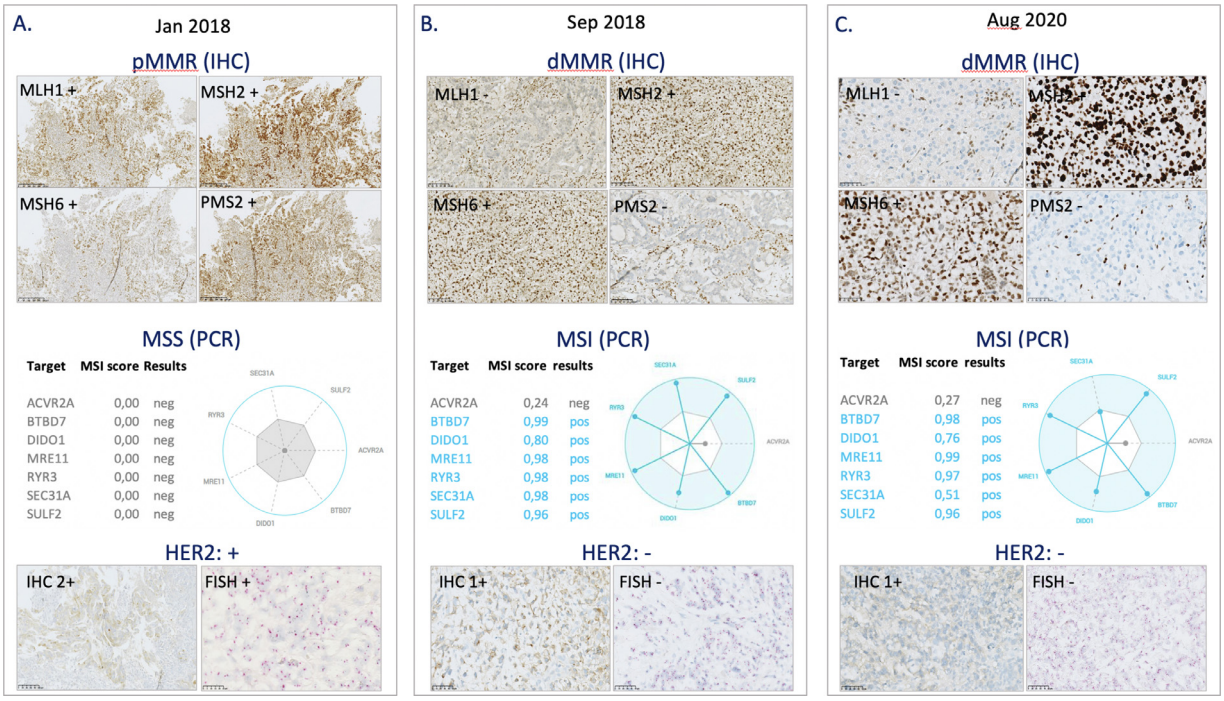


**Fig. 1. Overtime molecular characterization for MSI and HER2 status.** A: pMMR adenocarcinoma was characterized by IHC expression of *MLH1, MSH2, MSH6* and *PMS2* proteins. MSS was determined by PCR amplification and subsequent fragment analysis of seven monomorphic microsatellite biomarkers (*ACVR2A, BTBD7, DIDO1, MRE11, RYR3, SEC31A, SULF2*). No mutation has been found in the seven biomarkers (MSI score <0,5). HER2 had a weak to moderate membranous expression (IHC 2+) and was amplified (FISH: mean HER2 copy number of 5.75 and HER2/CEP17 ratio of 2.09). B and C: dMMR adenocarcinoma was characterized by loss of expression of *MLH1* and *PMS2* proteins at IHC staining. MSI was determined by PCR amplification and subsequent fragment analysis of seven monomorphic microsatellite biomarkers (*ACVR2A, BTBD7, DIDO1, MRE11, RYR3, SEC31A, SULF2*). The result is considered MSI-High because 6/7 biomarkers have a mutation detected (MSI score >0,5). HER2 had faint/barely perceptible membranous reactivity (IHC 1+) and was not amplified (FISH: mean HER2 copy number of 5.05 and HER2/CEP17 ratio of 1.38).
dMMR: MisMatch Repair deficiency; FISH: fluorescence in situ hybridization; IHC: immunohistochemistry; MSI: microsatellite instability; MSS: microsatellite stability; neg: negative; PCR: polymerase chain reaction; pMMR: MisMatch Repair proficiency; pos: positive.


**Fig. 2.** Nucleotide and amino acid sequence (IGV browser) of the MSH6 region carrying the two frameshift mutations acquired during cancer progression. The red lines delineate the nucleotidic repetitive region in which they are located.

studies including MSI cancers (GOAs and other tumor types) have reported higher treatment efficacy and survival benefit of anti-PD-1 immunotherapy, regardless of tumor origin [6].

MSI status has been correlated with older age, female gender, middle and lower gastric tumor localization, and poorly differentiated adenocarcinoma. MSI in GOAs is mainly related to germline mutations in a mismatch repair gene (Lynch Syndrome) or somatic methylation of a CpG island in the promoter region of *MLH1* and less frequently with somatic mutations of the mismatch repair machinery [1,2,6]. MSI status is generally considered an early genetic event rather than a status acquired through selective pressure. In our case, the evolution from MSS to MSI after platinum-based chemotherapy was demonstrated both at DNA and protein level. Nevertheless, we could not demonstrate the mechanism behind this evolution: *MLH1* promoter was not hypermethylated and DNA sequencing did not reveal a genetic alteration leading to the loss of expression of *MLH1* and *PMS2*, whereas the *MSH6* mutations did not lead to the loss of the corresponding protein expression. A post-translational mechanism is plausible, and we could hypothesize the treatment (platinum chemotherapy) led to reduced MLH1 expression, as already reported [7,8]. The exhaustion of the biopsies however precludes us from performing RNA sequencing analyses. The somatic *MSH6* variants must be considered as a consequence of acquired microsatellite instability, rather than the cause. Indeed, these genomic positions are located in a mononucleotidic or trinucleotidic repetitive region (Fig. 2). Heterogeneous loss of *MSH6* after chemotherapy has been described in literature in colorectal and endometrial cancer, whereas tumors with loss of *MLH1* and *PMS2* have been shown to acquire secondary *MSH6* alterations inside a microsatellite locus [9]. DNA sequencing furthermore confirmed the progressive lesion was phylogenetically related to the diagnostic cancer sample, given the persistence of the early *TP53* and *ERBB3* alterations.

Another hypothesis related to our observation could be the possibility of intra-tumoral heterogeneity of MMR status rather than acquired MSI tumor, as previously reported [10]. Heterogenous staining patterns of MMR proteins (restricted spatial loss restricted to some tumor area) associated with confirmed MSI status has been exceptionally reported in colorectal cancer [10,11]. In our case, we never observed a mixed histology or a spatially heterogenous MMR protein expression in the multiple biopsies performed overtime (5 time-points) at the same primary tumor site. Nevertheless, a mixed tumor compound (HER2 and MSI) could still have been missed by the initial biopsy. Clonal selection during exposure to trastuzumab is likely to occur in GOAs as reflected by poor efficacy of this treatment beyond progression [12]. Shared *TP53, ERBB3,* and *STK11* clonal and subclonal alterations suggested at least a common phylogenetic process in the tumor and exclude the presence of two different cancer types. The hypermutator-phenotype of MSI GOAs enables high evolvability producing ex-

**Table 1**
FoundationOne CDx assay findings.

| January 2018 | | | | | August 2020 | | | | |
|---|---|---|---|---|---|---|---|---|---|
| TMB | MS | Gene | Alteration | VAF (%) | TMB | MS | Gene | Alteration | VAF (%) |
| **5.0 muts/Mb** | **stable** | ***ERBB3*** | p.(V104M) | 3,4 | **23.9 muts/Mb** | **unstable** | ***ERBB3*** | p.(V104M) | 10,1 |
| | | ***TP53*** | p.(G279E) | 28,2 | | | ***TP53*** | p.(G279E) | 12 |
| | | / | | | | | ***TP53*** | p.(S362Afs*8) | 11,4 |
| | | ***INPP4*** | Exon 21–22 deletion | | | | ***INPP4*** | Exon 21–22 deletion | |
| | | ***PTEN*** | p.(T319*) | 24,2 | | | | | |
| | | ***STK11*** | P281fs*6 | 4,4 | | | ***STK11*** | P281fs*6 | 11,1 |
| | | | | | | | *MSH6* | G1070fs*9 | |
| | | | | | | | *MSH6* | F1088fs*5 | |
| | | | | | | | *PTCH1* | N97fs*20 | |
| | | | | | | | *PTCH1* | S1203fs*52 | |
| | | | | | | | *MLL2* | Q2932fs*10 | |
| | | | | | | | *QK1* | K134fs*14 | |
| | | | | | | | *CREBBP* | S976fs*22 | |
| | | | | | | | *ARID1A* | P146fs*86 | |
| | | | | | | | *CDK12* | 3761–2A>C | |
| | | | | | | | ***PTEN*** | loss | |
| | | | | | | | *ROS1* | amplification | |

VAF: Variant Allele Frequency, MS: Microsatellite, TMB: Tumor Mutational Burden.

treme intratumor heterogeneity [13]. Nevertheless, it seems highly unlikely that we faced a MSI GOA since the diagnosis, in which a subclone characterized by a driver alteration (HER2+) and a concomitant loss of MSI status was identified in the initial biopsy, definitively eliminated by trastuzumab and chemotherapy and not found in sequential biopsies overtime. Like MSI status, HER2 overexpression in premalignant gastric lesions suggests its potential involvement in the early steps of gastric carcinogenesis [1].

In conclusion, we described a rare case of MSI acquisition (and concomitant loss of HER2 positivity) in a patient with metastatic MSS gastro-esophageal junction cancer treated with platinum-based chemotherapy and trastuzumab. Despite our efforts, we were unable to identify the mechanism that led to MSI status. Biopsies performed at each stage of disease progression supported our decision on the best treatment option that allowed the patient to subsequently benefit from immunotherapy. This case also reinforces the need to rebiopsy progressive lesions to ensure optimal treatment selection.

## Compliance with Ethical Standards

All the authors of this manuscript declare no conflict of interest.

The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki (6th revision, 2008) as reflected in a priori approval by the institution's human research committee.

The authors obtained informed consent to publish the report from patient's legal representative (patient deceased).

## Conflict of interest

None declared.

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