Biomarkers and Lynch syndrome in patients with oesophago-gastric cancer

Clinical guidance document

Author: James Peaker, Consultant Biomedical Scientist, Cardiff & Vale UHB

Contributors: Dr Adam Boyde, Dr Adam Christian, Dr Alexandra Murray, Sian Nisbet, Sheila Palmer-Smith, Dr Betsan Thomas.

Reviewer: Dr Samantha Cox - Consultant Oncologist, Velindre Cancer Centre and Chair of the All Wales Genomics Onclogy Group (AWGOG)

Owner: All Wales Genomics Oncology Group (AWGOG)

Date: 06th September 2023	**Date of Review:** 06th September 2024	**Version:** 2e

Contents

1. Foreword

The aim of this document is to provide clinical and pathology staff with guidance on which biomarkers may be required in patients with oesophago-gastric (OG) cancer, how to order biomarker testing, and scenarios for when the patient should be referred to Clinical Genetics for suspected Lynch syndrome. This document applies to all Welsh patients.

2. Background

2.1 Treatment options in oesophago-gastric cancer

Currently, the National Institute for Health and Care Excellence (NICE) has approved three targeted treatments for patients with OG cancers: trastuzumab, pembrolizumab, and nivolumab, dependent upon location and tumour type (see figure 1).

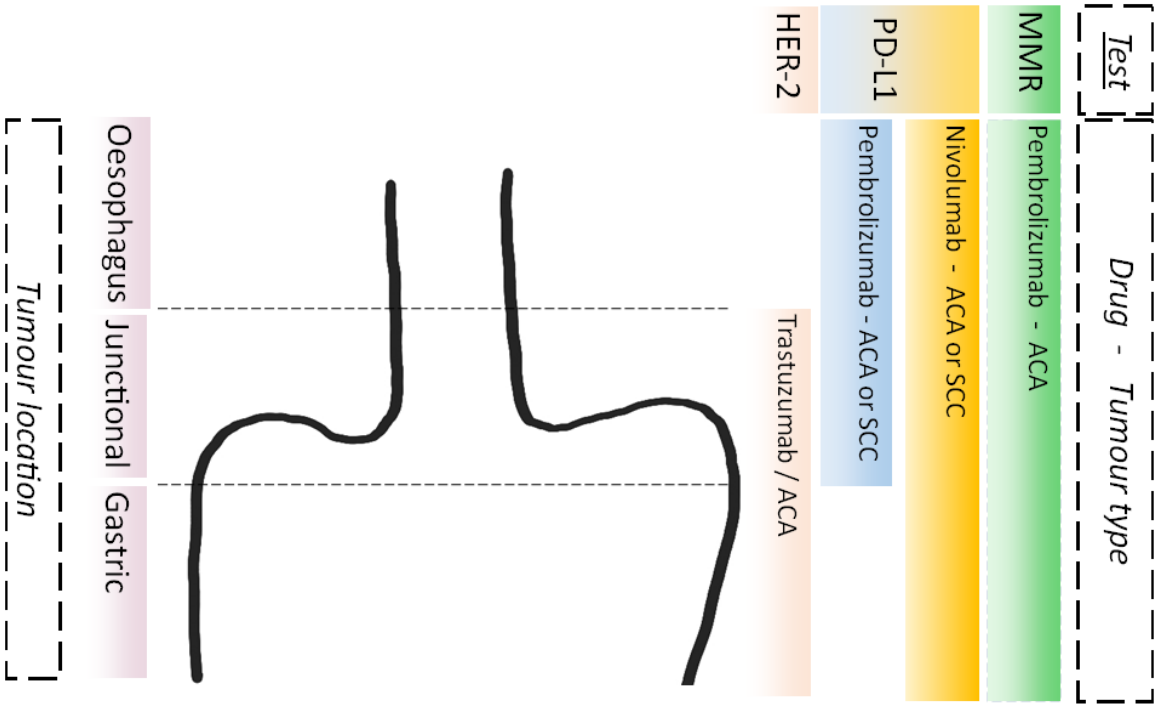

Figure 1.. Illustration of targeted agents available dependent upon anatomical site and tumour histology. ACA: adenocarcinoma; SCC: squamous cell carcinoma; PD-L1: programmed death ligand 1; HER2: human epidermal growth factor receptor 2; MMR: mismatch repair protein. Information adapted from NICE guidance.[1-5]

2.1.1 Treatment options for oesophago-gastric adenocarcinoma

Trastuzumab is approved in combination with chemotherapy for untreated, advanced, gastric and gastro-oesophageal junction cancer in adults, with human epidermal growth factor receptor 2 (HER2)-positive metastatic adenocarcinoma of the stomach or gastro-oesophageal junction.[1]

Pembrolizumab with platinum- and fluoropyrimidine-based chemotherapy is recommended, within its marketing authorisation, as an option for untreated locally advanced unresectable or metastatic carcinoma of the oesophagus or HER2-negative gastro-oesophageal junction

adenocarcinoma in adults whose tumours express programmed death ligand 1 (PD-L1) with a combined positive score (CPS) of 10 or more. [2]

Nivolumab with platinum- and fluoropyrimidine-based chemotherapy is recommended, within its marketing authorisation, as an option for untreated HER2-negative, advanced or metastatic gastric, gastro-oesophageal junction or oesophageal adenocarcinoma in adults whose tumours express PD-L1 with a CPS of 5 or more.[3]

Pembrolizumab is recommended as an option for treating tumours with high microsatellite instability (MSI) or mismatch repair (MMR) deficiency in adults with unresectable or metastatic gastric, small intestine or biliary cancer that has progressed during or after one prior therapy.[4]

2.1.1 Treatment options for oesophageal squamous cell carcinoma

Pembrolizumab with platinum- and fluoropyrimidine-based chemotherapy is recommended, within its marketing authorisation, as an option for untreated locally advanced unresectable or metastatic carcinoma of the oesophagus or HER2-negative gastro-oesophageal junction adenocarcinoma in adults whose tumours express PD-L1 with a CPS of 10 or more.[2]

Nivolumab with fluoropyrimidine-based and platinum-based combination chemotherapy is recommended as an option for untreated unresectable advanced, recurrent, or metastatic oesophageal squamous cell carcinoma in adults whose tumours show PD-L1 tumour proportion score (TPS) at a level of 1% or more. It is recommended only if pembrolizumab plus chemotherapy is not suitable.[5]

2.2 HER2 assessment

HER2 assessment is via assessing the staining intensity of immunohistochemical (IHC) protein expression in histological tumour material (see table 1). A low staining intensity (0 or 1+) is considered negative, equivocal staining 2+, and positive staining 3+.

Score	*Surgical specimen-staining pattern*	*Biopsy specimen-staining pattern*	*HER2 overexpression assessment*
0	No reactivity or membranous reactivity in <10% of tumor cells	No reactivity or no membranous reactivity in any tumor cell	Negative
1+	Faint/barely perceptible membranous reactivity in ≥10% of tumor cells; cells are reactive only in part of their membrane	Tumor cell cluster with a faint/barely perceptible membranous reactivity irrespective of percentage of tumor cells stained	Negative
2+	Weak to moderate complete, basolateral, or lateral membranous reactivity in ≥10% of tumor cells	Tumor cell cluster with a weak to moderate complete, basolateral, or lateral membranous reactivity irrespective of percentage of tumor cells stained	Equivocal
3+	Strong complete, basolateral, or lateral membranous reactivity in ≥10% of tumor cells	Tumor cell cluster with a strong complete, basolateral, or lateral membranous reactivity irrespective of percentage of tumor cells stained	Positive

Table 1. Human epidermal growth factor (HER2) scoring criteria for gastric and junction cancer.[6]

2.3 PD-L1 assessment

PD-L1 assessment is by assessing the IHC protein expression in histological tumour material.

Different scoring systems are available when assessing PD-L1 status for OG carcinomas. The TPS system looks at the percentage of positive tumour cells expressing PD-L1. This is only used when considered Nivolumab for squamous cell carcinoma's. The CPS system additional takes in to account PD-L1 expression of the host immune response, and is used in all other settings relating to OG PD-L1 testing. (see figure 2).

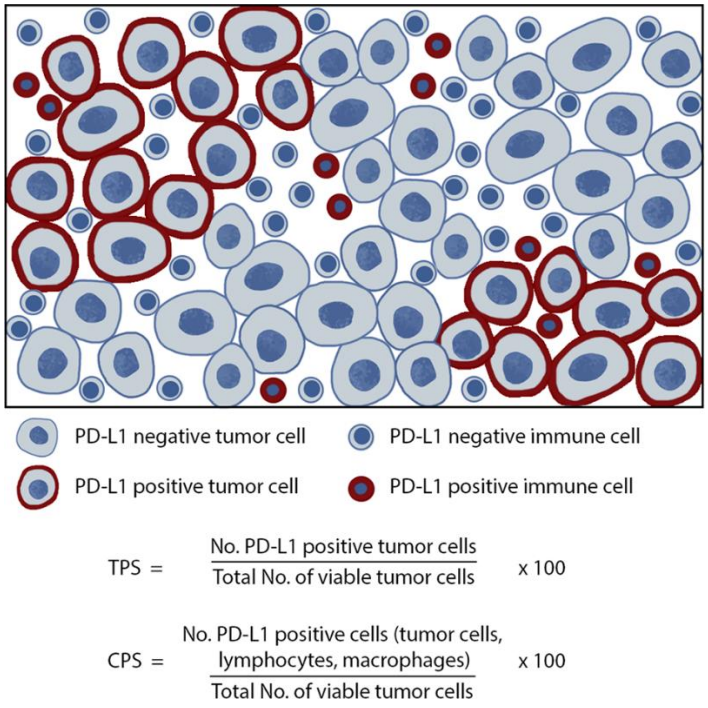

Figure 2 Schematic image of tumor specimen stained for PD-L1. Tumor proportion score (TPS) is defined as the number of positive tumor cells divided by the total number of viable tumor cells multiplied by 100%; combined positive score (CPS) as the number of positive tumor cells, lymphocytes and macrophages, divided by the total number of viable tumor cells multiplied by 100.[7]

Pembrolizumab and Nivolumab have each been developed with companion PD-L1 antibodies (dako 22C3 and dako 28.8 respectively). However, using the companion antibody is not a NICE requirement **(see section 3.1 and 4.4 on which PD-L1 antibody clone should be requested).**

2.4 Mismatch repair (MMR) protein assessment

MMR proteins form complexes which detect and correct mistakes made during DNA replication. There are four main MMR genes/proteins which we can assess (MLH1, PMS2, MSH2 and MSH6). Every cell contains two copies of each of these MMR genes. If all four MMR proteins show normal expression/function, a tumour is said to be mismatch repair proficient (pMMR).

MMR function is lost when both copies of an MMR gene are inactivated, which may be due to a pathogenic gene variant (*somatic* or *germline* (such as Lynch syndrome)) or *promoter*

methylation, which usually affects the MLH1 gene. If there is a loss of MMR protein expression/function, a tumour is said to be mismatch repair deficient (dMMR). In the presence of an MMR defect, mismatches can occur anywhere in the DNA but they are prone to occur in repetitive stretches of DNA called microsatellites, resulting in a state described as microsatellite instability (MSI). MSI is therefore a marker of dMMR. In the presence of a defective MMR system, the cells will become prone to acquiring large numbers of mutations which go uncorrected and can cause cancer to develop.

MSI/dMMR can be identified either using IHC to detect loss of MMR proteins or via molecular tests to show microsatellite alterations. MMR or MSI status has been shown to predict response to immunotherapy.

2.5 Microsatellite instability (MSI) assessment

Microsatellite instability (MSI) testing is **not** currently available in Wales via AWMGS for decision making in OG cancer management. MMR should instead be performed (in the appropriate scenario – see section 3).

2.6 Lynch syndrome and oesophago-gastric cancer

Lynch syndrome is an inherited cancer susceptibility syndrome with increased risk to certain types of cancer, including colorectal, endometrial, stomach, small bowel, gallbladder, hepatobiliary, pancreas, renal pelvis and/or ureter, bladder, kidney, ovary, brain, and prostate cancer[8]. It is usually due to a *germline pathogenic gene variant,* involving one of the mismatch repair (MMR) genes.

Routine screening for Lynch syndrome in UGI cancers is not currently recommended and identification of an dMMR tumour does not necessarily mean the patient has Lynch syndrome. However, Lynch syndrome may be incidentally detected during the assessment of MMR status in patients being considered for treatment with nivolumab. Identifying Lynch syndrome in such cases could allow patient surveillance, risk-reducing interventions (e.g. surgery) and early detection of Lynch syndrome-associated cancers in affected individuals and their family members.

This pathway details which patterns of dMMR require further assessment for Lynch syndrome.

3. Clinical pathway

3.1 Pathway flow chart for oesophageal and junctional tumours

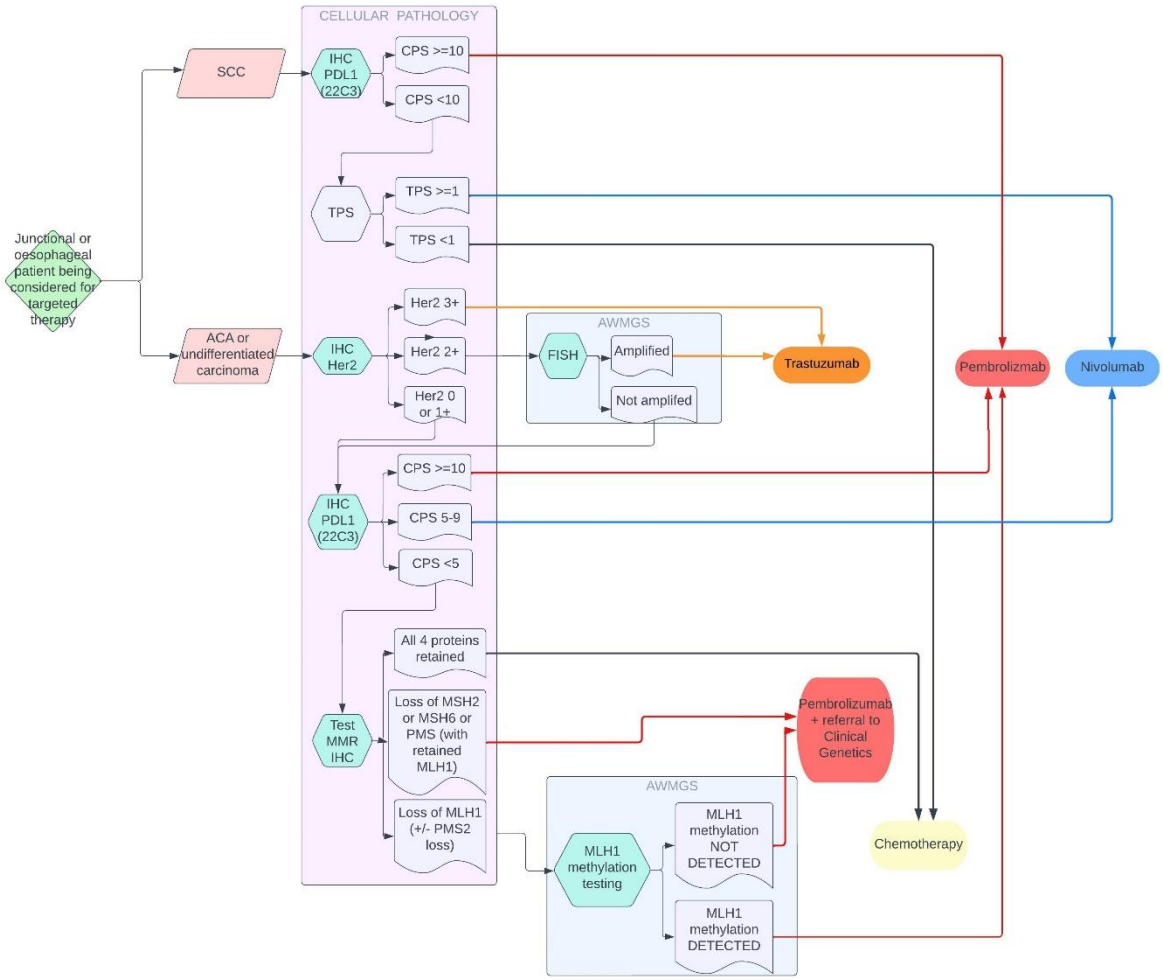

Figure 3. Biomarker pathway in untreated, advanced OG cancer. ACA: adenocarcinoma; SCC: squamous cell carcinoma; IHC: immunohistochemistry; PD-L1: programmed death ligand 1; (22C3): IHC test performed using PD-L1 Dako antibody 22C3; HER2: human epidermal growth factor receptor 2; AWMGS: All Wales Medical Genetics Service; FISH: fluorescence in-situ hybridization; MMR: mismatch repair proteins.

3.2 Pathway flow chart for gastric tumours

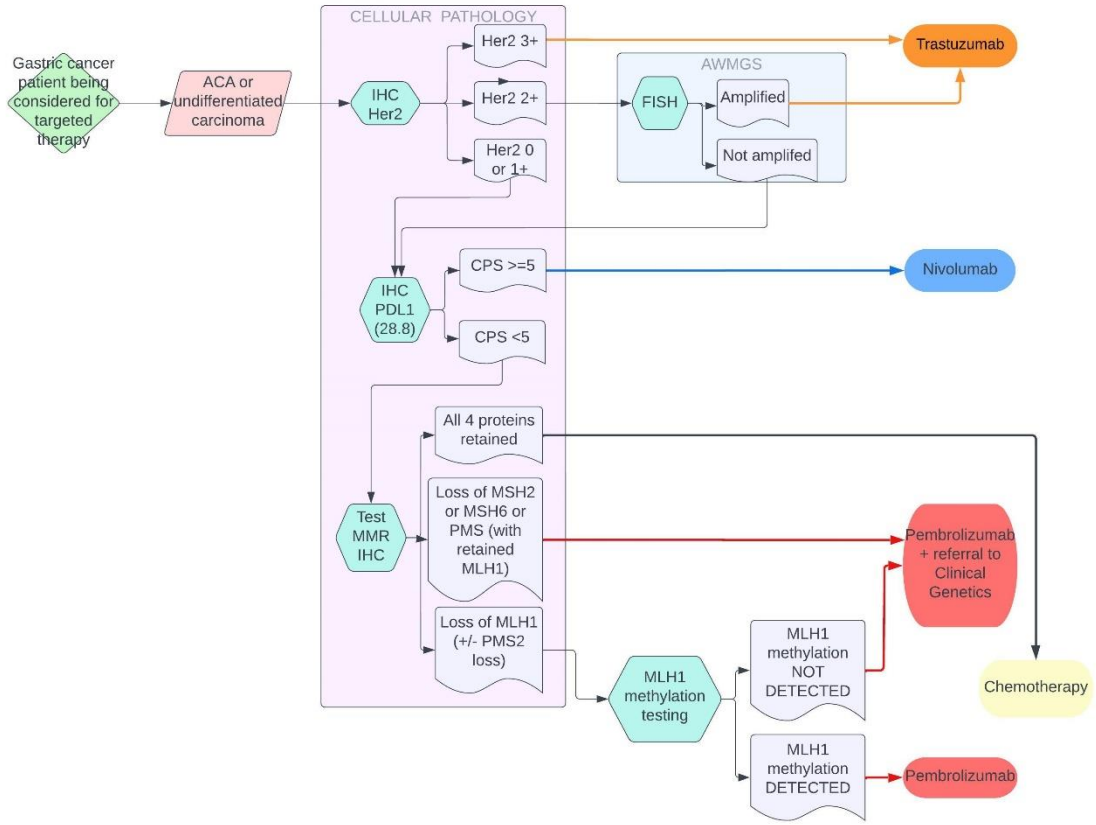

Figure 4. Biomarker pathway in untreated, advanced OG cancer. AdenoCa: adenocarcinoma; IHC: immunohistochemistry; PD-L1: programmed death ligand 1; (28.8): IHC test performed using PD-L1 Dako antibody 28.8; AWMGS: All Wales Medical Genetics Service; FISH: fluorescence in-situ hybridization; MMR: mismatch repair proteins.

4. Biomarker test requesting

4.1 Availability of biomarker testing

Immunohistochemistry for HER2, PD-L1 (dako antibodies 22C3 & 28.8; TPS and CPS scoring), and MMR protein retention are available to all laboratories and patients within Wales, by referring tissue to Cellular Pathology at Cardiff and Vale UHB (CVUHB):

Biomarkers,
Cellular Pathology,
University Hospital of Wales
Cardiff and Vale UHB
Cardiff
CF14 4XW

Telephone: +44(0)2920 7 42705
Email: Elaine.Cox@wales.nhs.uk

Alternatively, Betsi Cadwaladr are able to offer gastric HER2 for North Wales patients. Many Welsh histopathology laboratories also offer MMR IHC. Please contact your local laboratory for availability.

4.2 Availability of genetic testing

MLH1 methylation and FISH testing is offered by AWMGS to all eligible Welsh patients. MSI assessment is not currently offered. For guidance or queries related to MSI, MLH1 methylation, or FISH testing please see contact information below (or section 5.4 for further information on requesting details):

Telephone: +44(0)2921 8 44023
Email: Lab.genetics.cav@wales.nhs.uk

Postal Address
All Wales Genomics Laboratory
Institute of Medical Genetics
University Hospital of Wales
Heath Park
Cardiff
CF14 4XW

4.3 Who initiates biomarker testing

Initiation of biomarker testing can be requested by the oncologist either directly to the pathologist at MDT, or by submitting a request to the local histopathology laboratory. Reflex testing is **not** currently advised. The CVUHB test request form for HER2/PD-L1/MMR testing can be found here. For North Wales patients, please contact Betsi Cadwaladr directly (link: Betsi Cadwaladr). A link to AWMGS genetic test request forms for MLH1 methylation testing is available here (MLH1 methylation)

4.4 Which tests should be requested

The clinical pathway (see section 3.1) outlines required biomarkers. To reduce turnaround time, it is suggested HER2 and PD-L1 are requested at the same time, even though this will lead to ~30% of redundant PD-L1 results (HER2 positive cases). MMR may also be requested upfront, however will only be initiated by CVUHB if HER2 negative and PD-L1 CPS <5, unless explicitly stated on the request form.

The preferred pathway is for HER2/PD-L1/MMR testing to all be completed by CVUHB to reduce delays. However, the choice of testing strategy (location and technology) is at the discretion of the host pathology laboratories.

4.4.1 Which PD-L1 clone should be requested

For PD-L1 tests, Pembrolizumab and Nivolumab have each been developed with companion PD-L1 antibodies (dako 22C3 and 28.8 respectively). However, using the companion antibody is not a NICE requirement (11,12). The suggested Welsh approach is, where possible, the companion antibody is preferentially used, however *duplicate/repeat PD-L1 testing should **not** performed with the alternate PD-L1 antibody clone when considering an alternative PD-L1 targeting drug;* results using either PD-L1 antibody clone should be considered interchangeable.

Tumour location (irrespective of tumour histology)	*Preferential PD-L1 antibody clone*
Oesophageal or junctional	22C3
Gastric	28.8

Table 2. Preferential PD-L1 antibody clone to be used, by tumour location.

4.5 Tissue eligibility and requirements

Biomarker testing may be performed on all biopsy, endoscopic mucosal resections (EMR), or major UGI resections. A pragmatic approach whereby cases reported as suspicious may be tested, however this may lead to false negative or positive results. Interpretation within the clinicopathological context is required and repeat testing should be carried out on alternative tissue confirmed as cancer when available. Testing should **not** be performed on dysplasia only.

4.6 Material required for testing

Please send complete request form in to local histopathology laboratory, who will triage which tests are done in-house, and which are referred on to CVUHB Cellular Pathology. Please supply a copy of the original histopathology report, and one H&E stained slide, in addition to unstained spare slides (USS), with each request.

4.6.1 Specific HER2 requirements

4 x 4um unstained spares on FLEX slides. Cytology is not validated for HER2 testing, although may be requested if it is felt this may assist in further decision making.

4.6.2 Specific PD-L1 requirements

2 x 4um unstained spares on FLEX slides. Assessment can be made on adequately fixed biopsy or resection tissue containing ≥100 viable tumour cells. Any background dysplastic component is not included. Assessment of PD-L1 on cytological obtained material can lead to a false negative CPS, as the adjacent inflammatory response is lost in this sampling technique, and is not recommended.

4.6.3 Specific MMR requirements

4 x 4um unstained spares on FLEX slides.

5. Biomarker reports

The target for material referred to CVUHB is 7 calendar days from receipt for HER2 & PD-L1, and a further 5 calendar days if MMR is required.

Report will be made available as follows:

- Welsh Clinical Portal: An electronic report will be made available on the WCP.
- Referring pathologist: A paper copy along with any remaining tissue will be returned.
- Oncologist: Report will be emailed, and a separate paper copy sent. Please ensure appropriate details are supplied on the biomarker request form.

5.1 Interpretation of HER2 report

- 3+ staining is POSITIVE, or over expressed
- 2+ staining is equivocal, and requires FISH
- 0 or 1+ staining is NEGATIVE, or not overexpressed,

5.1.1 Sending material for FISH analysis

Cases of 2+ (equivocal staining) will require fluorescence in-situ hybridization (FISH) assessment. A H&E with the are of tumour showing 2+ staining should be circled, and 2 USS on FLEX be sent to AWMGS (see section 4.2 for postal address and link to departmental for request form).

- Where sufficient slides have been sent to cellular pathology C&V, FISH will be requested by C&V.
- For cases where insufficient slides have been sent to C&V, then a comment will be made in the biomarker report and returned to the host laboratory, requesting material to be sent.

The FISH report will be added to the biomarker report once completed.

5.2 Interpretation of PD-L1 reports

5.2.1 Interpretation of PD-L1 reports for oesophageal squamous cell carcinoma tumours

PD-L1 assessment is, in the first instance, assessed for pembrolizumab using the CPS system. If PD-L1 is less than 10, then the TPS system will be used for consideration of nivolumab. As such:

- Patients with PD-L1 CPS score of equal or greater than 10 are considered eligible for pembrolizumab in combination with chemotherapy.
- Patients with PD-L1 CPS of less than 10, but TPS equal or greater than 1, are considered eligible for nivolumab in combination with chemotherapy.

- Patients with PD-L1 CPS of less than 10 and TPS less 1 are not considered eligible for IO therapy.

5.2.2 Interpretation of PD-L1 reports for junctional or oesophageal adenocarcinoma tumours

When a patient's PD-L1 result gives the option of either nivolumab and pembrolizumab, then pembrolizumab is preferential. As such:

- Patients with PD-L1 CPS score of equal or greater than 10 are considered eligible for pembrolizumab in combination with chemotherapy.
- Patients with PD-L1 CPS of less than 10, but equal or greater than 5, are considered eligible for nivolumab in combination with chemotherapy.
- Patients with PD-L1 CPS of less than 5 are not considered eligible for IO therapy.

5.2.3 5.2.2 Interpretation of PD-L1 reports for gastric adenocarcinoma tumours

- Patients with PD-L1 CPS of greater than 5, then they are considered eligible for nivolumab in combination with chemotherapy.
- Patients with PD-L1 CPS of less than 5 are not considered eligible for IO therapy.

5.3 Interpretation of mismatch repair reports

- Loss of IHC staining in any of the MMR proteins is MMR deficiency
- MMR deficiency is comparable to MSI-H when considering eligibility for pembrolizumab

Loss of IHC staining in any of the MMR proteins is MMR deficiency (see appendix 1 for recommended reporting terminology). While the intended purpose for MMR or MSI assessment in this setting is eligibility for life prolonging treatment, this may also rarely indicate possible Lynch syndrome. Reports with IHC loss in any of the MMR proteins MLH1, PMS2, MSH2, or MSH6 will be issued with a report advising referral back to the oncologist to start the process of counselling for Lynch Syndrome testing (see section 6 for further guidance).

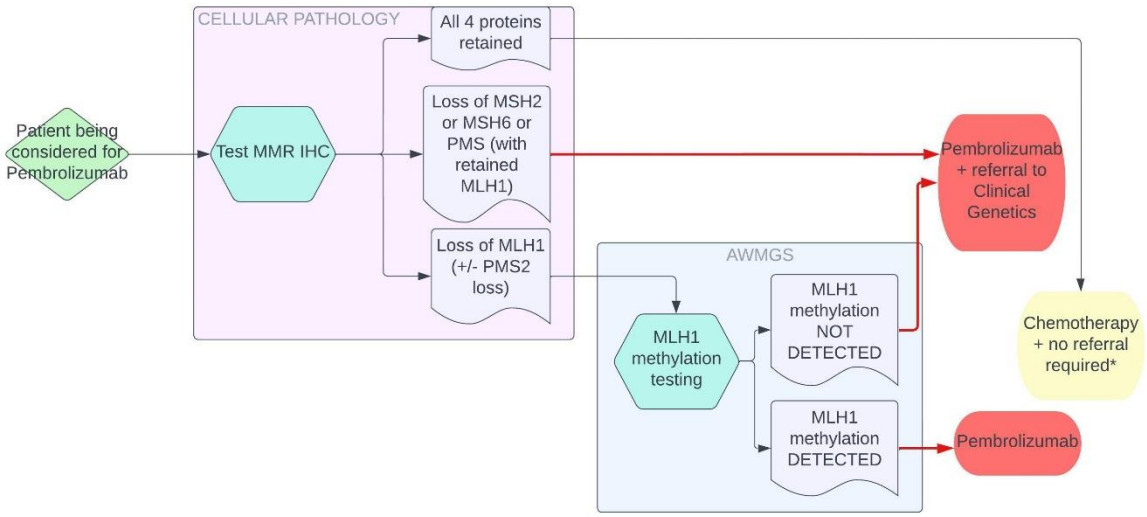

*Figure 5. Extract from clinical pathway detailing MMR/MSI results and when to refer to clinical genetics. *Please see section 6.3 for patient scenarios where referral to clinical genetics is advised when MLH1 methylation is detected.*

In patients with MLH1 and PMS2 loss, or loss of MLH1 alone, additional MLH1 methylation assessment will need to be requested (see figure 4). Where MMR assessment has been performed by CVUHB on behalf of a local histopathology laboratory, this additional testing requirement will be made clear on the biomarker report.

- Where sufficient slides have been sent to cellular pathology C&V, MLH1 methylation testing will be requested by C&V.

- For cases where insufficient slides have been sent to C&V, then a comment will be made in the biomarker report and returned to the host laboratory, requesting material to be sent.

Please note it will be the responsibility of the local laboratory to initiate MLH1 methylation assessment, unless sufficient slides have been sent to C&V. For CVUHB own cases, this duty will fall to the pathologist reporting the MMR.

It is the overall responsibility of the oncologist to ensure completion of appropriate of testing and referral to Clinical Genetics, where appropriate, for patients under their care.

5.4 MLH1 methylation testing

MLH1 methylation is usually sporadic and the patient does not require referral to Clinical Genetics. However, constitutional methylation can occur; please see section 6 for further information on referral. To request MLH1 methylation testing, the form is available **here.**

Please note, BRAF testing is not appropriate in this setting.

The results of MLH1 promoter methylation testing are provided in a report, which is issued by the All Wales Medical Genetics Laboratory. This report is uploaded to the Welsh Clinical

Portal (WCP) and is also sent by e-mail and paper copy to the requesting pathologist. Upon receipt of the sample, the All Wales Genetics Laboratory aims to complete MLH1 promoter methylation testing in 14 calendar days.

6. Referral to Clinical Genetics

Details on how to refer a patient to Clinical Genetics (AWMGS) is available here.

Patients should be provided with information, explaining why they are being referred to Clinical Genetics. Further information is available here.

Please refer to the AWMGS referral guidelines, when deciding if a patient should be referred to Clinical Genetics based on their personal or family history, even if there is no IHC evidence of an MMR deficiency.

6.1 For patients with a personal history of cancer:

Guidance is available here.

6.2 For patients with a family history of cancer:

Guidance is available here.

6.3 For patients where MLH1 promoter methylation is detected in the tumour:

If there is loss of MLH1 staining (±PMS2) on IHC and MLH1 promoter methylation is detected, the following patients should be referred to Clinical Genetics to be tested for constitutional MLH1 promoter methylation:

1. Any patient <50 years of age, diagnosed with an UGI cancer which shows MLH1 promoter methylation.

2. A patient of any age with MLH1 promoter methylation in their UGI tumour, who also has a first or second degree relative with another Lynch-associated cancer at any age.

3. A patient of any age with MLH1 promoter methylation in their tumour, who has had one or more other lynch-associated cancers.

Genetic counselling and informed consent are required for germline genetic testing for Lynch syndrome (including investigation of possible constitutional MLH1 promoter methylation). This will be undertaken by Clinical Genetics (AWMGS) and not by the referring oncologist.

References

1) NICE Technology appraisal guidance. Trastuzumab for the treatment of HER2-positive metastatic gastric cancer. [TA208]Published: 24 November 2010
2) NICE Technology appraisal guidance. Pembrolizumab with platinum- and fluoropyrimidine-based chemotherapy for untreated advanced oesophageal and gastro-oesophageal junction cancer. [TA737]Published: 20 October 2021
3) NICE Technology appraisal guidance. Nivolumab with platinum- and fluoropyrimidine-based chemotherapy for untreated HER2-negative advanced gastric, gastro-oesophageal junction or oesophageal adenocarcinoma. [TA857]Published: 11 January 2023
4) NICE Technology appraisal. Pembrolizumab for previously treated endometrial, biliary, colorectal, gastric or small intestine cancer with high microsatellite instability or mismatch repair deficiency [ID4036]. In development [GID-TA11038]. Expected publication date: 20 September 2023. Accessed 6/9/23.
5) NICE Technology appraisal guidance Nivolumab with fluoropyrimidine- and platinum-based chemotherapy for untreated unresectable advanced, recurrent, or metastatic oesophageal squamous cell carcinoma. [TA865]Published: 08 February 2023
6) Rüschoff J, Hanna W, Bilous M, Hofmann M, Osamura RY, Penault-Llorca F, van de Vijver M, Viale G. HER2 testing in gastric cancer: a practical approach. Mod Pathol. 2012 May;25(5):637-50. doi: 10.1038/modpathol.2011.198. Epub 2012 Jan 6. PMID: 22222640.
7) de Ruiter, E.J., Mulder, F.J., Koomen, B.M. et al. Comparison of three PD-L1 immunohistochemical assays in head and neck squamous cell carcinoma (HNSCC). Mod Pathol 34, 1125–1132 (2021).
8) Digestive System Tumours – WHO Classification. 5th edition. ISBN 9789283244998

Glossary

Autosomal dominant: One of the ways in which a genetic trait or condition can be inherited. In autosomal dominant inheritance, a genetic condition occurs when a pathogenic variant is present in only one allele (copy) of a given gene.

Germline pathogenic gene variant: A gene change in a body's germ cell (egg or sperm) that becomes incorporated into the DNA of every cell in the body of the offspring. These pathogenic gene variants are passed from parents to offspring.

Lynch-like syndrome: Where a person has an MMR deficient cancer and a personal or family history which is suggestive of Lynch syndrome, but where no germline pathogenic variants are found in one of the MMR genes.

Promoter methylation: The binding of methyl groups to the promoter region of a gene, which suppresses transcription of that gene and results in gene silencing (i.e. it switches the gene off).

Somatic pathogenic gene variant: Alterations in DNA which occur in any of the cells of the body except germ cells (egg or sperm) and are therefore not passed from parents to offspring.

Sporadic cancers: Cancers which are not due to inherited pathogenic gene variants.

Appendix 1- Summary of recommended terminology for reporting MMR IHC +/- MLH1 promoter methylation results[4].

MMR Result	Conclusion
MLH1: retention PMS2: retention MSH2: retention MSH6: retention	MMR IHC normal: There is no immunohistochemical evidence of a mismatch repair deficiency. Referral to Clinical Genetics is not indicated unless the patient has a personal or family history which is suggestive of Lynch syndrome.
MLH1: retention PMS2: retention MSH2: retention MSH6: loss	MMR IHC abnormal, MSH6 loss: This mismatch repair deficiency is associated with Lynch and related syndromes. This patient should be referred to Clinical Genetics.
MLH1: retention PMS2: loss MSH2: retention MSH6: retention	MMR IHC abnormal, PMS2 loss: This mismatch repair deficiency is associated with Lynch and related syndromes. This patient should be referred to Clinical Genetics.
MLH1: retention PMS2: retention MSH2: loss MSH6: loss	MMR IHC abnormal, MSH2 and MSH6 loss: This mismatch repair deficiency is associated with Lynch and related syndromes. This patient should be referred to Clinical Genetics.
MLH1: loss PMS2: loss MSH2: retention MSH6: retention	MMR IHC abnormal, MLH1 and PMS2 loss: This pattern of mismatch repair deficiency may be either sporadic or due to Lynch or related syndromes. The result of MLH1 promoter methylation testing will provide further information. A supplementary report will be issued when the results are available. IF MLH1 PROMOTER METHYLATION IS ABSENT While this mismatch repair deficiency could be sporadic, it is probable that this mismatch repair deficiency is due to Lynch or related syndromes. This patient should be referred to Clinical Genetics.

IF MLH1 PROMOTER METHYLATION IS PRESENT

The results indicate that this mismatch repair deficiency is almost certainly sporadic rather than due to Lynch syndrome. Referral to Clinical Genetics is not indicated unless the patient has a personal or family history which is suggestive of Lynch syndrome.

MLH1: subclonal loss PMS2: subclonal loss MSH2: retention MSH6: retention	MMR IHC abnormal, subclonal loss of MLH1 and PMS2: This pattern is likely to be sporadic, although it is possible that this mismatch repair deficiency is due to Lynch or related syndromes. Testing for MLH1 promoter methylation is recommended.
MLH1: loss PMS2: loss MSH2: retention MSH6: subclonal loss	MMR IHC abnormal, MLH1 and PMS2 loss with subclonal loss of MSH6: Report as for other cases of MLH1 loss.
MLH1: retention PMS2: retention MSH2: retention MSH6: subclonal loss	MMR IHC abnormal, subclonal loss of MSH6: This mismatch repair deficiency may be associated with Lynch and related syndromes. This patient should be referred to Clinical Genetics.

Appendix 2 - Links to test request forms

HER2/PD-L1/MMR test request form for assessment at Cellular Pathology Cardiff and Vale UHB is available here.
https://cavuhb.nhs.wales/our-services/laboratory-medicine/cellular-pathology/immunohistochemical-biomarkers/

MLH1 methylation test request form for assessment at AWMGS is available here.
https://medicalgenomicswales.co.uk/images/Request-Forms/LF-GEN-BRAFMLH1FUForm.pdf