A review of the current testing methodologies for the detection of mismatch repair deficiency in tumours

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Executive summary

- Microsatellite instability and/or loss of expression of the four mismatch repair proteins are both markers of a deficiency in the mismatch repair pathway.

- Cancers that have deficient mismatch repair pathways are characterised by a high mutation load ('neoantigenic'), with activated tumour infiltrating lymphocytes ('hot' tumours) and therefore, are a target for immunotherapeutic treatment strategies.

- In 2017, US FDA approved two PD-1 inhibitors for the treatment of MSI-high/dMMR colorectal cancer (nivolumab and pembrolizumab) with a pan-tumour approval for pembrolizumab for the treatment of MSI-high/dMMR cancers.

- Initially MSI PCR and IHC tests were developed largely as a prognostic test in colorectal cancer, and as a screening tool for the detection of Lynch syndrome (characterised by a germline mutation in one of five genes involved in the dMMR pathway, and an inherited predisposition to a range of cancers). These tests are complementary and have a high degree of concordance in colorectal cancer, but less so in other Lynch syndrome cancers.

- dMMR by MSI PCR is well-established and validated in colorectal cancer, and to a lesser extent in the other cancer types, including other Lynch syndrome cancers.

- dMMR detection by IHC is well-established and validated in colorectal cancers and endometrial cancers, but more data are required to confirm the sensitivity and specificity in the less common Lynch syndrome cancers and other rare cancer subtypes recently identified as MSI-high.

- Recent studies have identified 2.2% of a large, unselected series of tumours were MSI-high by NGS, with 16% having germline mutations in dMMR genes (Lynch syndrome). These included rarer, non-canonical Lynch syndrome cancers and the familial risks are not yet fully understood.

- Increased awareness of the potential range of dMMR tumours, together with a potential treatment option, has led to a new focus on developing and validating high through-put testing such as NGS to identify MSI-high/dMMR tumours, especially where these occur at lower frequency. NGS is evolving to facilitate rapid, large scale tumour assessments for MSI status, mismatch repair gene mutations, tumour mutation burden to predict responsiveness to immunotherapy, but require validation and determination to establish predictive clinical utility for patient selection for treatment.

- In Australia, IHC is recommended to be performed routinely on all colorectal specimens, and endometrial specimens and other tumour types or clinical situations which might indicate Lynch syndrome. This cheap test is available almost universally in Australian laboratories.

- MSI PCR is less widely available in Australian pathology laboratories.

- NGS detection of MSI is not routinely available in Australian pathology laboratories.

- No NGS panel tests for evaluating microsatellite instability are currently approved by the TGA.

Review report

The following document provides a summary of the key issues underlying the science, evidence and uncertainties regarding the use of microsatellite instability (MSI) testing and immunohistochemical analysis for the detection in cancers of mismatch repair deficiency (dMMR) in the Australian setting. The various test methodologies have been considered using the comparative, objective and subjective clinical utility framework proposed in a paper presented to the MSAC Executive in April 2019.

Background information

Scientific rationale

The DNA mismatch repair (MMR) pathway recognises and repairs mismatches that occur during DNA replication, principally through the activity of four key enzymes coded for by the following genes, *MLH1*, *MSH2*, *MSH6* and *PMS2*. Deficiency of mismatch repair (dMMR) occurs either through mutational inactivation in these four genes (or deletions in the *EPCAM* gene that cause allele-specific MSH2 inactivation) or through epigenetic inactivation, such as promoter methylation of *MLH1* leading to sporadic (i.e. non-germline mutated) MSI tumours. The proportion of sporadic versus germline mutations accounting for dMMR varies by cancer type. Both lead to the accumulation of short sequences of DNA repeated throughout the genome (MSI), and an increased risk of malignant transformation in certain tissues. These tumours have a higher frequency of somatic mutations compared with non-dMMR cancers (Le et al, 2017; Vanderwalde et al, 2018; Smyth et al, 2017), postulated to give rise to large range of tumour neoantigens (high tumour mutation burden), and a highly immunogenic signature, including a high proportion of tumour infiltrating lymphocytes (Le et al, 2017). These tumours are thought to evade the immune system through upregulation of immune inhibitory signals, such as PD-L1 expression. Thus, use of immune checkpoint inhibitors such as pembrolizumab is proposed to restore immune recognition and activity against both colonic and extracolonic tumours that exhibit dMMR, using this as the common signature, rather than tissue of origin.

Mismatch repair deficiency is a common cause of a range of cancers, including colorectal, endometrial, ovarian, cancers of the stomach, small intestine, pancreas, biliary tract and ureter. Recent studies using NGS to detect MSI have identified 2.2% of tumours were MSI-high in a much wider range of cancers than had previously been understood (Latham et al, 2018). This has been confirmed in other NGS-based studies, indicating that this may be a generalised cancer phenotype (Hause et al, 2016; Vanderwalde et al, 2018). The established purposes and clinical utility of dMMR testing, either by immunohistochemistry (absence of MMR proteins) or MSI by polymerase chain reaction (PCR) have been prognostic (and perhaps predictive of a negative outcome with adjuvant 5-FU treatment in colorectal cancer) and as pre-screening for Lynch syndrome, a hereditary cancer predisposition syndrome due to a germline mutation in any of the five genes mentioned above.

The FDA's tumour agnostic approvals in 2017 for the use of immunotherapy for the treatment of mismatch repair-deficient tumours, directed attention towards the clinical validity and utility as a predictive test for patient selection, and establishing diagnostic tests that would facilitate the required large scale tumour screening. There is extensive work underway to establish and validate the use of currently available tests for predictive purposes, but particularly of next generation sequencing for all three purposes, potentially as one-step process, rather than the current sequential testing approach.

Context of applications lodged with TGA and MSAC

- TGA has granted a provisional registration for pembrolizumab for the treatment of adult and paediatric patients with "**unresectable or metastatic microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) tumours that have progressed**

following prior treatment and when there are no satisfactory alternative treatment options".

- MSAC Application 1508 "The proposed medical service is an immunohistochemistry (IHC) test for identification of dMMR for access to pembrolizumab in patients with metastatic or unresectable solid tumours other than colorectal cancers." This has been **restricted by the applicant to use of IHC to demonstrate dMMR** for patient selection.
    - A codependent submission for the service for colorectal cancer was not required given IHC is used in routine clinical practice to identify patients with dMMR for prognostic and predictive information in early stage colorectal cancer, and as initial screening at any disease stage to identify patients who may carry a deleterious germline mutation in a mismatch repair gene (Lynch syndrome). The test is currently reimbursed under an MBS-listing for this service general Medicare Benefits Schedule (MBS) item number for immunohistochemical (IHC) staining (MSAC Executive minutes 1452, 3 March 2017).

MSAC Application 1452 is also relevant: "The proposed medical service is immunohistochemistry (IHC) test for identification of Mismatch Repair Deficiency (dMMR) for access to pembrolizumab in patients with Stage IV CRC who are receiving first-line treatment." This foreshadows submission for registration of pembrolizumab on the basis of the Keynote-177 trial.

Given both MSI-high and IHC testing to establish dMMR are included in the TGA registration of pembrolizumab, these two tests will be considered in this summary, although it is noted that MSAC at this time is not considering an application requesting use of MSI-high testing.

1. What are the tests proposed, and how do the proposed tests used support that? What is currently in use or available in Australia?

The tests

In the pivotal studies provided in support of the use of pembrolizumab for pan-tumour indication, dMMR was locally determined either by MSI by PCR, or absent staining of the mismatch repair proteins by immunohistochemistry (no other details provided).

Therefore, in the absence of a prespecified or restricted methodology, local methodologies for the detection of mismatch repair deficiency could include:

A. immunohistochemistry (IHC) testing to detect absence of expression of at least one of the mismatch repair gene proteins (MLH1, MSH2, MSH6, PMS2)
B. MSI testing – specific methodologies not proposed, but current technologies available include:
    a. polymerase chain reaction (PCR)
    b. next generation sequencing (NGS) – considered likely to replace current multistep process in Australia for diagnosing dMMR and Lynch syndrome (Yozu, 2019).

A. IHC

Best characterised for diagnosis of dMMR in colorectal cancer and endometrial cancer, but clinical validity less certain in other dMMR cancers.

Benefits of IHC testing

- High sensitivity (93%) and nearly perfect specificity in predicting MSI in colorectal cancer as determined by PCR (Lindor, 2002; Shia, 2015), but less certain in other cancer types.
- Cheap.
- Only requires tumour sample (not matched tumour/normal samples as PCR for MSI).
- Identifies the candidate protein/gene most likely to be affected (streamlines further investigation required for approximately 17% who will have Lynch syndrome).
- Available at most centres, technically relatively easy.

- In Australia, should already be routine for colorectal cancer, less established as routine in endometrial cancer diagnostic workup.

Limitations/risks with IHC testing

Analytical issues

- False negative results where protein function impaired but still present (eg some MLH1 promoter methylation cases which may show false-positive nuclear staining for MLH1; pathogenic missense mutations, dominant negative mutations); (56/57 MSI-H tumours demonstrated IHC abnormality (Latham et al, 2018). Higher rates of discordance reported with gastric cancer between MSI testing and IHC (Smyth et al, 2017).
- Variation in tissue fixation and other technical issues, especially with surgical specimens.
- May be subclonal loss (tumour heterogeneity) especially in endometrial cancer (Stelloo, 2017).
- Less reliable on small samples (Zhang et al, 2008).
- Requires sufficient sample to be available (reported as a problem in studies).

Clinical validity and clinical utility issues

- Heterogeneity for dMMR in primary vs metastatic specimen (Jung et al, 2017).
- Difficult to scale up where lower incidence of abnormality (eg pancreatic or ureteric cancers).
- Clinical validity uncertain as prognostic or predictive test in establishing dMMR status other than colorectal, and perhaps endometrial, and therefore clinical utility not established for proposed usage as pan-tumour assay.
- May be less MMR protein expression in tumours with lower proliferation rate, which may affect extra-colonic tumours particularly (Shia, 2015) so identifying dMMR deficiency may be difficult especially where laboratory calibration or cut-offs established using colonic cancers.

B. MSI testing – range of methodologies

a. PCR-based amplification compares the sizes of microsatellite marker sets in tumour DNA with corresponding DNA isolated from a normal tissue sample from the same patient via electrophoresis. Detects the phenotype of MSI without providing further information as to the cause of the MSI (eg germline, methylation etc). A range of markers may be used but core panel recommended to be 5 microsatellite markers consisting of 2 mononucleotide markers and 3 dinucleotide markers (Boland et al, 1998):
MSI-high: ≥2 of core panel, or >30% of markers for other panels showing instability
MSI-low: 1 of core markers, or <30% of markers for other panels showing instability
MSS: 0 markers showing instability.

b. Next generation sequencing (NGS): multiple reports of different panels demonstrating clinical validity of specific methodology and this approach for detection of MSI for Lynch syndrome (e.g. MSIsensor which is a software tool that quantifies MSI in paired tumour-normal genome sequencing data – high concordance with IHC, with 56/57 MSI-H tumours demonstrated IHC dMMR abnormality (Latham et al, 2018). Definition of MSI-high varies by panel used, and the reference standard depends upon the purpose of the test e.g. establishing MSI status, screening patients with Lynch syndrome.

Benefits of PCR method

- Complementary with IHC (IHC may not detect missense mutations as protein may be expressed but not functional).
- High concordance with IHC for MLH1 and MSH2 loss of protein expression in colorectal cancer (Lindor, 2002; Shia, 2015); less certainty regarding correlation with loss of MSH6

and PMS2 protein expression, especially in other dMMR-related cancers (NGS may be better for endometrial cancer MSI detection (Kunitomi et al, 2017)).
- Identifies if tumour associated with dMMR (important for detection of sporadic cancers in Lynch syndrome patients).
- Identifies MSI status regardless of protein function (cf IHC).
- Requires small sample (Zhang et al, 2008).
- High reproducibility (Zhang et al, 2008).

Benefits of NGS method
- Does not require tumour microdissection cf PCR.
- Requires smaller sample cf IHC.
- Potentially faster result.
- Potentially more accurate methodology than MSI PCR for detection of MSI-high status in some cancers (Hause et al, 2016; Kunitomi et al, 2017)?
- Allows large scale testing to be undertaken, especially where looking for dMMR where lower incidence cancers.
- Potentially removes stepwise approach by allowing direct initial inclusion or immediate reflex testing for MSI high/low patients for Lynch syndrome detection - subject to consent and resource considerations.
- Allows integration of results such as MSI status, tumour mutation burden within the same test.

Limitations of MSI PCR
- Not routinely performed in Australian pathology laboratories (Mascarenhas et al, 2015).
- Time-consuming -requires microdissection and molecular analysis.
- Requires normal and tumour tissue – may not be sufficient quantities in biopsy.
- Additional testing required to identify likely candidate gene where Lynch syndrome investigation required (approximately 17%) so IHC required in addition.
- dMMR tumour detection depends on cut-off used: not all tumours with dMMR are necessarily MSI-high (proposed indication) eg *MSH6* (Wu et al, 1999; Hu et al, 2018; Latham et al, 2018) and *PMS2* mutation-positive tumours (Latham et al, 2018; Smyth, 2017). Notably, the tumour mutation burden was reported to be high in the absence of MSI-high status associated with an *MSH6* germline mutation (Hu et al, 2018).
- Heterogeneity for dMMR in primary vs metastatic specimen (Ahn et al, 2000; Hu et al, 2018) which may support rebiopsy and testing of metastatic disease upon relapse.
- Difficult to scale up this standalone test where screening for treatment of cancers with lower incidence of MSI (eg pancreatic or ureteric cancers) or meet high throughput demand for pan-tumour testing.
- Clinical validity uncertain as evidence not provided to support it being a predictive test for response to immunotherapy other than for colorectal, and not considered to be as reliable as IHC in endometrial cancer, and therefore pan-tumour clinical utility not yet established.

Limitations of NGS
- Not established widely in Australia as yet for diagnostic, prognostic purpose for dMMR but would require extensive validation with established methodologies (described in Hause et al, 2016; Latham et al, 2018; Vanderwalde et al, 2018).
- No evidence currently available directly in support of predictive purpose for immunotherapy – evidence will be provided with confirmatory studies as part of FDA approval for nivolumab and pembrolizumab.
- Additional testing required to identify likely candidate gene where Lynch syndrome investigation required (approximately 17%), but potentially could add the five genes to the panel to be tested simultaneously where high risk of Lynch indicated. Ethical and

resource considerations, including counselling and consent issues with additional 'genetic' testing.
- Clinical validity uncertain as predictive test in establishing dMMR status other than colorectal, and perhaps endometrial, and therefore clinical utility not established for proposed usage as pan-tumour assay.

Tests available in Australia

Mascarenhas et al (2016) reported that 95% of laboratories routinely assess dMMR in colorectal and/or endometrial cancer tumours. The majority (77%) used IHC alone, 18% performed both tests and 5% lack in-house ability to screen for dMMR. Since that paper, the NHMRC is reported to have endorsed a universal approach to testing for dMMR in colorectal cancer (Yozu et al, 2019) and it is reasonable to assume that screening rates would increase in these cancers, and it is possible provision of services may widen. Additional Australian reports indicated the likely adoption of NGS-based approaches (Yozu et al, 2019).

IHC detection

ARTG entries for the IHC-based tests to detect dMMR are listed in the Sponsor's application to MSAC, currently as Class II IVDs, but with the proposed usage as a predictive test (and as a screening tool for Lynch syndrome, response to adjuvant chemotherapy), these ought to be Class III IVDs:

- *Type of therapeutic good: Class II in-vitro diagnostic test (GMDN CT1056)*
- *Manufacturer's name: various (see listing in next row)*
- *Sponsor's name: various (In house, Dako, Biospecifix, Roche, Thermo Fisher, MetaGene, Abacus ALS, Becton Dickinson, Beckman Coulter, Life Technologies, Leica, Diagnostic Solutions)*
- *ARTG listing, registration or inclusion number: 279628, 269420, 240833, 239099, 216549, 248292, 224218, 175635, 262536, 183436, 229929, 240833, 224829, 224373, 214553, 212747, 208140, 178442.*

MSI detection

No information is provided about current availability or entries in ARTG in the application to MSAC as this is not being requested for listing for the proposed application. The TGA was requested to provide this information and could not identify any tests on the ARTG. The ARTG cannot be searched by the intended purpose of the test and therefore no inclusions could be identified.

Next generation sequencing

This is reported as being used for this purpose in the Australian medical literature, but the extent and availability of these for the proposed usage is unclear. Likewise, whether these assays are developed in-house and the extent of any validation is unclear.

2. What uncertainties arise from the data provided supporting the tests and proposed usage?

No data are provided in support of the clinical validity and clinical utility of the tests themselves as used in the registration studies of pembrolizumab for the pan-tumour indication currently

sought. The uncertainties are addressed in the following review of tests for determining dMMR in a range of cancers and in the summaries about the tests above.

3. What evidence is currently available to support the clinical performance (including clinical validity and clinical utility) of the tests in the specific tumour types?

Colorectal cancer

12% of colorectal cancers are sporadic MSI:

- majority of these have *MLH1* promoter hypermethylation, 60% have *BRAF V600E* mutation
- somatic biallelic hits
- rate of detection of MSI higher in cancer than adenomas.

3% have Lynch syndrome (germline defect in *MLH1*, *MSH2*, *MSH6*, *PMS2*, *EPCAM*).

The clinical validity and clinical utility of MSI testing, ICH and more recently, NGS has been established for colorectal cancer, for the intended purposes of determining prognosis, possibly a predictive role for early stage adjuvant treatments (colorectal cancer) and as a screening method for detecting Lynch syndrome, (EGAPP, 2009; CADTH Assasi et al, 2016; Latham et al, 2018; Nowak et al, 2017). Hampel et al (2018) confirmed that direct sequencing rather than the traditional 6-step process for identifying Lynch syndrome was superior to existing methodologies: "Tumor sequencing alone had better sensitivity (100%; 95% CI, 93.8%-100%) than IHC plus BRAF (89.7%; 95% CI, 78.8%-96.1%; P = .04) and MSI plus BRAF (91.4%; 95% CI, 81.0%-97.1%; P = .07). Tumor sequencing had equal specificity (95.3%; 95% CI, 92.6%-97.2%) to IHC plus BRAF (94.6%; 95% CI, 91.9%-96.6%; P > .99) and MSI plus BRAF (94.8%; 95% CI, 92.2%-96.8%; P = .88)." In addition, a high proportion of positive results were obtained for other predictive biomarkers in the panel, including *KRAS*, *NRAS*, *BRAF* mutations as well as *DPYD* germline mutations, indicating an increased risk of toxicity with 5-FU.

Heterogeneity for dMMR determined by IHC, between the primary and metastatic specimens from the same patient, has been reported (Jung et al, 2017), raising uncertainty about the reliance on testing of the initial surgical resection specimen, particularly if morbidity and mortality are due to metastatic disease progression.

Moreira et al (2012) reported concordance of 97.5% between MSI and immunostaining performed on 5591 tumours from four large international cohorts of colorectal cancer patients. Ninety-four (94) cases [1.7%] showed MSI with retained protein expression and 49 [0.8%] exhibited loss of expression with microsatellite stability.

Buchanan et al (2017) reported similarly high levels of concordance in colorectal cancer tumours CRCs where MSI and MMR IHC testing were completed in 67.4% and 93.3% of participants in two Australian colorectal cancer study populations: these demonstrated 95.7% and 98.9% concordance for MMR-deficiency, respectively.

IHC is preferred in Australia due to cost, availability and identification of candidate protein for targeted assessment for Lynch syndrome in colorectal cancer screening recommendations (Yozu et al, 2019). However, these authors acknowledge the likely shift to NGS testing in the future.

Endometrial cancer

20-30% of endometrial cancers exhibit MSI/IHC loss of expression:

- mostly sporadic due to *MLH1* promoter methylation (Hampel, 2006; Stelloo, 2017)
- no correlation between *BRAF* mutations and *MLH1* promoter methylation, unlike colorectal cancer (Moreira et al, 2012)
- approximately 2-5% may be due to Lynch syndrome (Meyer, 2013).

There is no consensus on dMMR for prognostic utility (Powell, 2017).

Stelloo et al (2017) reported concordance rates of 94% between IHC and MSI PCR, with most instances of discordances being due to low MSI but absent MSH6 or PMS2 protein (also reported elsewhere for these 2 proteins). This suggests that IHC is the appropriate standard for establishing mismatch repair in endometrial cancer, given the frequency of *MSH6* and *PMS2* mutations as a cause of dMMR endometrial cancer and Lynch syndrome (Powell, 2017).

New methodologies for NGS detection of MSI may be more accurate in identifying MSI-high status than the traditional panels (Hause et al, 2016; Kunitomi et al, 2017).

No evidence is available directly in support of predictive purpose for immunotherapy.

Ovarian cancer

13% of ovarian cancers are MSI-high:

- defects in the MMR pathway an estimated 10%–12% of unselected ovarian cancers (Pal et al, 2008; Murphy and Wentzensen, 2011); 13% of ovarian cancer samples were MSI-high (Akbari et al, 2017)
- higher rates of non-serous and endometrioid subtypes among patients with Lynch syndrome suggest targeted screening of these tumour subtypes (Pal et al, 2012). Higher prevalence of *MSH6* germline mutations, which were also more likely to be MSI-indeterminate in the pan-tumour NGS screening using MSIsensor (Latham et al, 2018).

Lee et al (2014) reported 67.6% concordance between MSI-high and loss of MMR protein expression; 41 were classified as MSI-H with loss of expression (LoE) and 523 as microsatellite stable (MSS) with no loss of expression. Of the 270 discordant cases, 83 were MSI-H with no LoE and 187 were MSS with LoE. Both IHC staining method and reading pathologist were strongly associated with discordant results.

On the basis of the poor concordance, these authors consider the clinical validity and clinical utility of IHC as a method for identifying dMMR is uncertain, requiring further investigation to test different IHC methods and ensure inter-rater reliability in scoring methods.

Gastric cancer

Figures range from 8.5% (Smyth, 2017) to 22% of gastric cancers reported to be MSI-high (Ratti et al, 2018). Hypermutation was associated with MSI-high tumours (Ratti et al, 2018), MSI-high or MMRD appears to confer a better prognosis with surgery alone, but worse outcome with chemotherapy (Smyth et al, 2017). No gastro-oesophageal junction or oesophageal tumours were MSI-high (Smyth et al, 2017), but the numbers tested were relatively low (20).

While the overall concordance between IHC and MSI detection rates for dMMR was 97.6%, the concordance differed depending upon which dMMR protein was missing. In particular, 25% of tumours with absent PMS2 were declared MSI-stable or low, while tumours with absent MSH6 staining were reported as MSI-high (which contrasts with endometrial cancer where MSH6 absence is often not MSI-intermediate or stable) (Smyth, 2017). Loss of MMR protein expression was most commonly reported for PMS2 (6.2%) followed by MLH1 (5.1%), MSH2 (1.1%) and MSH6 (0.7%). No figures on the comparative sensitivity and specificity between the methods were reported.

Elsewhere, comparison of the results of immunohistochemical expression of the mismatch repair proteins MLH1 and MSH2 with microsatellite analysis showed concordant results in 95% of neoplasms, with a sensitivity of 82% and specificity of 98% (Beghelli et al, 2006). Given the propensity for PMS2 loss of expression to result in low MSI, this may not accurately capture the concordance when all dMMR proteins are included.

No evidence is provided for either test type of their clinical validity and clinical utility for treatment predictive purposes.

Small intestine carcinomas

Planck et al (2003) reported MSI was detected in 16/89 (18%) adenocarcinomas of the small intestine (12 MSI-high, 4 MSI-low based on 10-marker panel), and immunohistochemistry revealed loss of expression for MLH1 in 7/16 MSI tumours and in 2/73 MSS tumours, whereas all tumours showed normal expression for MSH2. Among the young patients, the authors identified MSI in 10/43 tumours (23%), and 6 of these 10 MSI tumours showed immunohistochemical loss of MMR protein expression (MLH1 in 3 cases and MSH2 in 3 cases).

IHC was only undertaken for MLH1 and MSH2 protein expression in these studies, but nonetheless, is suggestive of a low rate of concordance between the two tests of MMR and indicates that this needs further investigation to determine the clinical validity of the two tests in this cancer type and as a result, clinical utility is uncertain.

No evidence is provided for either test type of their clinical validity and clinical utility for treatment predictive purposes.

Pancreatic carcinoma

Eatrides et al (2017) reported that 24/109 (22%) pancreatic biopsies were MSI-H with a deficit of MLH1, PMS2, MSH2 or MSH6, based on tissue microarray. Ahn et al (2000) examined 13 pancreatic cancer specimens, two of which were MSI-high but only in the metastases of both specimens and not the primary tumours.

Hu et al (2018) reported low rates of dMMR in pancreatic adenocarcinoma (7/833; 0.8%) with strong correlation between IHC loss of protein expression and MSIsensor scores – all were found to have Lynch syndrome. MSI-high status also correlated strongly with a high tumour mutational burden.

Laghi et al (2012) also reported low rates of MSI-high in pancreatic cancer (0.3%; 1/338 cases) which indicates that sporadic dMMR is not a common cause of this type of cancer.

Bladder/urothelial cancer

Latham (2018) identified MSI – high or low status in 30% of samples analysed by NGS, with 37.5% found to have Lynch syndrome, and as expected, most were due to *MSH2* mutations. These high figures point to a potentially broad clinical utility of testing such tumours, and may support the selection of patients better than the current use of PD-L1. In a population selected to be potentially enriched for dMMR, MSI-high status only detected in 1/109 patients with bladder cancer <40 years of age, and did not correlate with IHC loss of expression (Giedl et al, 2014).

4. What steps are underway to address these uncertainties and what are the anticipated timeframes for delivery?

Most of the information to date regarding dMMR is for prognostic or hereditary predisposition and there is limited information regarding the optimal test for identifying dMMR in the rarer tumour types recognised as part of Lynch syndrome, as well as those newly found to be associated with mismatch repair deficiency (Latham, 2018). This is an area of intense clinical interest and

investigation, given the predictive role of such testing and the rapid development of new treatment option for such patients.

Regulatory postmarketing requirements

The FDA approved the use of two PD-1 inhibitors for tumours that are mismatch repair deficient with the following postmarketing commitments, as stipulated in the FDA approval letter of 23 May 2017 for pembrolizumab:
(https://www.accessdata.fda.gov/drugsatfda_docs/appletter/2017/125514Orig1s014ltr.pdf accessed 28 April 2019), with final reports for both commitments due in June 2019.

> *"Commitment to support the availability through an appropriate analytical and clinical validation study using clinical trial data that will support labeling of an immunohistochemistry based in vitro diagnostic device that is essential to the safe and effective use of pembrolizumab for patients with tumors that are mismatch repair deficient.*

The timetable you submitted on May 18, 2017, states that you will support the submission of a Premarket Approval (PMA) Application to FDA/CDRH according to the following schedule:
Final Report Submission: June 2019"

> *"Commitment to support the availability through an appropriate analytical and clinical validation study using clinical trial data that will support labeling of a nucleic acid-based in vitro diagnostic device that is essential to the safe and effective use of pembrolizumab for patients with tumors that are microsatellite instability high.*

The timetable you submitted on May 18, 2017, states that you will support the submission of a Premarket Approval (PMA) Application to FDA/CDRH according to the following schedule:
Final Report Submission: June 2019"

The same commitments were required in support of the accelerated approval for nivolumab granted on 21 July 2017, but with a final report date of September 2021[1].

On 3 January 2019, PMDA (Japan) approved pembrolizumab "As treatment for patients with advanced/recurrent MSI-H solid tumors that have progressed following chemotherapy, if refractory or intolerant to standard therapies." The Japan PMDA also approved the MSI-high FALCO kit as a companion diagnostic for MSI-H, which is a PCR assay developed by Promega.

[1] https://www.accessdata.fda.gov/drugsatfda_docs/appletter/2017/125554Orig1s034ltr.pdf accessed 20 June 2019

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