

***Discussion paper
on pan-tumour
biomarker testing
to determine
eligibility for
targeted treatment***

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EXECUTIVE SUMMARY

The aim of this discussion paper is to provide guidance on the evidence needed to evaluate immunohistochemistry (IHC) testing for mismatch repair deficiency (dMMR) in colorectal cancer (CRC) and many other types of tumour (pan-tumour or tumour agnostic), in order to access pembrolizumab treatment. Subsequent co-dependent technology submissions seeking subsidy for biomarker testing for treatments that have pan-tumour effects may also be assessed according to this guidance.

One key aim of companion testing is to determine whether the test accurately identifies the appropriate biomarker, in order for the clinician to commence the patient on treatment in the appropriate clinical context. Accuracy in this determination flows on to judging when the likelihood of harms from an incorrect test result would be outweighed by the likelihood of benefits from correctly targeted treatment. Four pieces of information are critical to assess this balance of benefits and harms:

1. The sensitivity and specificity of the proposed test compared to the reference standard or the evidentiary standard (which in this case is MSI-H phenotype testing).
2. The relationship between alternative biomarkers (which in this case is the dMMR biomarker and the evidentiary standard MSI-H phenotype).
3. The prevalence of these biomarkers, as determined by the proposed companion test, the reference standard, or the evidentiary standard.
4. The degree of harm likely to be experienced by the patient through subsequent treatment decisions if incorrectly identified as having the biomarker by the test (false positive) or through failing to identify the biomarker (false negative).

In addition, for pan-tumour testing applications the following information is recommended:

- **Recommendation 1:** A biological plausibility analysis to give the rationale as to why a therapeutic response to the treatment could be expected across diverse sites or organs.
- **Recommendation 2:** Any other biomarkers that may have predictive value for the proposed treatment should be discussed.
- **Recommendation 3:** The biomarker prevalence in the overall population should be reported, along with its prevalence in as many specific tumour types as possible.
- **Recommendation 4:** The biomarker prevalence may change during the course of disease, especially if the biomarker is unstable, or has a prognostic effect (as for dMMR in CRC). Thus, the prevalence rate of the biomarker should be considered in the specific stage(s) of disease being targeted for testing and treatment.
- **Recommendation 5:** The reference standard test and the evidentiary standard test should be nominated, see Section B3.1 and Item 5 in Appendix 7, respectively, of the MSAC Technical Guidelines for Investigative Services (MSAC 2017).

- **Recommendation 6:** If the proposed test is not the evidentiary standard test used in the supportive clinical trials assessing treatment efficacy, then bridging data should be provided to assess the comparability of the performance of the proposed test to the evidentiary standard test. Key differences that may affect or alter the eligibility/selection of patients for the proposed treatment should be identified, e.g., for pan-tumour use, this comparison would be dMMR as determined by IHC vs MSI-H as determined by either the PCR-based MSI test or, in the near future, a next generation sequencing (NGS) MSI computational algorithm.
- **Recommendation 7:** Data on the accuracy of the test across tumour types should be provided in Section B3 of the assessment to demonstrate that the test performance is consistent, or if not, to identify when other testing measures are required, e.g. varying diagnostic thresholds, at-risk patient populations etc.
- **Recommendation 8:** Test reproducibility is particularly important for pan-tumour assessments to demonstrate testing equivalence across different tumour types and for different diagnostic laboratories.
- **Recommendation 9:** It is important that the positive predictive value (PPV) and negative predictive value (NPV) for the biomarker test versus its reference standard is provided over the relevant biomarker prevalence range for the tumours being targeted to enable an assessment of the ratio of correct to incorrect test results.
- **Recommendation 10:** MSAC/PBAC may consider it prudent to ensure that testing for access to a pan-tumour medication is not undertaken before other viable treatment options are considered. Alternatively, each patient could be individually triaged for either standard of care or the pan-tumour medicine, based on the prevalence of the biomarker in that tumour type and/or the population level evidence supporting a potential treatment effect of the therapy in that patient.
- **Recommendation 11:** For tumour types with very low prevalence rates, MSAC could consider the use of sequential testing to reduce the number of false positive patients who would be eligible for targeted treatment.
- **Recommendation 12:** Should the prevalence of the biomarker change during the course of disease and in response to treatments such as chemotherapy or radiotherapy, a re-biopsy may be necessary which will have implications for patient safety, test uptake and costs.
- **Recommendation 13:** The evidence is likely to consist of single-arm phase II trials in pan-tumour applications. Thus, demonstrating a therapeutic benefit will rely on the use of a reference case (most common cancer) of the effect size of the treatment in biomarker-positive patients over the current standard of care. In the absence of randomised controlled trials, the comparison could be made using prognostic data from a historical data set with subgroup cohorts defined by having different test results (e.g. dMMR and proficient MMR), against which the results of single-arm trials across a pan-tumour population can be benchmarked.

If information is provided as recommended above, the clinical impact of pan-tumour treatment targeted by a biomarker may be estimated.

BACKGROUND

This discussion paper is supplemental to, and not a replacement of the MSAC Technical Guidelines for Investigative Services (MSAC 2017), which is available from the MSAC website at www.msac.gov.au, and the PBAC Guidelines Part B: product type 4 – Codependent technologies (PBAC 2016), which are available from <https://pbac.pbs.gov.au/>.

On 3 March 2017, the MSAC Executive met via teleconference to discuss MSAC Application 1452 – mismatch repair deficient (dMMR) immunohistochemistry (IHC) testing of colorectal carcinoma (CRC) for access to pembrolizumab. The MSAC Executive agreed with a proposal that the Department of Health to work with an assessment group to provide a discussion paper evaluating dMMR IHC testing for CRC that could be used as a benchmark against which subsequent co-dependent proposals for testing in other tumour types for access to a pan-tumour targeted treatment could be assessed.

At the 31 January 2017 MSAC Executive teleconference, the Executive had agreed with PASC that dMMR IHC testing in CRC is already routine practice and funded via the MBS general item numbers for IHC of biopsy material (72846-72850). The purpose of current dMMR IHC testing in CRC is to predict the efficacy of fluorouracil-based chemotherapy, especially in stage II disease, and/or as a triage test to identify patients who may have Lynch syndrome, a hereditary dMMR disease (where the causal mutation is germline, rather than a somatic mutation characteristic of the tumour).

It should be noted that there has been no previous formal evaluation of either routine dMMR IHC testing in CRC or for the usage of the MSAC general item numbers for IHC. Assessment of the analytical and clinical validity of the dMMR IHC test in a codependent application would help determine the validity of current testing practices, as well as the test's utility in determining eligibility for targeted therapies.

However, dMMR IHC testing is less likely to become routine practice for the purpose of identifying patients with Lynch syndrome for the majority of the foreshadowed pan-tumour testing population, as these tumours are associated with a much lower cumulative lifetime risk than CRC and endometrial cancer (Barrow et al. 2009). In 2015, routine dMMR IHC testing of endometrial cancer to identify patients with Lynch syndrome was only being performed in 26% of Australian diagnostic laboratories; since then the number of laboratories has most likely increased.

Tumours that are dMMR have defective DNA mismatch repair, due to loss of function in one of four proteins (MLH1, MSH2, MSH6, or PMS2), leading to an increased mutation rate. Microsatellites, or short tandem repetitive DNA sequences are particularly sensitive to mismatch errors, and accumulation of repeat length alterations due to dMMR leads to the microsatellite instability-high (MSI-H) phenotype. The loss of function is associated with either a mutation in one of the four genes encoding the MMR proteins, a deletion in the *EPCAM* gene leading to epigenetic inactivation of MSH2

(Lynch syndrome), or hyper-methylation of the *MLH1* gene promoter (sporadic tumours). Tumours that have no defect in their DNA mismatch repair system are referred to as MMR sufficient, and tend to be microsatellite stable.

Medicines that have pan-tumour capability are able to affect any tumour originating from any part of the body. In the United States, the Food and Drug Administration has referred to this indication as “tissue/site agnostic”¹. With respect to MSAC Application 1508 – pan-tumour dMMR IHC testing includes any unresectable or metastatic non-CRC solid tumour. CRC is omitted, as dMMR IHC testing of CRC tumours is included in a separate MSAC Application (1452, as discussed above).

SCOPE OF THIS DISCUSSION PAPER

It was recommended that this discussion paper should follow the MSAC Technical Guidelines for Investigative Services (MSAC 2017). The matters to be addressed for CRC that should set the benchmark for consideration of other tumours include:

- Analytical validity assessment of IHC dMMR testing against an accepted reference standard (in the case of CRC this would be MSI-H testing, including false positive and false negative rates)
- Relationship between the dMMR biomarker and the MSI-H biomarker
- Detailed laboratory procedures for dMMR IHC testing and associated Quality Assurance Programs (QAPs)
- Number needed to test of the defined tumour populations to detect one case of a true positive dMMR tumour
- Prevalence of dMMR in this population
- Whether other genetic mutations have similar or better predictive value for treatment outcomes than dMMR and, if so, the information outlined above for the testing for any such mutation.

These issues are addressed in appropriate sections of the assessment below.

¹ Available from URL: <https://www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm560040.htm>. Accessed 7 November 2017.

CLINICAL EVALUATION OF THE PROPOSED INVESTIGATIVE MEDICAL SERVICE

INTRODUCTION

The purpose of Section B of the MSAC Technical Guidelines for Investigative Services is to identify and present the best available clinical evidence for use of an investigative medical service that has the potential to change clinical management for the main indication, and thereby improve patient health outcomes. In the case of a co-dependent MSAC/PBAC application, the medical service is usually a diagnostic test that would determine eligibility for a targeted medicinal therapy.

The most important process for determining the evidence base for assessment of a medical service is the development of the PICO (population, intervention, comparator, outcomes) study selection criteria. This defines the relevant testing population, as well as the population eligible for treatment with the therapy. It also defines the reference standard test, the comparators (usually any current or alternative tests plus standard of care), and the clinical outcomes to demonstrate the clinical safety and effectiveness of the test. The studies that form the evidence base should meet these selection criteria. As an example, the PICO criteria for MSAC Application 1452 'dMMR IHC testing in CRC', which was slightly modified from those ratified by PASC, are shown below.

Component	Description
Patients	<p><u>Testing population:</u> Patients diagnosed with colorectal cancer (CRC) (any stage) not currently tested or Patients with Stage IV CRC who have not already been tested for a MMR deficiency (dMMR)</p> <p><u>Treatment population:</u> Patients with Stage IV dMMR CRC with disease progression following a first-line therapy for metastatic disease</p>
Prior tests	Routine histology, cytology and immunohistochemical (IHC) tests to confirm diagnosis and stage of CRC
Interventions	(1) dMMR IHC testing using antibodies directed against the four MMR proteins to detect a deficiency. If dMMR is identified patients are eligible for treatment with pembrolizumab on progression to Stage IV CRC (2) No dMMR IHC testing plus pembrolizumab (<i>to demonstrate the value of dMMR IHC testing</i>)
Reference standards	(1) Genomic sequencing of the four MMR genes and the EPCAM gene (Lynch syndrome) (2) Multiplex ligation-dependent probe amplification to detect hyper-methylation <i>MLH1</i> gene promoter (sporadic cases)
Comparators	<p>Diagnostic accuracy: polymerase chain reaction-based microsatellite instability testing, (<i>the NGS MSI computational algorithm tests would also be valid comparators</i>)</p> <p>Therapeutic effectiveness: no dMMR IHC testing plus standard of care (chemotherapy with a combination of two drugs)</p>

Component	Description
Outcomes	<p>Safety: harms from testing (including rates of re-biopsy required for testing); treatment-associated adverse events and tolerability</p> <p>Diagnostic performance: sensitivity and specificity (analytical validity), concordance, test-retest reliability</p> <p>Clinical validity: positive and negative predictive values</p> <p>Prognosis: prognostic effect of biomarker</p> <p>Clinical utility: % change in management plan (e.g. changes in treatment)</p> <p>Predictive validity: treatment effect modification by biomarker status</p> <p>Therapeutic effectiveness: critical outcomes^a: overall survival, progression-free survival, overall response rate; important outcomes^a: quality of life</p> <p>Cost-effectiveness: cost, cost per life year gained, cost per quality adjusted life year or disability adjusted life year, incremental cost-effectiveness ratio, cost per case identified</p>

^aOutcomes ranked as recommended by GRADE URL <<http://www.gradeworkinggroup.org/>>. Accessed 1 November 2017

CRC = colorectal cancer; dMMR = mismatch repair deficient; IHC = immunohistochemistry

TYPES OF EVIDENCE

Direct evidence:

The availability of randomised controlled trials (RCTs) that have been specifically designed to prove a linkage between the investigative medical service and the therapeutic outcome is unlikely. However, if these trial types are available, refer to the MSAC Technical Guidelines for Investigative Services (MSAC 2017) for further information on preparing an assessment of direct evidence.

Investigative medical services that have direct evidence of their effect on patient health outcomes still require additional information to inform whether the proposed service is superior or not to the main comparator (see Section B8 of the Guidelines). The following information (if not reported as part of any direct clinical trials) should also be presented as discussed below:

- The diagnostic performance and clinical validity of the investigative medical service
 - In particular any differences between the test(s) used in the key supporting studies and the test(s) that will be used in Australian clinical practice
- The relative clinical impact of false negatives and false positives arising from the test if this information cannot be extracted from direct evidence presented
- The impact of repeat testing (if relevant)
- The relative safety of performing the test.

If no direct evidence is available, the assessment will require a linked evidence approach.

Linked evidence:

A linked evidence approach requires evidence to determine the investigative medical service's impact on clinical management and health outcomes. In other words, different types of evidence from different sources are linked in a chain of argument to estimate the clinical impact of the test.

This approach is only meaningful when the evidence for the accuracy of the investigative medical service under consideration and the evidence supporting treatment considerations have been generated in relevant patient populations, as defined by the PICO criteria.

The evidence required includes:

- Prevalence of the biomarker in the population to be tested
- Stability/inducibility of the biomarker in the tumour type during the natural history and treatment of the disease
- Prognostic evidence showing whether the biomarker is a prognostic indicator or not, through the comparison of outcomes in patients receiving usual care conditioned on the presence or absence of the biomarker
- Comparative analytical performance of the proposed test, relative to the reference standard or evidentiary standard test
- Evidence to indicate that biomarker determination guides decisions about treatment with the medicine showing clinical utility or changes in patient management (therapeutic efficacy)
- Therapeutic effectiveness, which may include:
 - Single randomised controlled trial of medicine vs usual care in patients that are test positive in both arms
 - Prospective biomarker stratified randomised controlled trial of medicine vs usual care in patients with and without the biomarker (enabling an assessment of treatment effect modification by biomarker status)
 - Retrospective biomarker stratified randomised controlled trial of medicine vs usual care in patients with unknown biomarker status and then biomarker status determined (enabling an assessment of treatment effect modification by biomarker status)
 - An indirect comparison across two or more sets of randomised trials involving one or more common reference (indirect comparison of randomised trials)
 - A comparison of the results of single-arm studies to a reference case
- Relative safety of performing the investigative service, both immediate safety issues of directly performing the test and ‘flow on’ safety issues that arise from conducting the investigative service (false-positives and false-negatives).

How these matters should be addressed for dMMR IHC testing of patients with CRC or across multiple tumour types will be discussed below.

BIOLOGICAL PLAUSIBILITY

To justify the testing of the biomarker to identify biomarker positive or negative patients (depending on biomarker/test combination) who would be eligible for a treatment, a detailed analysis of the biological plausibility of the relationship between biomarker and treatment is required and should be included in Section A4 of the assessment.

In the case of dMMR IHC testing, this analysis would need to explain how the dMMR mutation leads to increased susceptibility to immune checkpoint inhibitors. One approach would be to link the following logical steps identified from the literature:

- Prevalence of dMMR tumours (Llosa et al. 2015)
- Increased expression of ‘foreign’ peptides on tumour cells (Llosa et al. 2015)
- Increase in tumour-infiltrating lymphocytes (Buecher et al. 2013; Drescher, Sharma & Lynch 2010)
- Increased expression of PD-L1 on tumour cells to avoid cell death (Rosenbaum et al. 2016)
- Susceptibility to immune checkpoint inhibitors (Le et al. 2015). In the case of pembrolizumab, the antibody binds to an epitope on the PD-1 molecule, and inhibits binding of PD-L1
- Inhibition of PD-1/PD-L1 binding results in the tumour-infiltrating lymphocytes being able to kill the ‘foreign’ tumour cell (Dudley et al. 2016).

For pan-tumour applications, this biological plausibility analysis would need to be expanded to provide a rationale as to why a therapeutic response to immune checkpoint inhibitors could be expected across diverse sites or organs. (**Recommendation 1**)

Current recommendations for dMMR IHC testing in CRC patients are partly driven by the relatively high incidence of Lynch syndrome patients presenting with the disease, the need for surveillance for the emergence of other Lynch syndrome-associated tumours and to identify family members at risk. Lynch syndrome is caused by a germline mutation in one of the *MMR* genes or the *EPCAM* gene. The cancer risks associated with Lynch Syndrome have been accurately determined for CRC and endometrial cancer, due to their relatively high frequency among those with predisposing mutations. However, the range of less common cancers recognised as part of the Lynch syndrome cancer spectrum and their prevalence rate will increase as incidental detection of Lynch syndrome cases occurs during somatic dMMR or MSI-H testing. The relatively low or uncertain frequency of the less common Lynch syndrome cancers has meant that routine dMMR IHC testing is not currently conducted beyond CRC and endometrial cancers (noting that uptake for the latter is still variable in Australian clinical practice). The cumulative lifetime risk (at 70 years of age) of having a non-CRC and non-endometrial dMMR tumour ranges from 0% to 15% compared with 20–100% for CRC and 20–71% for endometrial cancer; where the risk for individual Lynch syndrome patients varies according to which gene carries the mutation (Barrow et al. 2009). The potential for a treatment option for any or all of Lynch syndrome and/or MSI-H cancers agnostic of tumour histology, will result in a paradigm shift in how testing is conducted and an increase in the demand for such pan-tumour testing.

The dMMR IHC test is also used to predict efficacy of fluorouracil-based adjuvant chemotherapy for stage II CRC. In these patients, dMMR is associated with an excellent prognosis and fluorouracil-based chemotherapy is not indicated as it provides limited therapeutic benefit but additional harms (Buecher et al. 2013). This is also not relevant for the pan-tumour application.

Instead, the rationale for dMMR IHC testing of pan-tumours will mostly rely on the link between somatic dMMR status and the response to PD-1/PD-L1 inhibitor immunotherapy. Although spontaneous dMMR mutations can occur in sporadic tumours, nearly all are due to hyper-methylation of the *MLH1* gene promoter. dMMR testing in CRC patients as a means of identifying those tumours that will respond to immunotherapy treatments has not been definitively established. The biological plausibility of this relationship provides a less robust benchmark against which to measure the currently available evidence to support dMMR testing of non-CRC tumours in order to determine access to pembrolizumab.

The picture for sporadic dMMR is further complicated by an association between hyper-methylation of the *MLH1* gene promoter and *BRAF* V600E mutations, noting that the *BRAF* protein is also almost never mutated in Lynch syndrome CRC tumours. In fact, the Royal College of Pathologists of Australasia (RCPA) CRC Reporting Protocol indicates that the presence of a *BRAF* V600E mutation in CRC tumours exhibiting loss of *MLH1* expression can effectively exclude Lynch syndrome, but its absence is unhelpful as the tumour could be either sporadic or caused by Lynch syndrome (RCPA 2016).

The highly conserved MMR proteins function to restore DNA integrity after the occurrence of mismatching errors resulting from DNA replication and recombination or damage from external stimuli such as chemical or radiation (Scarpa, Cataldo & Salvatore 2016). Thus, it is logical to assume that there are likely to be little to no differences in the expression and function of these genes in different tumour cell types. However, evidence to support this function across tumour types is still required.

This will not be the case for all biomarkers. Many proteins will have different expression levels in different cell types as a consequence of their basic function; this may affect the biomarker expression levels in different tumour cell types. Differences in expression may alter the ability of the test to detect the biomarker and/or the effectiveness of the treatment for different tumour types. This will have important implications for pan-tumour applications and will need to be addressed.

ALTERNATIVE PREDICTIVE BIOMARKERS

Any other biomarkers that may have predictive value for treatment outcomes should be discussed. **(Recommendation 2)** This includes their comparative abilities to predict response to the treatment.

Microsatellite instability

The most important alternative predictive biomarker is MSI-H. Tumours that are dMMR have defective DNA mismatch repair leading to an increased mutation rate. Microsatellites, or short tandem repetitive DNA sequences are particularly sensitive to mismatch errors, and accumulation of repeat length alterations due to dMMR leads to the MSI-H phenotype.

There are two different tests that can be used to determine MSI status. The PCR-based MSI test has been used to determine the MSI-H phenotype and eligibility for all or nearly all check-point inhibitor trials to date, including the KEYNOTE-158 trial.

This test involves PCR amplification of specific microsatellite markers, usually by multiplex PCR, and can be performed on fresh, frozen or formalin-fixed paraffin-embedded (FFPE) tumour material (Buecher et al. 2013). A MSI-H phenotype is defined by at least two out of five unstable markers being identified as having insertions or deletions (or ≥30% of unstable markers if a larger panel is used). All other tumours, with 0–30% unstable markers, are considered as being microsatellite stable owing to their clinical, histological and outcome similarities.

However, the use of NGS MSI computational algorithms to determine MSI status is quickly approaching. The algorithms currently being discussed in the literature include MSIsensor (Niu et al. 2014), MANTIS (Kautto et al. 2016) and MSI-ColonCore (Zhu et al. 2018). Each method uses different criteria to select the loci, including setting thresholds and creating baselines using normal samples. Two of these algorithms (MSIsensor and MANTIS) use a normal reference standard against which the repeat lengths of different mononucleotide repeat microsatellite locus are directly compared. The MSI-ColonCore algorithm uses predetermined baseline coverage ratios and categorise a locus as unstable if the coverage ratio is less than a given threshold. The MSI status of a sample is determined by the percentage of unstable loci in the given sample. NGS MSI analysis using these computational algorithms is highly accurate compared with PCR-based MSI testing (Kautto et al. 2016; Zhu et al. 2018); with sensitivities of 97.9% (MSI-ColonCore), 97.18% (MANTIS), and 96.48% (MSIsensor) and specificities of 100% (MSI-ColonCore), 99.68% (MANTIS), and 98.73% (MSIsensor).

The biological plausibility of the relationship between the biomarkers of dMMR and MSI-H should be discussed for tumours beyond their more widely understood relationship in the context of Lynch syndrome and CRC.

Other possible biomarkers

Other MSAC applications have requested programmed cell death-ligand 1 (PD-L1) testing, not dMMR IHC testing, in other cancers to determine access to pembrolizumab. PD-L1 status is determined using an IHC PD-L1 test and involves a number of different scoring methods relating to the proportion of tumour cells that express detectable levels of PD-L1, and a number of different threshold proportions, depending on the PD-L1 antibody used in the test.

Thus, a discussion on the reason why the PD-L1 biomarker is not used for the CRC or the pan-tumour application is also warranted. This discussion should answer potential questions such as:

- Once the potential problems and limitations of PD-L1 testing have been overcome, could PD-L1 testing be used as an alternative biomarker in the CRC and pan-tumour populations?
- Can the over-expression of PD-L1 by dMMR tumour cells be detected by the PD-L1 IHC test?

- Would the PD-L1 test identify a similar, broader or entirely different sub-population that would be eligible for pembrolizumab?
- How does the relationship between dMMR and PD-L1 overexpression vary in different tumour types?
- How informative is biomarker testing in non-CRC pan-tumours, given that pembrolizumab access in at least one tumour type (melanoma) requires no biomarker testing for treatment? (presumably as a consequence of the prevalence of the PD-L1 biomarker in melanoma)
- Is sequential testing an option, i.e. PD-L1 testing, and if negative, dMMR testing or *vice versa*?
- One study by Rosenbaum et al. (2016) found that only 9% of patients with MSI-H (a marker for dMMR) CRC tumours had IHC-detectable PD-L1 expression levels. Why would pembrolizumab, which directly inhibits the PD-1/PD-L1 signal transduction pathway, be effective in dMMR tumours with undetectable PD-L1 levels, but not MMR-proficient tumours? Especially MMR-proficient tumours with detectable PD-L1 expression levels.
- It is feasible that PD-L1 testing may be a more clinically relevant test for determining eligibility for pembrolizumab in patients with solid tumour types that have a very low dMMR prevalence rate. A study by Mills et al. (2017) reported that 12% of breast cancers tested (32% of triple-negative breast cancers) expressed PD-L1 while dMMR was found in only 1 case out of 285 samples (giving a 0.4% prevalence rate). The dMMR case was also PD-L1 positive, indicating that the PD-L1 test was sufficient to detect all patients likely to benefit from pembrolizumab in this study. However, further testing would be needed to determine if this scenario is true for all dMMR breast cancers.

MULTIPLE PREDICTIVE BIOMARKERS THAT PREDICT RESPONSE TO THE TARGETED THERAPY

Although the focus of the current pan-tumour application is to identify patients who will likely respond to immune checkpoint inhibitors due to their dMMR phenotype, there are many other known (and as yet unknown) genetic mutations that lead to tumours with an increased tumour mutation burden (TMB). Next generation sequencing (NGS) panels are currently being used in the research setting to identify these tumours, and the causative activating gene mutations. Rizvi et al. (2018) reviewed 240 patients with NSCLC who had been treated with immune checkpoint inhibitors (anti-PD-1/PD-L1 alone or in combination with anti-CTLA-4). In addition, these patients had had NGS molecular profiling to determine TMB, PD-L1 testing to determine proportion of tumour cells with membranous staining and radiologically evaluation for their clinical response. The authors found that the TMB was significantly greater in patients with a durable clinical benefit from immune checkpoint inhibitors than in those with no durable benefit ($p=0.006$). They also found that TMB and PD-L1 expression were independent variables, and a composite of these two variables further enriched for patients who would benefit from immune checkpoint inhibitor therapy. They reported that 50% of NSCLC patients with a high TMB and high PD-L1 expression (defined as $\geq 1\%$ of tumour cells) had a durable clinical benefit compared to 35%, 29% and 18% of patients with high PD-L1 alone, high TMB alone and low TMB/PD-L1, respectively.

Other mutations known to lead to increased TMB

Tumours with an increased TMB or hyper-mutated tumours can have either MSI-H or microsatellite stable phenotypes. Cancers mutated in the exonuclease (proofreading) domain of the catalytic subunits of the DNA polymerase delta and epsilon (POLD1 and POLE) have hyper-mutated but microsatellite stable cancer phenotypes, with large numbers of CD8+ tumour infiltrating lymphocytes (Briggs & Tomlinson 2013). BRCA1/2 proteins participate in double-stranded DNA break repair via homologous recombination, and mutations in these genes, especially BRCA2, lead to hyper-mutated MSI-H cancers with significantly increased CD3+ and CD8+ tumour-infiltrating lymphocytes, as well as elevated expression of PD-1 and PD-L1 in tumour-associated immune cells (Strickland et al. 2016). EGFR and NRTK mutations have also been strongly associated with the MSI-H phenotype in CRC (Gokare, Lulla & El-Deiry 2017). Tumours expressing these hyper-mutated phenotypes are all likely to benefit from immune checkpoint inhibitors, but targeted therapies directed against the activating mutations are also available in some cases, such as EGFR. Should an EGFR mutation-positive MSI-H CRC tumour initially receive targeted treatment for the EGFR mutation or the MSI-H phenotype, or both? Additionally, does the same association occur in other cancer types? For example, in the study by Rizvi et al. (2018) discussed above, NSCLC patients with an EGFR mutation rarely experienced a durable clinical benefit when treated with an immune checkpoint inhibitor; however, the authors did not comment on whether or not these patients also had a high TMB.

Thus, the identification of tumours likely to respond to immune checkpoint inhibitors and/or other targeted therapies will become much more complex, probably leading to complementary NGS and IHC screening panels to identify patients suitable for immune checkpoint inhibitors and/or other targeted therapies. Kim et al. (2017) used NGS and IHC panels on 81 patients with advanced/metastatic gastric cancer and identified 19 patients who received targeted therapies. Two of these patients had dMMR tumours and received treatment with a PD-1 inhibitor. Although this type of approach is likely to be the focus of pan-tumour applications in the future, this discussion paper will focus on dMMR being the causative agent of the MSI-H phenotype.

The tests listed in Table 1 have been used to determine the status of biomarkers whose presence or absence could affect the outcomes of pembrolizumab therapy in patients with CRC and/or pan-tumours in the trials listed in the MSAC applications for dMMR testing in CRC and non-CRC populations.

Table 1 Biomarker tests that have been used to evaluate response to pembrolizumab in patients with CRC or pan-tumours in clinical trials

Trial	PD-L1 positive	dMMR	MSI-H	High TMB
KEYNOTE-158	IHC	IHC	PCR-based	-
KEYNOTE-012	IHC	-	PCR-based-	-
KEYNOTE-016	-	-	PCR-based	-
KEYNOTE-028	IHC	-	-	NGS
KEYNOTE-164	-	IHC	PCR-based	-
KEYNOTE-177	-	Locally confirmed Test not specified	Locally confirmed Test not specified	-

dMMR = mismatch repair deficiency; IHC = immunohistochemistry; MSI-H = microsatellite instability – high; PCR = polymerase chain reaction; PD-L1 = programmed cell death ligand 1; TMB = tumour mutation burden

PREVALENCE OF THE BIOMARKER IN THE POPULATION TO BE TESTED

The prevalence of the biomarker in the testing population, relevant to the Australian setting, should always be included in Section A4 of the assessment, as outlined in the MSAC Technical Guidelines for Investigative Services (MSAC 2017). For a pan-tumour application, the prevalence estimate should include the biomarker prevalence in the overall population and the prevalence for each of the many specific tumour types, if possible. **(Recommendation 3)** The biomarker prevalence may change during the course of disease, especially if the biomarker is unstable, or has a prognostic effect (as for dMMR in CRC). The prevalence of dMMR in the total CRC population is around 15%, but in stage IV CRC it is only 4%. The lower prevalence in Stage IV CRC is most likely due to dMMR CRC having a favourable prognosis compared to MMR sufficient CRC. Thus, the prevalence rate of the biomarker should be considered in the specific stage(s) of disease being targeted for testing and treatment. **(Recommendation 4)**

For example, Table 2 shows the estimated prevalence rates of dMMR for various tumours, however, the stage of disease was not always specified. For the purpose of this discussion paper, the rates are assumed appropriate for stage IV disease. It should be noted that patients with melanoma currently have access to pembrolizumab without requiring testing for either PD-L1 or dMMR, and dMMR testing of CRC patients for access to pembrolizumab is covered by a separate MSAC application (1452) to the pan-tumour application (1508). The importance of the prevalence of dMMR (based on MSI-H prevalence) for the remaining pan-tumour types is discussed further in the ‘Clinical validity’ section.

Table 2 Prevalence of the most common sporadic solid tumours cases in Australia, proportion that are Stage IV, and MSI-H prevalence per tumour type

Carcinoma	Proportion of all cancers	Number of patients that are Stage IV	Proportion of cancers that are MSI-H
Breast cancer	26.5%	2,521	1%
Prostate cancer	24.5%	2,678	4–12%
Lung cancer	9.0%	7,495	1%
Endometrial cancer	4.1%	295	17–33%
Renal cell carcinoma	2.6%	835	1–4%
Thyroid cancer	2.6%	123	23%
Head and neck cancer	2.5%	1,007	1–3%
Pancreatic cancer	2.4%	2,408	1–4%
Ovarian cancer	2.3%	811	10–11%
Gastric cancer	1.7%	1,007	7.5–15%
Cervical cancer	1.5%	221	5–9%
Glioma	1.5%	1,278	0–33%
Oesophageal cancer	1.2%	1,179	5–10%
Colorectal cancer	12.3%	3,784	4–15%
Melanoma	10.6%	1,352	11–77%

* Assumed that all prevalence rates are for Stage IV disease for the purposes of this discussion paper

dMMR = mismatch repair deficient; MSI-H = microsatellite instability-high

Sources: Scarpa, Cataldo & Salvatore (2016); Cancer in Australia 2017, PICO confirmation for MSAC Application 1508; Australian Institute of Health and Welfare. Available from URL <<https://www.aihw.gov.au/reports/cancer/cancer-in-australia-2017/data>>. Accessed 11 October 2017

DIAGNOSTIC PERFORMANCE

THE IHC dMMR TEST

IHC dMMR testing uses antibodies directed against each MMR protein (MLH1, MSH2, MSH6 and PMS2) and IHC staining to detect the expression of these proteins in the tumour cells to determine eligibility for treatment with pembrolizumab. The test uses four FFPE tumour tissue sections (one for each antibody) from either a surgical resection or a biopsy (if unresectable). The sample would be obtained as part of normal diagnostic work-up, and patients are unlikely to require a new biopsy for the specific purpose of IHC dMMR testing. Evidence of the stability of these proteins in FFPE tissue blocks should be provided if archival tissue is likely to be retrieved for testing.

The results for the four individual antibodies are combined to provide an overall positive or negative test result. However, due to the nature of these proteins, the four individual “tests” can also act as internal controls for each other. This is because the proteins form heterodimers (MLH1/PMS2 and MSH2/MSH6), and as the loss of one protein usually affects the expression of its partner; most dMMR tumours usually show loss of expression of both proteins in the affected heterodimer. Loss of protein expression should be complete, with the absence of nuclear staining of all cancer cells and unequivocal positive staining of the nuclei of surrounding non-cancer cells and infiltrating lymphocytes. The loss of expression of MSH2/MSH6 is highly suggestive of a MSH2 germline mutation, and loss of expression

of MLH1/PMS2 may result either from a MLH1 germline mutation or from acquired somatic hypermethylation of the MLH1 gene promoter. Patients whose tumours show a lack of expression of any one of these proteins would be classed as dMMR and would be eligible for treatment with pembrolizumab at diagnosis of or progression to Stage IV disease.

It should be noted that in rare cases, dMMR cannot be detected by IHC. Apparent intact expression of all four proteins by IHC cannot entirely exclude dMMR and Lynch syndrome as missense mutations can lead to a non-functional protein with retained antigenicity (Buecher et al. 2013). Additionally, the occurrence of a tumour in a patient with Lynch syndrome does not guarantee that the cancer was caused by dMMR. Lotsari et al. (2012) reported that only 13/20 (65%) of breast cancers from Lynch syndrome patients were dMMR and/or MSI-H. Thus, new tumour types occurring in patients known to have Lynch syndrome should be tested for suitability for treatment with pembrolizumab.

REFERENCE OR EVIDENTIARY STANDARD

Determine if there is a reference standard test (as outlined in Section B3.1) and evidentiary standard test (as outlined in Item 5 in Appendix 7 of the MSAC Technical Guidelines for Investigative Services, MSAC 2017). **(Recommendation 5)**

In the case of dMMR IHC testing for CRC, there are potentially three reference or evidentiary standards. The PICO confirmation for this application identified two potential reference standards:

- Next generation sequencing (NGS) of the *MMR* genes in a germline DNA sample (peripheral blood) for the diagnosis of Lynch syndrome
 - Lynch syndrome is caused by a mutation in one of the *MMR* genes or the *EPCAM* gene (typically, *MLH1*, *MSH2*, *MSH6*, *PMS2*, *EPCAM* are the key genes sequenced).
 - Over 3,100 unique DNA variants associated with Lynch syndrome have been described with 57% classed as pathogenic (cancer-causing) or likely pathogenic, 32% as uncertain, 4% as likely not pathogenic and 7% as not pathogenic (Da Silva et al. 2016).
- Methylation specific multiple ligation-dependent probe amplification (MS-MLPA) to detect hyper-methylation of the *MMR* gene promoters for detection of sporadic cases of dMMR.
 - This test can be used to detect methylation in the promoter of several MMR genes, including *MLH1*, *MSH2*, *MLH3*, *PMS2*, and *MSH6*.
 - Epigenetic inactivation (methylation) of the *MLH1* promoter causes dMMR in nearly all sporadic cases.

However, the MSAC Executive agreed on 3 March 2017 that PCR-based microsatellite instability (MSI) testing would be an accepted reference standard for dMMR IHC testing. MSAC Application 1452 originally included MSI testing as a comparator to dMMR IHC testing, but the applicant withdrew it as it was no longer widely available nor routinely performed in Australia. Lab Tests Online² (a website

² Available from URL: <https://www.labtestsonline.org.au>. Accessed 8 November 2017.

that is part of the Quality Use of Pathology Program) states that MSI testing for Lynch Syndrome has been largely replaced by IHC dMMR testing. Nevertheless, the RCPA reported that although MSI testing “is used less commonly in the diagnostic pathology setting, it continues to have a role in problematic cases”.

- The MSI test detects both sporadic and Lynch syndrome dMMR cases. Laboratories may implement one of several different biomarker panels for this purpose. Typically, an MSI phenotype is defined by the presence of at least two unstable markers, identified as having insertions or deletions among five tested markers, or ≥30% of unstable markers if a larger panel is used. A clear definition of the panel and cut-off used for determining the MSI-H phenotype must be reported to enable appropriate comparisons of the IHC dMMR test with the “same” reference standard.

Given the sporadic nature of most non-CRC tumours, most will result from hyper-methylation of the *MLH1* promoter. Thus, NGS of the *MMR* genes, which detects germline DNA mutations causing Lynch syndrome, is unlikely to be a relevant reference standard for pan-tumour dMMR IHC testing. The relevant reference standards for MSAC Application 1508 would be MS-MLPA testing of the *MLH1* promoter and MSI testing.

If the proposed test is not the evidentiary standard test used in the supportive clinical trials assessing treatment efficacy, then bridging data should be provided to assess the comparability of the performance of the proposed test to the evidentiary standard test. Key differences that may affect or alter the eligibility/selection of patients for the proposed treatment should be identified, e.g., for pan-tumour use, this comparison would be dMMR as determined by IHC vs MSI-high as determined by either the PCR-based MSI test or, in the near future, an NGS MSI computational algorithm.

(Recommendation 6)

ANALYTICAL VALIDITY

Analytical validity (sensitivity and specificity) refers to the assessment of a test against an accepted reference standard. A true or ‘gold’ reference standard enables patients who have the target condition or clinical information of interest to be distinguished from those who do not. However, for most genetic companion tests, the reference standard is either imperfect or an evidentiary standard is used as no true reference standard can be identified.

In the case of dMMR IHC testing of CRC, reasonable analytical validity in determining the dMMR phenotype and eligibility for pembrolizumab might be assumed, given the routine nature of the test for CRC, even though there has been no formal MSAC evaluation of this test and it is not currently subjected to a QAP. The RCPA CRC structured reporting protocol (RCPA 2016) only includes *KRAS* and *NRAS* tests (consistent with current PBS restrictions for panitumumab and cetuximab). Additionally, the RCPA currently offers QAPs for IHC testing for specific indications for breast cancer and lymphoma

but not CRC³. It should also be noted that dMMR IHC testing is not used as a stand-alone test for the diagnosis of Lynch syndrome. Confirmatory DNA sequencing is usually undertaken to identify a germline DNA mutation.

The importance of a QAP with detailed laboratory procedures to train and guide pathologists can be illustrated using dMMR IHC testing as an example. The dMMR phenotype is identified by the absence of an MMR protein leading to faulty DNA repair. Thus, negative staining represents a *positive* dMMR test result. Lack of adequate training and reporting guidelines could lead to negative staining being incorrectly reported as a negative test result. In fact, the feedback on MSAC Application 1508 received from Lynch Syndrome Australia⁴ indicated that in their experience, pathologists and/or clinicians do misinterpret the ‘negative result’ of a dMMR test (i.e., indicating loss of staining in proteins and the dMMR phenotype) as a negative result requiring no further action. This has serious implications for test accuracy and the proportion of patients who would receive false positive and false negative test results.

Therefore, in addition to the implementation of a QAP, appropriate training of pathologists and clear reporting guidelines should be mandated before implementation of pan-tumour dMMR IHC testing to determine access to pembrolizumab, even though the test is already widely used in current clinical practice for detecting Lynch Syndrome. The reporting guidelines should provide a uniform and unambiguous way of reporting dMMR results to the clinicians.

Hence, any studies that provide data to assess the analytical validity of dMMR IHC testing compared with the two potential reference standards that have been identified for the pan-tumour application should be provided, as it will enable an assessment of rates of false positive and false negative test results. These are important for determining any adverse health outcomes associated with the test when used to direct treatment with pembrolizumab.

It should be noted that even if MSAC considers dMMR IHC testing to have acceptable analytical validity for CRC, reasonable analytical validity cannot necessarily be assumed for the pan-tumour application, and it would be appropriate to assess whether the test could have altered sensitivity across different tumour types. Inherent differences in tumour biology may result in altered expression levels of the biomarker, and/or expression of other proteins that interfere with detection of the biomarker. Tissue fixation and processing variables, especially with large surgical samples, may also result in differential IHC staining in different tumour types. This can affect any biomarker that is detected using an IHC test.

Thus, some data on the accuracy of the test across tumour types should be provided in Section B3 of the assessment to demonstrate that the test performance is consistent, or if not, to identify when other testing measures are required, e.g. diagnostic thresholds, at-risk patient populations, etc.

³ Available from URL: <<https://www.rcpaqap.com.au/product-catalogue/2017>>. Accessed 14 November 2017

⁴ Lynch Syndrome Australia website <<https://lynchsyndrome.org.au/>>. Accessed 3 November 2017

(Recommendation 7) To support the analytical validity of dMMR IHC testing for both CRC and other tumours, the assessments would need to include:

- False positive and false negative rates against all reference and evidentiary standards
- Detailed laboratory procedures for dMMR IHC testing and associated QAPs to ensure correct reporting of results
- Number needed to test in the defined populations to detect one true positive case of dMMR CRC or dMMR in other tumours.

EXTENDED ASSESSMENT OF RELIABILITY EVIDENCE

Evidence of the reliability of the test should be supplied as described in Section B3.7 of the MSAC Technical Guidelines for Investigative Services. Evidence on test inter-rater reproducibility may also be available from a reliable QAP. Test reproducibility is particularly important for pan-tumour assessments to demonstrate testing equivalence across different tumour types and for different diagnostic laboratories. **(Recommendation 8)**

CONCORDANCE ANALYSIS

In the absence of a suitable reference standard, measures of agreement, such as positive percent agreement and negative percent agreement, and/or kappa coefficients, comparing the proposed investigative medical service and a comparative method should be provided in Section B3.8 of the assessment. This is especially important if more than one type of test could be used in the Australian clinical setting to identify a specific biomarker (i.e. either two different commercially available tests, or two different testing methods such as NGS and a PCR-based assay).

CLINICAL VALIDITY

Even if the analytic validity of the dMMR test when used for diagnosis of other tumour types is assumed to be equivalent to that determined for CRC, the clinical validity is unlikely to be the same as this depends on the prevalence (or pre-test probability) of having the target condition or outcome of interest. The prevalence of dMMR among the different tumour types is variable (Table 2). This has implications for the clinical effectiveness of testing. For tumour types with very low prevalence rates, there may be more patients with false-positive results than true positive results that would be treated with pembrolizumab, potentially resulting in a net effect of poorer health outcomes for the patient, i.e. the toxicity of the treatment outweighing the survival gains as there is no biomarker available to target.

The key measures used to determine clinical validity are the positive and negative predictive values (PPV and NPV). The PPV is the percentage of patients with a positive test who actually have the condition (in this case, the biomarker), and is equivalent to the post-test probability of having the biomarker if test positive (or true positive). The NPV is the percentage of patients with a negative test

who do not have the biomarker; therefore, 1-NPV is equivalent to the post-test probability of having the biomarker despite being test-negative, or the post-test probability of being false negative.

Generally, for a given sensitivity and specificity, the PPV increases as the prevalence increases while the NPV decreases. The rate of the increase or decrease with increasing prevalence depends on the sensitivity and specificity of the test. Figure 1 shows the relationship between PPV and 1-NPV, with respect to the post-test probability (y-axis) of having the biomarker for prevalence rates 0–100% (x-axis) after testing with a hypothetical test having 95% sensitivity and specificity (red and blue solid lines). In this scenario, a positive test result can reliably predict the presence of the biomarker in at least 90% of patients if the prevalence rate is at least 30%, and conversely, the number of patients who are biomarker positive despite having a negative test result only increases markedly at very high prevalence rates ($\geq 70\%$).

Therefore, if the prevalence of the biomarker was around 15%, as is the case for dMMR prevalence in CRC at any stage (as per current testing), the post-test probability of having the biomarker with a positive test result (PPV) is 77%. This indicates that about three quarters of patients with a positive test result would truly have the biomarker and one quarter would be false positives. For endometrial cancer, with a dMMR prevalence of up to 33%, nine out of ten patients with a positive test result would be true positives. However, if the prevalence of the biomarker was 1%, as for dMMR in breast cancer, the post-test probability of having the biomarker with a positive test result would be 16%. This indicates that the number of false positives would far outweigh the number of true positives (5:1). This will greatly impact both the treatment effectiveness (taking into account the large number of false-positive patients who would be treated with pembrolizumab) and the cost-effectiveness of the test.

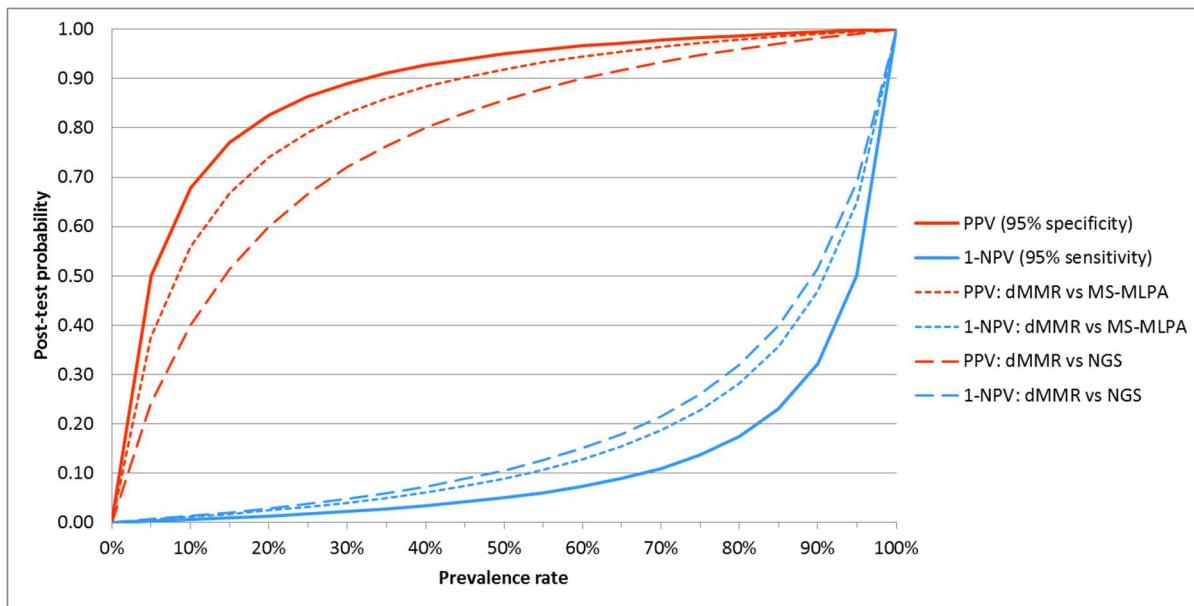


Figure 1 The post-test probability of having the biomarker (dMMR) after receiving a positive test result (PPV) and after receiving a negative test result (1-NPV) for prevalence rates from 0–100%

The solid lines relate to the post-test probability of having the biomarker using a hypothetical test with 95% sensitivity and specificity. The dashed lines relate to the post-test probability of having a dMMR tumour using the dMMR IHC test compared with the reference standards MS-MLPA of the *MLH1* promoter and NGS of the *MMR* genes, with sensitivities of 90% and 91%, and specificities of 81% and 92%, respectively.

dMMR = mismatch repair deficient; NGS = next generation sequencing; NPV = negative predictive value; MS-MLPA = methylation specific multiple ligation-dependent probe amplification; PPV = positive predictive value

Conversely, for biomarker prevalence rates of 1% and 20%, the post-test probability of having the biomarker with a negative test result (1-NPV) would be 0% and 8%, respectively. This indicates that no more than one in eight patients with a negative test would be false negative, making the test very useful in *ruling out* the presence of the biomarker. In cases where a targeted treatment relies on the absence of a biomarker, the NPV would be very helpful in instances where the mutation rate is low.

The sensitivity and specificity of dMMR IHC testing varied in the literature, depending on the reference standard used. For example, the sensitivity and specificity of dMMR IHC testing compared with MSI-H testing was reported to be 92.3% and 100%, respectively by Lindor et al. (2002). In this case, there would be no false positives and the low prevalence rate would be of no concern, except for the number of tests that would be needed to identify one test-positive dMMR patient. When compared against MS-MLPA testing of the *MLH1* promoter, the sensitivity and specificity for detecting sporadic disease were 91% and 92%, respectively (Poynter et al. 2008). When using NGS of the *MMR* genes as the reference standard, the sensitivity for detection of Lynch syndrome was similar (90%), but there was a reduction in the specificity (85%) of dMMR IHC testing (CADTH 2016). Thus, the post-test probability of having a dMMR tumour would be reduced compared with the hypothetical case discussed above. How this reduction alters the post-test probability of having dMMR is shown in Figure 1 (dashed lines).

It is important that the PPV and NPV for a test versus its reference standard is provided over the relevant biomarker prevalence range for the tumours being targeted in a pan-tumour application to

enable an assessment of the likely proportion of false-positive and false-negative tests. **(Recommendation 9)** An example of the number of true positive and false-positive patients with pan-tumour dMMR testing, who would be eligible for treatment with pembrolizumab, is given in Table 3.

Firstly, the most appropriate reference standard was determined. Using NGS of the *MMR* genes as the reference standard is not relevant for the pan-tumour application because of the very low likelihood of the patients having Lynch syndrome. Therefore, the most relevant reference standard for determining the diagnostic accuracy of dMMR IHC testing may be MS-MPLA testing of the *MLH1* promoter. However, as this test was not used in any of the pivotal trials, using the PCR-based MSI test as the evidentiary standard may provide the most accurate link between diagnostic and clinical outcomes.

For the purposes of this discussion, the sensitivity (91%) and specificity (92%) of dMMR IHC testing compared with MS-MLPA testing of the *MLH1* promoter was used to calculate the PPV for various tumour types included in the pan-tumour application (Table 3). The MSI-H prevalence rate as estimated in Table 2 was used as a surrogate measure for dMMR prevalence, given the high level of agreement between dMMR and MSI-H reported above. The dMMR prevalence rate was used to determine the total number of true positives in the testing population, and according to the sensitivity of the test, 91% of true-positive patients would have a positive test result. The number of patients with positive test results who would be true positive or false positive were calculated using both the dMMR prevalence rate and the inverse of the specificity. The number of true positive patients who had a negative test result was also determined from dMMR prevalence rate and the test sensitivity (9% of true positives).

The next step is to consider the clinical impact for both false-positive and false-negative patients (as well as true-positives and true-negatives). If there are more false-positive patients than true-positive patients receiving targeted treatment for some tumour types, as illustrated in Table 3, health outcomes may be inferior for most of the treated patients. Treatment with the targeted medicine due to an incorrect test result may be acceptable if these patients have no other treatment options, and the side effects are not significant. However, if they are forgoing viable alternate treatments due to the incorrect test result, this is likely to detrimentally impact on their health and their survival.

Patients eligible for pan-tumour medication could also be eligible for several different standard of care treatment options depending on several factors. Different tumour types, such as endometrial cancer and pancreatic cancer, will have different treatments available. Additionally, different subgroups of the same cancer type, such as endocrine receptor-positive breast cancer and triple-negative breast cancer, will also have different treatment options. Furthermore, the patient's overall health will determine eligibility for the types of treatment available. As more treatment options fail, the patient's performance status will decrease, and they may become ineligible for some treatment options.

This has implications for patients with false-positive test results. These patients will forgo a potentially beneficial standard of care treatment to receive a treatment that is unlikely to be effective. Their

health status may decline, making them ineligible to receive subsequent potentially beneficial treatments, thus shortening their survival.

Table 3 Number of patients with dMMR IHC positive test results for various non-CRC solid tumours types who would be eligible for pembrolizumab

Carcinoma	MSI-H prevalence	Number of Stage IV patients (TPs)	Post-test probability of being TP (PPV)	Total number of TP:FP eligible for treatment	Number of TPs with negative test result
Breast cancer	1%	2,521 (25)	10.3%	23:200	2
Prostate cancer	12%	2,678 (321)	60.8%	292:188	29
Lung cancer	1%	7,495 (75)	10.3%	68:592	7
Endometrial cancer	33%	295 (97)	84.9%	88:16	9
Renal cell carcinoma	4%	835 (33)	32.2%	30:63	3
Thyroid cancer	23%	123 (28)	77.3%	25:7	3
Head and neck cancer	3%	1,007 (30)	26.0%	27:77	3
Pancreatic cancer	4%	2,408 (96)	32.2%	87:183	9
Ovarian cancer	11%	811 (89)	58.4%	81:58	8
Gastric cancer	15%	1,007 (151)	66.8%	137:68	14
Cervical cancer	9%	221 (20)	52.9%	18:16	2
Glioma	33%	1,278 (422)	84.9%	384:68	38
Oesophageal cancer	10%	1,179 (118)	55.8%	107:85	11

Source: Table 2 using the highest estimate of the MSI-H prevalence rate (best-case scenario) for each tumour type. The PPV was calculated using the sensitivity and specificity for dMMR IHC testing, compared with MS-MLPA testing of the MLH1 promoter as the reference standard (91% and 92%, respectively).

dMMR = mismatch repair deficient; FP = false positive; PPV = positive predictive value; TP = true positive

MSAC/PBAC may consider it prudent to ensure that testing for access to a pan-tumour medication is not undertaken before other viable treatment options are considered. Alternatively, each patient could be individually triaged for either standard of care or the pan-tumour medicine according to their tumour type and the prevalence of the biomarker in that tumour type and/or the population level evidence supporting a potential treatment effect of the therapy in that patient, in a fashion similar to the algorithm shown in Figure 2. **(Recommendation 10)** The prevalence rate used to triage patient eligibility would be pre-determined by MSAC/PBAC, and would depend on the sensitivity and specificity of the test, such that the post-test probability of being truly positive is acceptable with respect to any potential harms arising from inappropriate treatment of false-positive and false-negative patients.

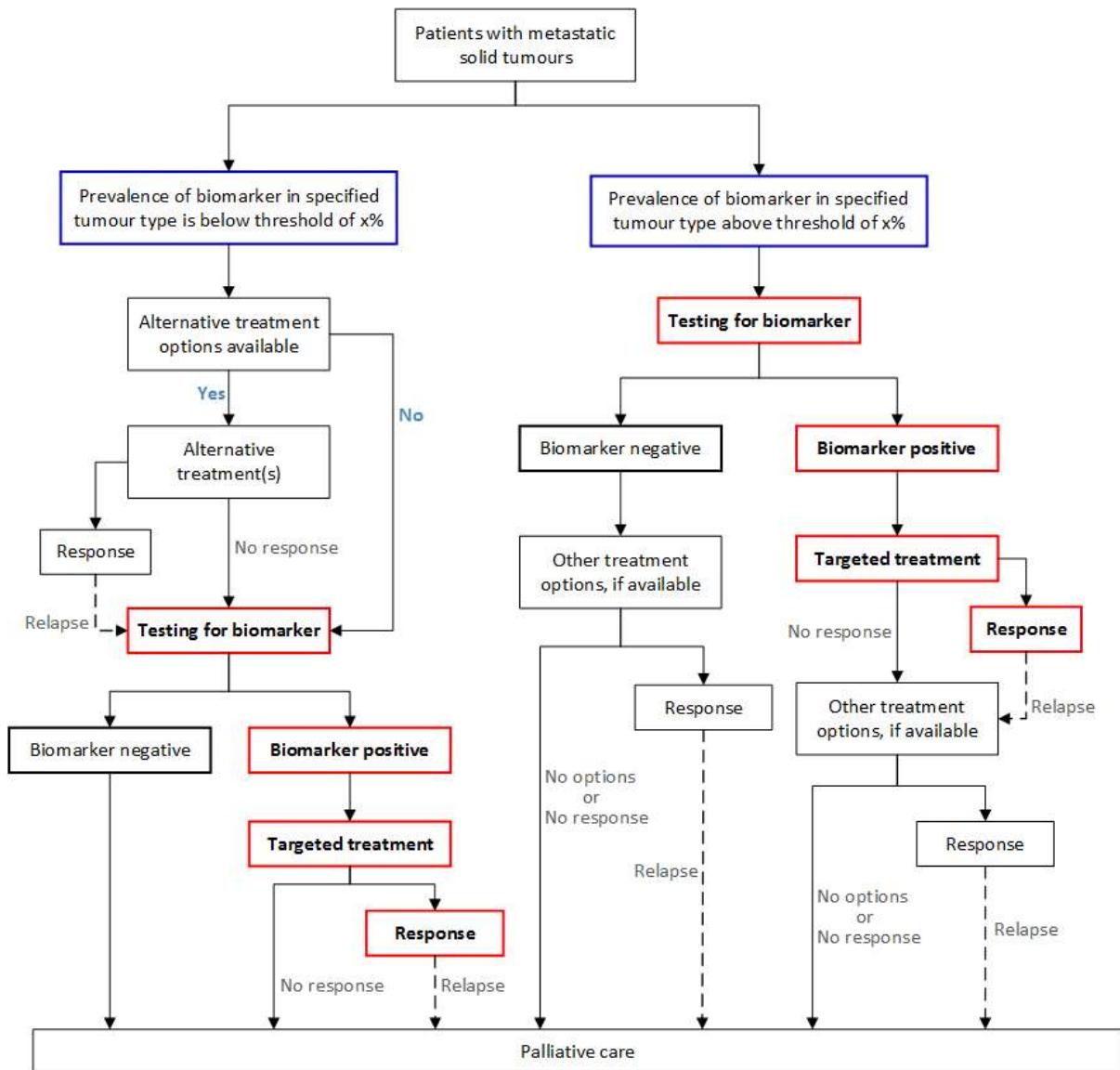


Figure 2 An algorithm to guide testing and treatment for a pan-tumour population to minimise harm in the event of false-positive test results

The prevalence rate used to triage patients would be pre-determined by MSAC/PBAC depending on the sensitivity and specificity of the test, and with respect to any potential harms arising from inappropriate treatment.

Alternative treatment options will depend on the tumour type (e.g. endometrial cancer versus pancreatic cancer), tumour subtype (e.g. endocrine receptor-positive breast cancer versus triple-negative breast cancer), and the patients performance status (overall health).

Confirmatory testing of IHC dMMR positive test results could also be implemented in one or both arms of the algorithm to reduce the number of false-positive patients treated inappropriately (green boxes). This would be especially useful for tumour types with low dMMR prevalence (left arm of the algorithm), although the additional confirmatory test will have cost implications.

For tumour types with very low prevalence rates, MSAC could also consider the use of a different test to triage patients for eligibility to pembrolizumab. For example, one study found that IHC PD-L1 testing was of greater clinical relevance than IHC dMMR testing in breast cancer (Mills et al. 2017). In addition to identifying patients who had dMMR tumours, the IHC PD-L1 test identified additional patients whose tumours expressed PD-L1 and would also likely benefit from pembrolizumab treatment. Alternatively, a number of tests could be conducted sequentially to confirm a positive IHC dMMR test result. (**Recommendation 11**) In the case of sporadic dMMR, the most relevant confirmatory tests would be MSI-H and/or MS-MLPA.

It should be noted that in the future, these tests will be superseded by NGS panels that can detect any tumours that would benefit from immune checkpoint inhibitors.

The timing of the test

The timing of testing is also important. The number of patients tested will be lower if tested when the patient would become eligible for the treatment (after failure of first-line treatment for Stage IV disease), compared with testing of the entire population at initial diagnosis of the tumour. In most cases, testing for access to PBS subsidy for a medicine is only funded on the MBS for the stage of disease relevant to treatment. In the case of CRC, dMMR IHC testing is currently done at initial diagnosis, due to its prognostic value. If dMMR testing for access to pembrolizumab is listed, testing will still occur early even though the patient will not be eligible for treatment with pembrolizumab until progression to Stage IV disease. However, in the case of pan-tumour dMMR IHC testing (except endometrial cancer and maybe ovarian cancer), the prognostic value is less important and dMMR IHC testing is likely to occur at diagnosis of, or progression to, Stage IV disease to reduce the number of tests required and the costs involved.

As the dMMR phenotype is stable during the course of disease, dMMR IHC testing on progression to Stage IV can be performed using archived formalin-fixed paraffin-embedded tissue blocks from biopsies obtained at diagnosis. However, this is not the case for all biomarkers. Should the prevalence of the biomarker change during the course of disease or in response to treatments such as chemotherapy or radiotherapy, a re-biopsy may be necessary which will have implications for patient safety, test uptake and costs. (**Recommendation 12**)

CLINICAL EVALUATION

PROGNOSIS OR PREDISPOSITION

All assessments, for both specific tumour (e.g. CRC) and pan-tumour applications, should report on whether or not the diagnostic test has prognostic value or generates information about predisposition in the targeted population, as per instructions in Section B4.2 of the MSAC Technical Guidelines for Investigative Services. This information may impact on the interpretation of the size of effect in the 'Therapeutic effectiveness' section.

CLINICAL UTILITY

Clinical utility refers to how likely the test is to significantly impact on patient management and health outcomes. In this section, it is important to identify the relative clinical impact of false negatives and false positives arising from the test. Any major health concerns for patients receiving incorrect test results should be discussed. It should also be noted if the clinical outcomes of patients treated on the basis of false positive or false negative test results or both are accounted for in the health outcomes from the clinical trials included in the evidence base that has been presented.

Any adverse effects from the treatment compared with standard of care are likely to be the same for both CRC and pan-tumour dMMR populations. However, the balance between harms and benefits may shift due to the increased proportion of false positive compared to true positive patients in very low dMMR prevalence pan-tumour subpopulations.

The degree of harm experienced by false-positive patients will depend on the effectiveness of the treatment compared with other standard of care treatments the patient would have been eligible to receive.

THERAPEUTIC EFFECTIVENESS (INCLUDING IMPACT OF EFFECT MODIFICATION)

Health outcomes from clinical studies of patients based on their biomarker status should be reported in this section. The evidence required to assess the effectiveness of treatments is provided in detail in the PBAC Guidelines (PBAC 2016).

For applications involving pan-tumour populations, the evidence is likely to consist of single-arm phase II trials. Thus, demonstrating a therapeutic benefit will rely on the use of a reference case (the most common cancer) of the effect size of the treatment in biomarker-positive patients over the current standard of care. In the absence of randomised controlled trials, the comparison could be made using prognostic data from a historical data set with subgroup cohorts defined by having different test results (e.g. dMMR and proficient MMR), against which the results of single-arm trials across a pan-tumour population can be benchmarked. (**Recommendation 13**)

In the case of dMMR IHC testing to determine eligibility for pembrolizumab in treatment naïve Stage IV CRC patients, the evidence base (reference case) will include:

- one RCT in which patients who have stage IV MSI-H or dMMR CRC (tested by IHC for dMMR or PCR for MSI-H) and have not received prior systemic treatment are randomised to either pembrolizumab monotherapy or standard of care chemotherapy
- single-arm phase II trials that enrolled MSI-H or microsatellite stable CRC patients to receive treatment with pembrolizumab.

The outcomes of the RCT will provide a direct comparison of health outcomes for patients whose tumours had dMMR treated with pembrolizumab and those treated with standard of care chemotherapy in the first-line setting. However, this trial provides no evidence for the effectiveness of pembrolizumab in patients whose tumours were MMR sufficient (to determine the need for testing), or for those being treated in later-line settings. If earlier signal-finding studies addressed this in the seamless oncology trial approach, whereby patients for whom no signal is observed are not subsequently enrolled into clinical studies, then these studies should be provided as the best available evidence.

In the case of dMMR IHC testing across many tumours, the available evidence is more limited, and insufficient to determine pembrolizumab effectiveness in each tumour type. The expected evidence base will include:

- two single-arm phase II trials that enrolled both CRC and non-CRC patients whose tumours were dMMR and treated with pembrolizumab
- two single-arm phase II trials of patients with any advanced non-CRC solid tumour that is MSI-H and have received at least one prior therapy
- two single-arm phase II trials of patients with advanced solid tumours expressing PD-L1 and treated with pembrolizumab, who were retrospectively tested for MSI-H status.

Assuming pembrolizumab was found to have superior effectiveness to standard of care for dMMR metastatic CRC (mCRC) in the first-line setting, the reported treatment effect size for patients treated with pembrolizumab can be naively compared to that of other tumours treated with pembrolizumab, as shown in Figure 3A. It is important to note, however, that the pan-tumour application is for second-line treatment. This complicates the interpretation of the relevance of any difference in the size of the clinical effect between CRC and pan-tumour studies. The likely incremental gain over the relevant comparator therapies can then also be informed by naïve comparison with the reported results of their trials (Figure 3B). It is important to note that these trials would involve biomarker unselected populations, so the prevalence and the prognostic value of the biomarker is likely to be influential to this comparison. This will likely differ for the different treatment options available across different cancers (comparators), as well as the differing life expectancies across different cancers. Data would be required to estimate the clinical benefits gained from the standard of care for the other non-CRC tumour types which could then be indirectly compared (assuming the populations are transitive) to the single-arm pembrolizumab studies to determine the incremental gain in treatment effect. There would be a number of comparators with different efficacies, making it difficult to determine the incremental gain of pembrolizumab against all other treatments.

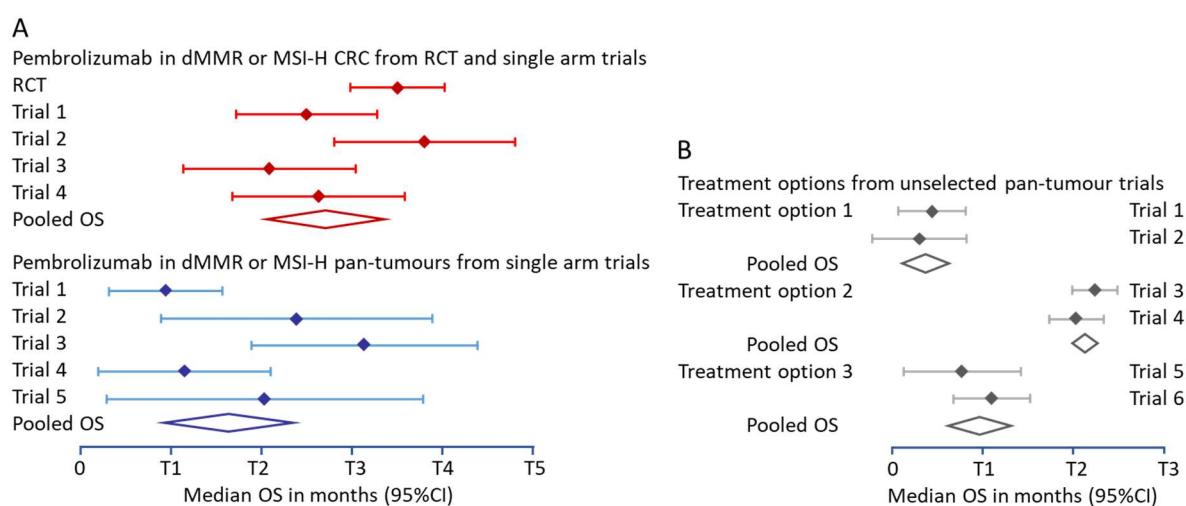


Figure 3 Indirect comparison of the effectiveness of pembrolizumab versus standard of care in dMMR CRC compared with dMMR pan-tumours (A) and standard of care treatment options in biomarker unselected populations

CI = confidence interval; CRC = colorectal cancer; dMMR = mismatch repair deficiency; MSI-H = high microsatellite instability; OS = overall survival; RCT = randomised controlled trial

However, if pembrolizumab is to be offered as last-line therapy rather than second-line therapy, then this unadjusted indirect comparison may be sufficient, as all cancers would be terminal and the comparator would be the same (palliative care) across all pan-tumour patients. The comparison would essentially be adjusted for a common reference (comparator), standardising the incremental benefit across tumour types.

It is also likely that determining the incremental harm of forgoing potentially beneficial treatment in false-positive patients will be equally as difficult. Whereas information on the effectiveness of the various standard of care treatment options is likely to be available in a biomarker-unselected population, there may be no information about the effectiveness of pembrolizumab in biomarker-negative patients.

COMPARATIVE COSTS

Although it is estimated that only 4% of Stage IV CRC patients will have dMMR tumours, the cost of testing in this population will not increase greatly because many patients are already tested for both diagnostic (Lynch syndrome) and prognostic (sensitivity of Stage II CRC to fluorouracil-based chemotherapy) purposes. For patients with other solid tumour types, who are currently not tested, a large number of patients will need to be tested to identify the small proportion of patients with dMMR tumours who would be eligible for pembrolizumab. Thus, the costs of testing will greatly increase for the pan-tumour application.

To determine the total cost of the test per positive result, the number needed to screen (test) to detect one biomarker-positive patient can be calculated. This is defined as the total number of patients tested divided by the number of patients who tested positive. This was estimated for the tumour types listed in Table 4, using the data presented in Table 3. The numbers of patients to be tested were divided by the total number of true positives and false positives eligible for treatment with pembrolizumab (i.e. tested positive). The number needed to screen to detect one dMMR-positive patient ranged from three to 11 for the different tumour types. This appears to be a reasonable ratio, but it is misleading with respect to the potential effectiveness of the treatment received by these patients. The number needed to screen includes patients with false positive results, who may have experienced harm from receiving an incorrect treatment.

In the case of pan-tumour applications with low prevalence rates, the number needed to screen to identify one true positive patient who would benefit from targeted treatment may be a more appropriate measure by which to balance testing and treatment outcomes. As shown in Table 4, this number ranged from three to 37 for all tumour types listed, except breast cancer and lung cancer, which were much higher at 110. Thus, although one out of every 11 patients with breast or lung cancer who were tested would be dMMR-positive and treated with pembrolizumab, only one out of every 110 patients who were tested would truly have the dMMR biomarker and would expect to benefit

from treatment. This may have serious consequences for the cost-effectiveness of the treatment as well as for the test.

It may be possible to minimise the cost of IHC dMMR testing in tumours with low dMMR prevalence rates by implementing a triage plan that takes into account the histopathology (eg. high numbers of tumour infiltrating lymphocytes, triple-negative breast cancer) and the genetic profile of the tumour (eg. BRAF, EGFR, BRCA1/2, ALK, RAS mutational status) and the availability of other targeted and non-targeted treatment options. Although the use of confirmatory MSI-H or MS-MLPA testing of IHC dMMR-positive tumours will increase costs, the benefit to the patients not receiving inappropriate treatment may outweigh this additional cost.

The costs due to potential re-biopsy and any complications arising have not been considered here as dMMR testing in CRC is performed at diagnosis/presentation. However, this would potentially be an additional cost for testing in non-CRC tumours where dMMR testing may not have been performed at diagnosis/presentation.

Table 4 Number needed to screen to detect one patient with a dMMR IHC positive test result for various non-CRC solid tumours

Carcinoma	dMMR prevalence	Number of Stage IV patients (TPs)	Total number of TP:FP eligible for treatment	NNS to detect one test-positive patients	NNS to detect one TP patient
Breast cancer	1%	2,521 (25)	23:200	11	110
Prostate cancer	12%	2,678 (321)	292:188	6	9
Lung cancer	1%	7,495 (75)	68:592	11	110
Endometrial cancer	33%	295 (97)	88:16	3	3
Renal cell carcinoma	4%	835 (33)	30:63	9	28
Thyroid cancer	23%	123 (28)	25:7	4	5
Head and neck cancer	3%	1,007 (30)	27:77	10	37
Pancreatic cancer	4%	2,408 (96)	87:183	9	28
Ovarian cancer	11%	811 (89)	81:58	6	10
Gastric cancer	15%	1,007 (151)	137:68	5	7
Cervical cancer	9%	221 (20)	18:16	7	12
Glioma	33%	1,278 (422)	384:68	3	3
Oesophageal cancer	10%	1,179 (118)	107:85	6	11

Source: Table 3

The calculations are based on the sensitivity and specificity for dMMR IHC testing, compared with MS-MLPA testing of the MLH1 promoter as the reference standard (91% and 92%, respectively).

dMMR = mismatch repair deficient; FP = false positive; NNS = number needed to screen; TP = true positive

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