

SPECIAL ARTICLE

JSCO—ESMO—ASCO—JSMO—TOS: international expert consensus recommendations for tumour-agnostic treatments in patients with solid tumours with microsatellite instability or *NTRK* fusions

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A Japan Society of Clinical Oncology (JSCO)-hosted expert meeting was held in Japan on 27 October 2019, which comprised experts from the JSCO, the Japanese Society of Medical Oncology (JSMO), the European Society for Medical Oncology (ESMO), the American Society of Clinical Oncology (ASCO), and the Taiwan Oncology Society (TOS). The purpose of the meeting was to focus on what we have learnt from both microsatellite instability (MSI)/deficient mismatch repair (dMMR) biomarkers in predicting the efficacy of anti-programmed death-1 (PD-1)/programmed death ligand-1 (PD-L1) immunotherapy, and the neurotrophic tyrosine receptor kinase (*NTRK*) gene fusions in predicting the efficacy of inhibitors of the tropomyosin receptor kinase (TRK) proteins across a range of solid tumour types. The recent regulatory approvals of the anti-PD-1 antibody pembrolizumab and the TRK inhibitors larotrectinib and entrectinib, based on specific tumour biomarkers rather than specific tumour type, have heralded a paradigm shift in cancer treatment approaches. The purpose of the meeting was to develop international expert consensus recommendations on the use of such tumour-agnostic treatments in patients with solid tumours. The aim was to generate a reference document for clinical practice, for pharmaceutical companies in the design of clinical trials, for ethics committees in the approval of clinical trial protocols and for regulatory authorities in relation to drug approvals, with a particular emphasis on diagnostic testing and patient selection.

Key words: microsatellite instability, mismatch repair, *NTRK*, tumour-agnostic, recommendations

INTRODUCTION

The last 2 years have seen a paradigm shift in the regulatory approval of cancer treatments with the approval of the first two agents, pembrolizumab and larotrectinib, for the treatment of solid tumours based on the presence of

specific biomarkers rather than on tumour site, and thus establishing the precedent of tumour-agnostic therapies.

The first of these agents, pembrolizumab, is a well-known anti-programmed death-1 (PD-1) T-cell receptor antibody.^{1–3} In 2015, a small investigator-initiated study (KEYNOTE-016) showed colorectal cancer (CRC) patients with deficient mismatch repair (dMMR) treated with pembrolizumab to achieve immune-related objective response (ORR) and progression-free survival (PFS) rates at 20 weeks of 40% and 78%, respectively.⁴ In May 2017, the US Food and Drug Administration (FDA) approved pembrolizumab for the treatment of adult and paediatric patients with unresectable or metastatic, microsatellite instability-high (MSI-H) or dMMR

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solid tumours, based on data from 149 patients from five single-arm studies.⁵ Thus, pembrolizumab became the first drug to receive a tumour-agnostic approval.⁶ In December 2018, the Japan Pharmaceuticals and Medical Devices Agency (PMDA) approved pembrolizumab for the treatment of adult patients with advanced MSI-H tumours.⁷

These approvals in turn are supported by the results of an expanded proof-of-concept study which showed MSI/dMMR predicts response to PD-1 blockade across a range of solid tumour types⁸ and by a review of immune checkpoint blockade therapies in patients with MSI/dMMR tumours.⁹ Additionally, another monoclonal antibody that targets the PD-1 receptor, nivolumab, had previously been approved by the FDA for the treatment of adults and children with MSI or dMMR metastatic CRC that had progressed following treatment with fluoropyrimidine, oxaliplatin, and irinotecan, as a single agent and subsequently in combination with ipilimumab.^{10,11}

In November 2018, larotrectinib, a tyrosine kinase inhibitor of the tropomyosin receptor kinase (TRK) proteins, TRKA, TRKB, and TRKC, encoded for by the neurotrophic tyrosine receptor kinase genes *NTRK1*, *NTRK2* and *NTRK3* respectively, became the second drug to receive tumour-agnostic FDA approval for the treatment of adult and paediatric patients with solid tumours with *NTRK* gene fusions.^{12,13} In 2019, larotrectinib became the first tumour-agnostic cancer treatment to be approved in the European Union.

Following on from these first approvals, in 2019, Japan and subsequently the FDA approved entrectinib, a selective tyrosine kinase inhibitor that targets TRKA, TRKB and TRKC, and the ROS1 and ALK proteins¹⁴ for patients with *NTRK* fusion-positive advanced, recurrent solid tumours.¹⁵ These tumour-agnostic agent approvals pose several clinical questions regarding not only MSI/MMR/*NTRK* testing but also the sequence of administration of these agents in the treatment pathways of patients with MSI/dMMR or *NTRK* fusion-positive solid tumours. Also, going forward, should all cancer patients be tested, and if so, when, and using which test(s)?

The European Society for Medical Oncology (ESMO) recommendations on MSI testing for immunotherapy, and for the detection of patients with tumours with *NTRK* fusions, were published in May 2019¹⁶ and July 2019,¹⁷ respectively. Also, the Japan Society of Clinical Oncology (JSCO) published 'provisional clinical opinion' guidelines for the diagnosis and use of immunotherapy in patients with dMMR tumours, in July 2019.¹⁸ In order to respond to the potential changes in clinical practice envisaged following the tumour-agnostic agent approvals described above, and those anticipated for other agents in the future, the JSCO convened a face-to-face meeting in Japan, in October 2019, of international experts in the field of oncology representing the oncology societies of Europe (ESMO), the United States (ASCO), and two additional Asian societies namely, the Japanese Society of Medical Oncology (JSMO) and the Taiwan Oncology Society (TOS). The ultimate aim of the meeting was to develop the present international expert

consensus recommendations on tumour-agnostic therapies based on the results of expert voting on a series of pre-formulated recommendations focussing on patients with advanced (unresectable or metastatic) MSI/dMMR and *NTRK* fusion-positive solid tumours, as outlined below.

Aim

The aim of the meeting was to generate a document that could provide guidance for the use and management of the currently approved tumour-agnostic therapies in patients with solid tumours, and to aid clinical trial design for both these agents and those currently under development, going forward.

Scope

The meeting focused exclusively on the tumour-agnostic therapies associated with MSI/dMMR and *NTRK* fusions.

METHODOLOGY

Composition of the expert panel and aims

This manuscript represents the opinion of 19 experts in oncology, representing JSCO and JSMO, ESMO, ASCO and TOS, who took part in a survey of clinical questions (CQs) devised to test our thinking on the management of patients with MSI/dMMR and *NTRK* fusion-positive tumours in the era of tumour-agnostic drug approvals.

Clinical questions and proposed recommendations

In preparation for the meeting, six identical CQs relating to the MSI/dMMR and *NTRK* precision agnostic therapy approaches were formulated by Drs T. Yoshino, S. Mishima, Y. Naito, H. Taniguchi and J.-Y. Douillard and approved by all the experts (Table 1). The evidence to support the two sets of recommendations proposed in response to these CQs was provided by searching the PubMed and Cochrane databases using the search terms listed in [supplementary](#)

Table 1. The six identical clinical questions (CQs) formulated for the treatment and management of patients with MSI/dMMR or *NTRK* fusion-positive tumours from which two separate series of recommendations were developed, i.e. one series of clinical recommendations for each clinical situation

CQ no.	CQs
CQ1	Should all patients with solid tumours be tested for MSI/MMR or <i>NTRK</i> fusions?
CQ2	When is the optimal timing for tests for MSI/MMR or for <i>NTRK</i> fusions?
CQ3	Which tests are recommended for determining MSI/MMR status or <i>NTRK</i> fusions?
CQ4	What is the appropriate biospecimen for testing for MSI/MMR or <i>NTRK</i> fusions?
CQ5	Which treatment is recommended for MSI/dMMR patients or patients with <i>NTRK</i> fusions?
CQ6	Where in the treatment algorithm should immunotherapy be used in the treatment of patients with MSI/dMMR solid tumours or a TRK inhibitor be used in the treatment of patients with <i>NTRK</i> fusion-positive solid tumours?

dMMR, deficient in (DNA) mismatch repair; MSI, microsatellite instability; *NTRK*, neurotrophic tyrosine receptor kinase; TRK, tropomyosin receptor kinase.

Tables S1 and S2 (available at *Annals of Oncology* online) for MSI/MMR and *NTRK*, respectively. The details of the number of records identified in response to each clinical question during the systematic review and the number of records finally used in the synthesis of the recommendations are presented in supplementary Tables S3 and S4 (available at *Annals of Oncology* online) for MSI/MMR and *NTRK*, respectively. The two sets of proposed recommendations made in response to each CQ, relating to MSI/dMMR and *NTRK* fusion-positive tumours together with the proposed levels of evidence (LoE) and grades of recommendation (GoR), based on an adapted version of the 'Infectious Diseases Society of America-United States Public Health Service Grading System' (supplementary Table S5, available at *Annals of Oncology* online),¹⁹ were then circulated to all 19 experts to gather their acceptance or otherwise of the recommendations made (see supplementary Tables S6 and S7, available at *Annals of Oncology* online). The responses of the experts had to represent science-based opinion assuming that all drugs, diagnostic and testing modalities were available to them.

Final consensus statements

Where there was full agreement between all voting parties for the recommendations made in response to each CQ no further discussion was required. Where there was an absence of full agreement, however, a modified Delphi process was used during the final voting process at the face-to-face working meeting to develop each of the disputed recommendations towards a consensus. The experts present were asked to vote on their level of agreement (LoA) for a particular recommendation based on the evidence available, on a scale of A to E, where A = accept completely, B = accept with some reservation, C = accept with major reservation, D = reject with some reservation and E = reject completely (supplementary Table S5, available at *Annals of Oncology* online).¹⁹ A consensus was considered to have been achieved when $\geq 80\%$ of experts voted to accept completely (A) or accept with some reservation (B), a specific recommendation made in response to a particular CQ. A recommendation was considered to have been rejected when $>80\%$ of the voting members indicated 'reject completely' (E) or 'reject with some reservation' (D).

RESULTS AND MEETING OUTCOMES

In the initial pre-meeting surveys, the 19 experts reported on the applicability of the 10 recommendations developed in response to the six CQs (Table 1) in relation to MSI/dMMR tumours (supplementary Table S8, available at *Annals of Oncology* online) and on the applicability of the 13 recommendations developed in response to the same six CQs for the treatment of patients with tumours with *NTRK* gene fusions (supplementary Table S9, available at *Annals of Oncology* online).

Of the 23 recommendations developed in response to the six CQs across both biomarker categories, 13 were fully agreed upon during the pre-meeting surveys. An

unqualified response of YES in the pre-meeting survey equated with 'accept completely' in the final voting, giving an LoA of A = 100%. The remaining 10 draft recommendations, four for MSI/MMR (supplementary Table S8, available at *Annals of Oncology* online), and six for *NTRK* (supplementary Table S9, available at *Annals of Oncology* online), were discussed and voted upon at the face-to-face meeting. Each of the four groups/organisations represented at the face-to-face meeting (i.e. JSCO/JSMO, ESMO, ASCO, TOS) had the right to one vote each per recommendation. Where changes to the text of the original recommendations were made, these are indicated in bold both in the main text of the manuscript and in the two summary tables of the final consensus recommendations, Tables 2 and 3. In addition, the final voting patterns, in terms of GoR, LoE and LoA, were recorded for each recommendation.

Table 2. Summary of the expert recommendations for the treatment of patients with MSI/dMMR solid tumours

CQ1. Should all patients with solid tumours be tested for MSI/MMR?	
1-1	Patients with advanced (unresectable or metastatic) solid tumours with a high incidence of MSI/dMMR should be tested for their MSI/MMR status. [LoE: III, GoR for testing: A, LoA: A = 100%]
1-2	Patients with advanced (unresectable or metastatic) solid tumours with a low incidence of MSI/dMMR should be considered for MSI/MMR testing. [LoE: III, GoR for testing: B, LoA: A = 100%]
1-3	Patients with localised resectable non-colorectal tumours should not be considered for MSI/MMR testing outside of a clinical trial, unless Lynch syndrome is clinically suspected . [LoE: V, GoR for testing: D, LoA: A = 100%]
CQ2. When is the optimal timing for tests for MSI/MMR?	
	MSI/MMR should be tested before or during the standard treatment of advanced (unresectable or metastatic) solid tumours. [LoE: V, GoR: A, LoA: A = 100%]
CQ3. Which tests are recommended for determining MSI/MMR status?	
3-1	IHC is highly recommended for testing. [LoE: III, GoR for testing: A, LoA: A = 100%]
3-2	PCR is recommended for testing either upfront or when IHC is equivocal or not available . [LoE: III, GoR for testing: B, LoA: A = 75%, B = 25%]
3-3	Validated NGS is recommended for testing either upfront or when IHC is equivocal or not available . [LoE: III, GoR for testing: B, LoA: A = 75%, B = 25%]
CQ4. What is the appropriate biospecimen for testing for MSI/MMR?	
	Formalin-fixed, paraffin-embedded tissue blocks are appropriate for testing. [LoE: V, GoR: A, LoA: A = 100%]
CQ5. Which treatment is recommended for MSI/dMMR patients?	
	PD-1/PD-L1 inhibitors are strongly recommended for patients with MSI/dMMR tumours. [LoE: III, GoR: A, LoA: A = 100%]
CQ6. Where in the treatment algorithm should immunotherapy be used in MSI/dMMR solid tumours?	
	We recommend immunotherapy for patients with MSI/dMMR during the course of their therapy when no other satisfactory treatment options exist depending on the clinical context. [LoE: III, GoR: A, LoA: A = 100%]

dMMR, deficient in (DNA) mismatch repair; GoR, grade of recommendation; IHC, immunohistochemistry; LoA, level of agreement; LoE, level of evidence; MSI, microsatellite instability; MMR, mismatch repair; NGS, next generation sequencing; PCR, polymerase chain reaction; PD-1 programmed (cell) death protein-1; PD-L1, programmed death ligand-1.

Table 3. Summary of the expert recommendations for the treatment of patients with solid tumours with *NTRK* fusions

CQ1. Should all patients with solid tumours be tested for <i>NTRK</i> fusion?	
1-1	Patients with advanced (unresectable or metastatic) solid tumours without actionable and driver gene mutations/fusions/amplifications should be tested for <i>NTRK</i> fusion. [LoE: V, GoR: B, LoA: A = 100%]
1-2	Patients with advanced (unresectable or metastatic) solid tumours which are highly likely to harbour <i>NTRK</i> fusions should be tested for <i>NTRK</i> fusion, especially <i>ETV6-NTRK3</i> fusion. [LoE: V, GoR: A, LoA: A = 100%]
1-3	Patients with advanced (unresectable or metastatic) solid tumours other than above (CQ1-1 and 1-2) should be considered for testing for <i>NTRK</i> fusions. [LoE: V, GoR: A, LoA: A = 100%]
1-4	Patients with locally-advanced tumours with a high incidence of <i>NTRK</i> fusions should be tested when considering neoadjuvant therapy before resection. [LoE: V, GoR: B, LoA: A = 100%]
CQ2. When is the optimal timing for tests for <i>NTRK</i> fusion?	
<i>NTRK</i> fusion testing should be considered before or during the standard treatment of advanced (unresectable or metastatic) solid tumour. [LoE: V, GoR: B, LoA: A = 100%]	
CQ3. Which tests are recommended for determining <i>NTRK</i> fusions?	
3-1	IHC is not recommended for confirming <i>NTRK</i> fusion. It may be used for screening to enrich patients with <i>NTRK</i> fusion. [LoE: V, GoR: B, LoA: A = 100%]
3-2	<i>In situ</i> hybridisation (ISH, e.g. FISH) for <i>ETV6-NTRK3</i> fusion is recommended for patients with tumours which are highly likely to harbour <i>NTRK</i> fusions. ISH is not recommended for patients other than the above. [LoE: V, GoR: B, LoA: A = 100%]
3-3	RT-PCR for <i>ETV6-NTRK3</i> fusion is recommended for patients with tumours which are highly likely to harbour <i>NTRK</i> fusions. [LoE: V, GoR: B, LoA: A = 100%]
3-4	NGS which detects <i>NTRK</i> fusion is recommended for testing for <i>NTRK</i> fusion. [LoE: V, GoR: C, LoA: A = 100%]
CQ4. What is the appropriate biospecimen for testing for <i>NTRK</i> fusions?	
Both fresh samples as well as archival tissue samples properly fixed and preserved are appropriate for testing. [LoE: V, GoR: B, LoA: A = 100%]	
CQ5. Which treatment is recommended for patients with <i>NTRK</i> fusions?	
TRK inhibitors are strongly recommended for patients with <i>NTRK</i> fusions. [LoE: III, GoR: A, LoA: A = 100%]	
CQ6. Where in the treatment algorithm should a TRK inhibitor be used in the treatment of patients with <i>NTRK</i> fusion-positive solid tumours?	
We recommend TRK inhibitors for patients with <i>NTRK</i> fusions during the course of therapy, when no other satisfactory treatment options exist, depending on the clinical context. [LoE: III, GoR: A, LoA: A = 100%]	

GoR, grade of recommendation; IHC, immunohistochemistry; ISH, *in situ* hybridisation; LoA, level of agreement; LoE, level of evidence; NGS, next generation sequencing; *NTRK*, neurotrophic tyrosine receptor kinase; PCR, polymerase chain reaction; RT-PCR, reverse transcriptase PCR; TRK, tropomyosin receptor kinase.

Background to development of MSI/dMMR status as a predictive biomarker

Cancers deficient in MMR (dMMR) are associated with short tandem-repeat sequences (microsatellites) and are characterised by exceptionally high numbers of somatic mutations due to errors in DNA MMR. Such cancers are classified as exhibiting MSI, which is the phenotype of dMMR. Tumour dMMR status is the consequence of mutations in the *MLH1*, *MSH2*, *MSH6*, *PMS2*, or *EPCAM* genes.

Historically, tumour MSI/MMR status has been used to guide prognosis for patients with stage II CRC and to potentially predict the efficacy of chemotherapy in patients with CRC.²⁰ MSI/dMMR is also found to varying degrees in other tumour types.^{21–23} This together with the recent evidence that MMR deficiency is predictive of response to immune checkpoint inhibitors^{8,24} and the agnostic approval of pembrolizumab, based on tumour MSI/MMR status, points to MSI/MMR status becoming increasingly important in the management of cancer patients in the era of precision therapy. It therefore seems prescient to determine in which patients MSI/MMR testing is appropriate, and when and which tests for MSI/MMR tumour status should be carried out.

Recommendations in response to the CQs for MSI/MMR

Six of the 10 draft recommendations made in response to the six CQs in relation to MSI/MMR (Table 1) were accepted completely in the pre-meeting survey, i.e. LoA A = 100% (supplementary Table S8, available at *Annals of Oncology* online). Thus, theoretically four recommendations (CQs1-1 and 1-3, CQ3-2 and CQ6) had to be discussed at the face-to-face meeting. In reality, some of the other recommendations were revised. All 10 recommendations are discussed in the text below and changes made to the original recommendations (supplementary Table S6, available at *Annals of Oncology* online) indicated in **bold** text.

CQ1: Should all patients with solid tumours be tested for MSI/MMR?

*Recommendation CQ1-1. Patients with **advanced (unresectable or metastatic) solid tumours with a high incidence of MSI/dMMR should be tested for their MSI/MMR status.***

[LoE: III, GoR for testing: A, LoA: A = 100%]

*Recommendation CQ1-2. Patients with **advanced (unresectable or metastatic) solid tumours with a low incidence of MSI/dMMR should be considered for MSI/MMR testing.***

[LoE: III, GoR for testing: B, LoA: A = 100%]

*Recommendation CQ1-3. Patients with **localised resectable non-colorectal tumours should not be considered for MSI/MMR testing outside of a clinical trial unless Lynch syndrome is clinically suspected.***

[LoE: V, GoR for testing: D, LoA: A = 100%]

All the experts agreed with and accepted completely 'recommendation CQ1-2' above in the pre-meeting survey (supplementary Table S8, available at *Annals of Oncology* online). However, the experts thought the tumours highly likely to harbour MSI/dMMR in 'recommendation CQ1-1' should be better defined, together with the definition of early disease as it applied to 'recommendation CQ1-3', as early disease is not included in the label.

A pooled-data analysis of four large population-based cohorts of CRC patients has shown universal screening of CRC patients using tumour MMR testing to be more sensitive than clinical criteria²⁵ in diagnosing Lynch syndrome. Thus, patients with tumours which may be MSI/dMMR, and

for whom MSI/MMR testing is generally recommended, should include patients clinically suspected of having Lynch syndrome and elderly female CRC patients with tumours with a mucinous component or with a *BRAF p.V600E* mutation.²⁶ A summary of tumours highly likely to harbour MSI/dMMR is provided in [supplementary Table S10](#) (available at *Annals of Oncology* online), based on data from a study of 15 045 patients with >50 different cancer types (NCT01775072).²² The wording of ‘*recommendation CQ1-1*’ was revised to specify ‘**advanced (unresectable or metastatic) solid tumours with a high incidence of MSI/dMMR**’ and the GoR revised to an A. All the experts agreed with and accepted completely [A = 100%] the revised recommendation.

Also, although all the experts agreed with and accepted completely ‘*recommendation CQ1-2*’ above during the pre-meeting survey, at the face-to-face meeting there was considerable discussion about the cost/economic issues of testing patients with solid tumours associated with a low incidence of MSI/dMMR. However, because the efficacy of PD-1/PD-L1 inhibitors has been clearly and consistently demonstrated in advanced solid tumours with MSI/dMMR,^{8,10,11,27} the expert opinion was that MSI/MMR testing should be **considered** to determine eligibility for treatment with PD-1/PD-L1 inhibitors for all patients with advanced solid tumours (‘*recommendation CQ1-2*’). Clearly, in principle, it is not necessary to perform MSI/MMR testing for solid tumours for which PD-1/PD-L1 inhibitors can be used in the second- or later-line treatment settings irrespective of MMR functionality. MSI/MMR testing may be considered if it provides predictive value for PD-1/PD-L1 inhibitors and may prompt their use earlier in the treatment path minimising the percentage of patients who will miss out on immunotherapy as a result of rapid clinical deterioration. Thus, the ‘*recommendation CQ1-2*’ that ‘*patients with advanced solid tumours should be tested for MSI/MMR*’ was revised to read ‘**Patients with advanced (unresectable or metastatic) solid tumours with a low incidence of MSI/dMMR should be considered for MSI/MMR testing**’, making it optional depending on treatment location and cost concerns. The GoR for testing was revised to **B**, and the experts present agreed with and accepted completely [A = 100%] the revised recommendation. In the case of ‘*recommendation CQ1-3*’ where the recommendation was that patients with early-stage disease should not be tested outside of a clinical trial setting, the experts expressed concern over the definition of early disease, and thought that general testing needed to be separated from testing in situations where Lynch syndrome was suspected, and the text was revised accordingly (see above and [Table 2](#)). The use of immune checkpoint inhibitors in MSI/dMMR early-stage colon cancer is presently being evaluated in clinical trials. Furthermore, it is known that MSI/dMMR status is a favourable prognostic factor for CRC, particularly for stage II CRC^{20,28,29} in which MSI/dMMR status has negative implications in terms of benefit from 5-fluorouracil (5-FU) adjuvant chemotherapy.^{20,29} As a consequence, it is considered desirable to perform MSI/MMR testing to assess

the requirement for adjuvant chemotherapy in patients with early-stage (stage II) CRC, although not in the early stages of any other tumour type. After the revisions highlighted in bold text above, all the experts agreed with and accepted completely ‘*recommendation CQ1-3*’ [A = 100%]. The GoR for testing was revised to D.

CQ2. When is the optimal timing for tests for MSI/MMR?

Recommendation CQ2. MSI/MMR status should be tested before or during the standard treatment of advanced (unresectable or metastatic) solid tumours.

[LoE: V, GoR: A, LoA: A = 100%]

Since the turnaround time for MSI/MMR testing is 1–2 weeks, MSI/MMR testing should be carried out early to determine a patient’s eligibility for treatment with PD-1/PD-L1 inhibitors. Additionally, in the case of solid tumours for which the applicability of PD-1/PD-L1 inhibitors is judged appropriate based on a biomarker other than MSI/MMR status, such as PD-L1 expression, and that biomarker is negative, MSI/MMR testing is recommended, because these drugs are expected to be effective if the tumour is MSI/dMMR.¹⁸ The general feeling of the experts was that the ideal scenario would be to test at the time of diagnosis and tissue availability, when there may be only one chance at biopsy. All the experts agreed with and accepted completely ‘*recommendation CQ2*’ [A=100%].

CQ3. Which tests are recommended for determining MSI/MMR status?

Recommendation CQ3-1. IHC is highly recommended for testing.

[LoE: III, GoR for testing: A, LoA: A = 100%]

Recommendation CQ3-2. PCR is recommended for testing either upfront or when IHC is equivocal or not available.

[LoE: III, GoR for testing: B, LoA: A = 75%, B = 25%]

Recommendation CQ3-3. Validated NGS is recommended for testing either upfront or when IHC is equivocal or not available.

[LoE: III, GoR for testing: B, LoA: A = 75%, B = 25%]

All the experts agreed with and accepted completely ‘*recommendations CQ3-1*’ and ‘*CQ3-3*’ in the pre-meeting survey. However, there was a query over the suggestion in ‘*recommendation CQ3-2*’ that polymerase chain reaction (PCR) is highly recommended for testing.

Tumour MSI/MMR status can be tested using immunohistochemistry (IHC), PCR and more recently by next generation sequencing (NGS) techniques.³⁰ The expression of MMR proteins (MLH1, MSH2, MSH6 and PMS2) in tumour tissue is typically examined by IHC in the first instance to evaluate whether the tumour is dMMR and is the approach recommended in the recently published ESMO recommendations on MSI (MMR) testing for immunotherapy in cancer.¹⁶ If IHC expression of at least one protein is lost, the tumour is considered to be dMMR. If the IHC results are equivocal, the ESMO recommendation is to use MSI-PCR, based on PCR amplification of microsatellite markers.¹⁶

There was considerable discussion amongst the experts at the face-to-face meeting about the use of PCR ('*recommendation CQ3-2*'). For example, it was agreed that conventional MSI-PCR, which was developed and validated for colon cancer, was an excellent approach for patients with CRC but that its accuracy was inferior in other tumour types such as endometrial and prostate cancers.^{21,31} A five poly-A panel comprising five poly-A mononucleotide repeats is the panel recommended by ESMO for MSI-PCR testing, due to its higher sensitivity and specificity,³² with MSI defined as 'loss of stability in ≥ 2 of the five microsatellite markers'.¹⁶ In addition, IHC is not reimbursed in all countries and MSI-PCR is the upfront test of choice, and is also generally indicated for the assessment of dMMR in cancers belonging to the spectrum of Lynch syndrome cancer types. The MSI-PCR test kit FALCO has been approved in Japan as a companion diagnostic for pembrolizumab.¹⁸ After discussion, the experts from Japan, Taiwan and ESMO agreed with and accepted completely [A = 75%] the revised '*recommendation CQ3-2*' (see revisions in bold text above), while the representatives of ASCO could only accept the revised recommendation with some reservation [B = 25%].

NGS represents an alternative molecular test for the detection of tumour MSI status^{21,33} and includes several techniques.^{21,34,35} NGS also has the potential to determine tumour mutation burden (TMB). Interestingly, in the clinical trials conducted for the application to the FDA for the approval of pembrolizumab, the screening tests for MSI/MMR did not include NGS. However, the reported concordance rates between NGS testing and MSI-PCR testing and between NGS and IHC are both extremely high.³⁶ NGS testing has the potential to become the test of choice going forward for determining patient eligibility for treatment with PD-1/PD-L1 inhibitors, but should only be carried out at selected specialist centres or through validated central laboratory methods. It might also offer the potential to assess tumour response during anti-PD-1 therapy.^{37,38} Experts from three of the four groups/organisations represented agreed with and accepted completely [A = 75%] the revised '*recommendation CQ3-3*', while those of the fourth could only accept the revised recommendation with some reservation [B = 25%]. The GoR for testing was revised to a **B**.

CQ4. What is the appropriate biospecimen for testing for MSI/MMR?

Recommendation CQ4. Formalin-fixed, paraffin-embedded (FFPE) tissue blocks are appropriate for testing.

[LoE: V, GoR: A, LoA: A = 100%]

All the experts agreed with and accepted completely '*recommendation CQ4*' and the supporting evidence in the pre-meeting survey.

Thus, the expert opinion was that the recommended specimens for MSI/MMR testing should be FFPE tissue blocks of surgical specimens. Also, since MLH1 and MSH6 protein expression is possibly lost after cisplatin-containing

therapy^{39,40} and MSH6 protein expression is reported to be lost after neoadjuvant radiation,⁴¹ it is desirable to use specimens for testing that have not been exposed to cisplatin or radiation therapy. A freshly frozen tissue specimen may be used if it is histologically confirmed that there are sufficient tumour cells, for the specific testing method, contained in the specimen. As stated previously (CQ2), the general feeling was that ideally testing should be done at the time of diagnosis and tissue availability, when there may be only one chance at biopsy.

CQ5. Which treatment is recommended for MSI/dMMR patients?

Recommendation CQ5. PD-1/PD-L1 inhibitors are strongly recommended for patients with MSI/dMMR tumours.

[LoE: III, GoR: A, LoA: A = 100%]

All the experts agreed with and accepted completely [A = 100%] '*recommendation CQ5*' and the supporting evidence in the pre-meeting survey.

PD-1 inhibitors are strongly recommended for the treatment of patients with MSI/dMMR solid tumours based on the evidence from the clinical trials of pembrolizumab.^{4,5,8,42,43} In addition, both nivolumab monotherapy and nivolumab/ipilimumab combination therapy have demonstrated activity in MSI/dMMR metastatic CRC patients^{10,11} and more recently nivolumab has been shown to be effective in non-colorectal tumours that are dMMR.⁴⁴ The PD-L1 inhibitor durvalumab has also demonstrated efficacy in two ongoing studies (a phase II trial in MSI/dMMR CRC and a phase I/II trial in patients with MSI/dMMR solid tumours).²⁷

CQ6. Where in the treatment algorithm should immunotherapy be used in the treatment of patients with MSI/dMMR solid tumours?

Recommendation CQ6. We recommend immunotherapy for patients with MSI/dMMR during the course of their therapy when no other satisfactory treatment options exist depending on the clinical context.

[LoE: III, GoR: A, LoA: A = 100%]

All the experts except one agreed with and accepted completely '*recommendation CQ6*', and the supporting evidence in the pre-meeting survey, but eventually the recommendation was reworded to be less prescriptive in terms of the timing of immunotherapy.

PD-1 inhibitors have demonstrated efficacy in patients with previously-treated MSI/dMMR solid tumours.^{4,5,8,10,42,45} Thus, pembrolizumab and nivolumab can be considered for second- or later-line treatment in patients with MSI/dMMR solid tumours. Also, a recent case report describes dual immune checkpoint blockade with ipilimumab plus nivolumab, following sequential therapy with the PD-1 and PD-L1 inhibitors pembrolizumab and atezolizumab, in a patient with Lynch syndrome and metastatic colon and localised urothelial cancers.⁴⁶ This suggests that, for some patients with MSI/dMMR tumours, multiple sequential immune checkpoint therapies may be beneficial. The GoR was revised to **A**.

Background to the development of NTRK fusions as a biomarker for TRK inhibitors

Oncogenic *NTRK* gene fusions induce tumour cell proliferation and activate various cancer-related downstream signalling pathways.^{13,17,47} *NTRK1* gene fusions were first identified in colon cancer^{48,49} but have since been identified in a range of adult and paediatric tumours together with gene fusions involving the *NTRK2* and *NTRK3* genes.^{50–54} Although *NTRK* gene fusions are common in a small number of rare adult and paediatric tumour types, they also occur at lower frequencies in many common tumour types (supplementary Table S11, available at *Annals of Oncology* online).^{17,55} Nearly always the 3' region of the *NTRK* gene is joined with the 5' region of an unrelated fusion partner gene.^{13,17,55} Currently, approximately 80 different 5' fusion partners have been identified but the best known of the *NTRK* fusions is the *ETV6-NTRK3* gene fusion which occurs in >95% of secretory carcinomas of the breast.⁵⁶

Larotrectinib and entrectinib are TRK inhibitors and are currently being investigated in patients with oncogenic *NTRK* 1, 2, and 3 gene fusions.^{12,15,57–59} Their recent approval for the tumour-agnostic treatment of patients with *NTRK* fusions means that there is a need for guidance on the diagnosis and treatment of patients with tumours with *NTRK* fusions. The ESMO has recently published recommendations on the standard methods to detect *NTRK* fusions in daily practice and also for clinical research. Two other key publications on *NTRK* fusion detection across multiple assays^{60–62} and the molecular characterisation of cancers with *NTRK* fusions⁶³ have also recently been published. It is hoped that these publications will help inform the consensus recommendations generated below in response to the CQs in Table 1.

CQ1: Should all patients with solid tumours be tested for NTRK fusion?

Recommendation CQ1-1. Patients with advanced (unresectable or metastatic) solid tumours without actionable and driver gene mutations/fusions/amplifications should be tested for NTRK fusion.

[LoE: V, GoR: B, LoA: A = 100%]

Recommendation CQ1-2. Patients with advanced (unresectable or metastatic) solid tumours which are highly likely to harbour NTRK fusions should be tested for NTRK fusion, especially ETV6-NTRK3 fusion.

[LoE: V, GoR: A, LoA: A = 100%]

Recommendation CQ1-3. Patients with advanced (unresectable or metastatic) solid tumours other than above (CQ1-1 and 1-2) should be considered for testing for NTRK fusions.

[LoE: V, GoR: A, LoA: A = 100%]

Recommendation CQ1-4. Patients with locally-advanced tumours with a high incidence of NTRK fusions should be tested when considering neoadjuvant therapy before resection.

[LoE: V, GoR: B, LoA: A = 100%]

All the experts agreed with and accepted completely 'recommendations CQ1-1 and CQ1-2' above in the pre-

meeting survey (supplementary Table S9, available at *Annals of Oncology* online). They thought that the wording of 'recommendations CQ1-1 to CQ1-3' should be revised to specify **advanced (unresectable or metastatic)** solid tumours, to better define advanced disease, and the wording of 'recommendation CQ1-4' refined to better define early disease. These changes are highlighted in bold text in 'recommendations CQ1-3 and CQ1-4' above.

At the face-to-face meeting, the experts' recommendation was that the wording of 'recommendation CQ1-1' was revised to 'patients without actionable and driver gene mutations/fusions/amplifications should be tested', as the original wording was felt to be confusing, as currently, there are no published data showing the coexistence of an *NTRK* fusion and certain actionable drivers (*EGFR*, *ALK* and *ROS1* in NSCLC, *KIT* in gastrointestinal stromal tumour, and *BRAF* in NSCLC and malignant melanoma).^{53,64} Also, an independent analysis of the available datasets for any overlap between *NTRK* fusions and other mutations, in particular oncogenes/driver gene mutations, according to tumour type (GENIE dataset), identified an overlap with certain in-frame mutations but not with key actionable mutations.^{65,66}

NTRK fusions have been reported to occur with a frequency of 75%–100% in infantile fibrosarcoma (congenital fibrosarcoma),^{67–71} secretory carcinoma of the breast,^{56,72,73} MASC^{74–77} and congenital mesoblastic nephroma,⁷¹ mostly as *ETV6-NTRK3* fusions, and these patients should therefore be tested ('recommendation CQ1-2'). In common tumours which harbour *NTRK* fusions at low frequency^{50–52,78} various partner genes have been reported. Since TRK inhibitors have been shown to have excellent activity in patients with *NTRK* fusions, with acceptable toxicity,^{12,13,15,79,80} all patients with unresectable or metastatic advanced solid tumours, other than those described in 'recommendations CQ1-1 and 1-2' above, should be considered for testing for *NTRK* fusions to avoid missing the opportunity of treatment with a TRK inhibitor ('recommendation CQ1-3').

Finally, although there is only limited evidence to support the clinical utility of TRK inhibitors in patients with early-stage solid tumours,⁸¹ it was felt that the high response rate of TRK inhibitors in tumours harbouring *NTRK* fusions meant that the use of a TRK inhibitor in the neoadjuvant setting could be considered, with complete rewording of the initial recommendation to better define early-stage solid tumours (see bold text 'recommendation CQ1-4' above) and the GoR revised to **B**. All the experts agreed with and accepted completely [A = 100%] the revised recommendation.

CQ2. When is the optimal timing for tests for NTRK fusion?

Recommendation CQ2. NTRK fusion testing should be considered before or during the standard treatment of advanced solid tumours.

[LoE: V, GoR: B, LoA: A = 100%]

The experts queried the initial recommendation in the pre-meeting survey. The general feeling was that testing for *NTRK* fusions should be considered before or during standard first- or

subsequent-line therapy for advanced solid tumours characterised by a high frequency of *NTRK* fusions, and otherwise only in the context of a larger NGS panel that is being conducted to identify other mutations. Thus, the recommendation was reworded (see bold text above) and the GoR revised to **B** and accepted completely [A = 100%] by all the experts present.

CQ3. Which tests are recommended for determining *NTRK* fusions?

*Recommendation CQ3-1. IHC (immunohistochemistry) is not recommended for confirming *NTRK* fusion. It may be used for screening to enrich for patients with *NTRK* fusions.*

[LoE: V, GoR: B, LoA: A = 100%]

*Recommendation CQ3-2. In situ hybridisation [ISH, e.g. fluorescence ISH (FISH)] for *ETV6-NTRK3* fusion is recommended for patients with tumours which are highly likely to harbour *NTRK* fusions. ISH is not recommended for patients other than the above.*

[LoE: V, GoR: B, LoA: A = 100%]

*Recommendation CQ3-3. Reverse transcriptase (RT)-PCR for *ETV6-NTRK3* fusion is recommended for patients with tumours which are highly likely to harbour *NTRK* fusions.*

[LoE: V, GoR: B, LoA: A = 100%]

*Recommendation CQ3-4. Next generation sequencing (NGS) which detects *NTRK* fusion is recommended for testing *NTRK* fusion.*

[LoE: V, GoR: C, LoA: A = 100%]

All the experts agreed with and accepted completely [A = 100%] the four recommendations listed above without revision. A fifth recommendation, originally CQ3-4, regarding the predictive value of nanostring technology was deleted due to a paucity of data and the original 'recommendation CQ3-5' (supplementary Table S9, available at *Annals of Oncology* online) became 'recommendation CQ3-4'.

IHC examines the expression of the TRK proteins but does not directly detect *NTRK* fusions.^{82–84} Thus, negative protein expression determined by TRK IHC only predicts a lack of *NTRK* fusions.⁸⁵ Consequently, IHC, when positive, may be used to enrich for patients with *NTRK* fusions as part of a two-step process for their detection. It is noted that IHC shows lower sensitivity for *NTRK3* fusions, and both sensitivity and specificity were poor in sarcomas in one report.⁶² ISH is also not recommended for the routine detection of *NTRK* fusions in all patients but can be used in patients with tumours which are highly likely to harbour *ETV6-NTRK3* fusions. RT-PCR^{77,86} is designed to identify only known fusion partners and breakpoints and is not recommended for routine detection of *NTRK* fusions in all patients, although it could be used for patients with tumours that are highly likely to harbour *ETV6-NTRK3* fusions. DNA-based NGS, on the other hand, is effective for the detection of *NTRK* fusions.^{52,54} Although, not all the *NTRK* fusions can be identified, especially those involving *NTRK2* and *NTRK3* where large intronic regions can render DNA-based detection challenging. RNA sequencing does however^{85,87} offer

an approach for the *de novo* detection of transcribed fusion genes. Thus, validated NGS methods which cover *NTRK* fusions regardless of fusion partner are recommended.⁸⁸ The application of all these techniques is described in detail in the ESMO recommendations.¹⁷ The challenge in terms of diagnosis is to find a method that allows the rapid, accurate testing of a large number of patients.

CQ4. What is the appropriate biospecimen for testing for *NTRK* fusions?

Recommendation CQ4. Both fresh samples as well as archival tissue samples properly fixed and preserved are appropriate for testing.

[LoE: V, GoR: B, LoA: A = 100%]

Three studies were included in the qualitative synthesis of this recommendation,^{62,79,89} and all the experts agreed with and accepted completely [A = 100%] 'recommendation CQ4' without revision.

Archival FFPE tissue sections are appropriate for IHC, FISH, RT-PCR and anchored multiplex (PCR) NGS if properly fixed and preserved.⁸⁵ The quality of the archival material to be tested is crucial, and FFPE RNA in particular is known to be labile. In the basket study of entrectinib, both fresh and archival tissue was used.¹⁵ It may be necessary to recommend that, when necessary, patients should be re-biopsied to obtain appropriate tissue for examination.

CQ5. Which treatment is recommended for patients with *NTRK* fusions?

*Recommendation CQ5. TRK inhibitors are strongly recommended for patients with *NTRK* fusion.*

[LoE: III, GoR: A, LoA: A = 100%]

Although there has been no study comparing the two TRK inhibitors (larotrectinib, entrectinib) approved for tumour-agnostic therapy, with other standard treatment options, they have shown high and durable responses^{13,15,59,79} coupled with relatively mild toxicity profiles. Thus, based on the available evidence, TRK inhibitors are strongly recommended for patients with *NTRK* fusions.

CQ6. Where in the treatment algorithm should a TRK inhibitor be used in the treatment of patients with *NTRK* fusion-positive solid tumours?

*Recommendation CQ6. We recommend TRK inhibitors for patients with *NTRK* fusions during the course of therapy when no other satisfactory treatment options exist depending on the clinical context.*

[LoE: III, GoR: A, LoA: A = 100%]

The Japanese (JSCO, JSMO), TOS and ASCO experts agreed with and accepted completely the initial recommendation (supplementary Table S9, available at *Annals of Oncology* online) in the pre-meeting survey, but the ESMO experts thought that the recommendation should only apply to patients with tumours known to frequently harbour *NTRK* fusions for whom there was no other effective first-line treatment. In the case of tumours with an

alternative effective first-line treatment option and an *NTRK* fusion, some physicians may opt for the use of TRK inhibitors in later line settings. 'Recommendation CQ6' was reworded to reflect this and the GoR revised to **A**, and all the experts accepted [A = 100%] the revision. Currently, despite the efficacy of TRK inhibitors, including in the first-line setting, there is no study comparing a TRK inhibitor with standard of care for patients with *NTRK* fusion-positive solid tumours.

Implications of prevalence of MSI and *NTRK* fusions in adult and paediatric tumours on recommendations for testing

These recommendations, particularly those developed in response to the CQs1 above for testing patients for both MSI/dMMR and known/likely *NTRK* fusions are made in the knowledge that the prevalence of MSI/dMMR is low in most common solid tumours and the prevalence of known/likely *NTRK* fusions in most common tumour types is extremely low. We investigated the prevalence of MSI, *NTRK* rearrangements and high TMB (>20 mutations/Mb) in solid tumours from adult (age ≥ 18 years) and paediatric (age <18 years) patients. Comprehensive genomic profiling of >300 cancer-related genes was carried out by Foundation Medicine (Cambridge, USA) as previously described in detail.^{90,91} Analysis was carried out on 217 086 samples across different solid tumour types, which already had their MSI status and TMB score determined^{92,93} (supplementary Tables S12 and S13, available at *Annals of Oncology* online). To avoid overestimation of prevalence in rare cancers, the figures were reported only for those tumour types with data for >500 adult patients and >100 paediatric patients.

These data support the low prevalence of MSI and known/likely *NTRK* fusions in common tumours and show that MSI is more prevalent in adult (as high as 15.09% in endometrial tumours, 1.65% overall in 212 704 adult profiles) than in paediatric solid tumours (as high as 0.84% in kidney tumours, 0.23% overall in 4382 paediatric profiles) and that conversely known/likely *NTRK* fusions are more prevalent in paediatric (as high as 4.7% in soft tissue sarcomas, 1.10% overall in 4382 paediatric profiles) than in adult (highest at 2.49% in salivary gland tumours, 0.20% overall in 212 704 adult profiles) tumours. The percentage of patients with a high TMB was much higher than for either MSI or known/likely *NTRK* rearrangements in adult tumours (as high as 54.60% in skin tumours, 6.32% overall in 212 704 adult profiles) but was low in paediatric patients (maximum 2.25% in gliomas, 0.91% overall in 4382 paediatric profiles).

CONCLUSION

The results of the voting by the experts from Asia, Europe and the United States, both before (supplementary Tables S8 and S9, available at *Annals of Oncology* online) and after (Tables 2 and 3) the face-to-face meeting, showed high concordance across the different geographical regions for the testing for, and treatment of, patients with either MSI/dMMR tumours or solid tumours with *NTRK* fusions.

Thus, these recommendations can be considered to be international expert consensus recommendations for the treatment of patients with either MSI/dMMR tumours or solid tumours with *NTRK* fusions. The ESMO Magnitude of Clinical Benefit Scale (MCBS) score for pembrolizumab and TRK inhibitors in the agnostic therapy setting have not been confirmed, but the preliminary scores are 3 for both, the highest score attainable for efficacy evaluated on single-trial data.

As the numbers of clinically relevant predictive biomarkers for the treatment of solid tumours increases, it is likely that NGS will become the key diagnostic tool to inform our treatment decisions. Genomic profiling of tumours to identify other potentially targetable alterations (such as *ALK*, *BRAF*, *BRCAness*, *FGFR*, *HER2*, *HER3*, homologous recombination deficiency (HRD), *KRAS*, *RET*, *ROS1* and TMB-high), which can be used in tumour-agnostic treatment approaches, is ongoing. Thus, the era of focussing on a tumour's molecular biology has arrived and will alter our approach to future drug development.

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