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SPECIAL ARTICLE

Recommendations for the use of next-generation sequencing (NGS) for patients with advanced cancer in 2024: a report from the ESMO Precision Medicine Working Group

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Background: Advancements in the field of precision medicine have prompted the European Society for Medical Oncology (ESMO) Precision Medicine Working Group to update the recommendations for the use of tumour next-generation sequencing (NGS) for patients with advanced cancers in routine practice.

Methods: The group discussed the clinical impact of tumour NGS in guiding treatment decision using the ESMO Scale for Clinical Actionability of molecular Targets (ESCAT) considering cost-effectiveness and accessibility.

Results: As for 2020 recommendations, ESMO recommends running tumour NGS in advanced non-squamous non-small-cell lung cancer, prostate cancer, colorectal cancer, cholangiocarcinoma, and ovarian cancer. Moreover, it is recommended to carry out tumour NGS in clinical research centres and under specific circumstances discussed with patients. In this updated report, the consensus within the group has led to an expansion of the recommendations to encompass patients with advanced breast cancer and rare tumours such as gastrointestinal stromal tumours, sarcoma, thyroid cancer, and cancer of unknown primary. Finally, ESMO recommends carrying out tumour NGS to detect tumour-agnostic alterations in patients with metastatic cancers where access to matched therapies is available.

Conclusion: Tumour NGS is increasingly expanding its scope and application within oncology with the aim of enhancing the efficacy of precision medicine for patients with cancer.

Key words: next-generation sequencing (NGS), advanced cancer, precision medicine, ESCAT

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INTRODUCTION

Considering the evolving field of drug development and genomic data, the European Society for Medical Oncology (ESMO) Precision Medicine Working Group (PMWG) sought to update recommendations for the use of next-generation sequencing (NGS) for patients with metastatic cancers published in 2020. After several discussions among the members of the group, a consensus was reached to adopt the same methodology as per the previous publication.[1] Moreover, the group made the decision to encompass rare cancers, including gastrointestinal stromal tumours (GISTs), sarcomas, thyroid cancers, and cancer of unknown primary (CUP). This expansion was motivated by increased understanding of the molecular landscape of these tumour types in recent years and the large patient population they represent. The PMWG concentrated its efforts on genomic alterations classified as ESMO Scale for Clinical Actionability of molecular Targets (ESCAT) level I, since they are the key determinants for recommending the use of NGS for routine practice within specific cancer types. In addition, it was unanimously agreed to report ESCAT level II genomic alterations to facilitate patient enrolment in clinical trials and promote drug development. Updated alterations are highlighted in red in the respective tables. Notably, genomic alterations classified as ESCAT level III/IV were not reported in the manuscript since they should not be used for routine practice, and they frequently undergo updates and reclassification with limited impact on patient clinical outcomes (Supplementary Table S1, available at https://doi.org/10.1016/j.annonc.2024.04.005). Acknowledging the diverse technologies available for detecting fusions and the potential for incidental findings when employing NGS, the PMWG opted to include a dedicated chapter addressing specific considerations for medical oncologists when ordering NGS tests. The following recommendations are for routine practice only. In addition, as for 2020 recommendations, ESMO recommends that clinical research centres carry out multigene sequencing for patients with metastatic cancers in order to accelerate clinical research. Finally, ESMO acknowledges that patients and their oncologists can order a multigene sequencing outside these recommendations if the patient is informed about the likelihood of benefit, if it does not generate substantial extra cost to the public health system, and if it does not lead to off-label use of drugs without sufficient evidence.

UPDATED RECOMMENDATIONS

Tumour-agnostic biomarkers

Table 1 provides a list of tumour-agnostic genomic alterations.

NTRK1,2,3 fusions, *RET* and *FGFR1/2/3* fusions/mutations, *BRFA*^{V600E} mutations, MSI-H, and tumour mutation burden-high (TMB-H) are designated as tumour-agnostic biomarkers, categorised as level IC. This classification is based on the clinical improvement of patient outcomes in basket trials.[2-11] Prospective randomised or open-label phase II studies have been carried out for certain tumours, enabling the categorisation of some of these biomarkers into level IA/IB in the respective section.

Summary of recommendations

ESMO recommends carrying out multigene NGS in patients with advanced cancers in countries where tumour-agnostic targeted therapies are accessible. Cost-effectiveness should be assessed at the local level and the decision to implement NGS should be taken accordingly. It is important for clinicians to ensure that fusions are integrated in the panel.

Genomic alterations according to ESCAT in advanced non-squamous non-small-cell lung cancer (NSCLC)

Table 2 provides a list of genomic alterations level I/II according to ESCAT in advanced non-squamous NSCLC.

Table 1. List of tumour-agnostic genomic alterations

Gene/Signature[a]	Alteration	Estimated prevalence (illustration of tumours with high prevalence of the alteration)	ESCAT score	Drug class matched	References
NTRK1/2/3	Fusions	80%-90% secretory breast cancer 15%-20% Spitzoid melanoma	IC	TRK inhibitors	Hong et al., *Lancet Oncol* 2020[2] Demetri et al., *Clin Can Res* 2022[3]
MSI-H/dMMR[a]	MSI-H/dMMR	15%-20% endometrial cancer 15%-20% gastric adenocarcinoma	IC	PD-1 checkpoint inhibitors	Marcus et al., *Clin Can Res* 2019[4]
RET	Fusions	7% thyroid papillary cancer 2% salivary gland cancer	IC	RET inhibitors	Subbiah et al., *Lancet Oncol* 2022[5] Subbiah et al., *Nat Med* 2022[6]
BRAF	Mutations (p.V600E)	40%-45% melanoma 5%-6% small intestinal adenocarcinoma	IC	BRAF inhibitors + MEK inhibitors	Subbiah et al., *Cancer Discov* 2020[7] Salama et al., *J Clin Oncol* 2020[8]
FGFR1/2/3	Fusions Mutations	20%-40% bladder cancer 3% glioblastoma multiforme 10%-20% urothelial carcinoma 10% endometrial cancer	IC	Pan-FGFR TKIs	Pant et al., *Lancet Oncol* 2023[9]
TMB-H[a]	TMB-H	40% small-cell lung cancer	IC	PD-1/PD-L1 checkpoint inhibitors	Valero et al., *JAMA Oncol* 2021[10] Friedman et al., *Cancer Discov* 2022[11]

dMMR, mismatch repair deficient; ESCAT, ESMO Scale for Clinical Actionability of molecular Targets; FGFR, fibroblast growth factor receptor; MSI-H, microsatellite instability-high; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; TKIs, tyrosine kinase inhibitors; TMB-H, tumor mutation burden-high; TRK, tropomyosin receptor kinase.
[a]Signature; TKIs, tyrosine kinase inhibitors.

Table 2. List of genomic alterations level I/II according to ESCAT in advanced non-squamous non-small-cell lung cancer

Gene	Alteration	Estimated prevalence	ESCAT score	Drug class matched	References
EGFR	Common mutations (deletion exon 19, p.L858R)	15% Caucasian 50% Asian 30% LATAM	IA	First-, second- and third-generation EGFR TKIs EGFR-MET bispecific antibodies + third-generation EGFR TKIs EGFR-MET bispecific antibodies + chemotherapy ± EGFR TKIs (after PD on third-generation EGFR TKIs)	Midha et al., *Am J Can Res* 2015[12] Arrieta et al., *J Thorac Oncol* 2015[13] Soria et al., *N Engl J Med* 2018[14] Ramalingam et al., *N Engl J Med* 2020[15] Cho et al., *Ann Oncol* 2023[16] Passaro et al., *Ann Oncol* 2024[17]
	Acquired p.T790M mutation in exon 20	60% after first- or second-generation EGFR TKIs	IA	Third-generation EGFR TKIs	Mok et al., *N Engl J Med* 2017[18] Papadimitrakopoulou et al., *Ann Oncol* 2020[19]
	Exon 20 insertions	2%	**IA**	EGFR-MET bispecific antibodies or TKIs	Park et al., *J Clin Oncol* 2021[20] Zhou et al., *N Engl J Med* 2023[21]
	Uncommon mutations (p.G719 variants in exon 18, p.L861Q in exon 21, p.S768I in exon 20)	10%	IB	Second- and third-generation EGFR TKIs	Cho et al., *J Clin Oncol* 2020[22] Yang et al., *Front Oncol* 2022[23]
ALK	Fusions	5%	IA	ALK TKIs	Mok et al., *Ann Oncol* 2020[24] Shaw et al., *N Engl J Med* 2020[25] Camidge et al., *J Thorac Oncol* 2021[26] Horn et al., *JAMA Oncol* 2021[27] Solomon et al., *Lancet Respir Med* 2023[28]
KRAS	Mutations (p. G12C)	12%	**IA**	KRAS ^{G12C} TKIs	Jänne et al., *N Engl J Med* 2022[29] de Langen et al., *Lancet* 2023[30]
RET	Fusions	1%-2%	**IA**	RET TKIs	Subbiah et al., *Clin Can Res* 2021[31] Griesinger et al., *Ann Oncol* 2022[32] Drilon et al., *J Clin Oncol* 2023[33] Zhou et al., *N Engl J Med* 2023[34]
ROS1	Fusions	1%-2%	IB	ROS1 TKIs	Shaw et al., *Ann Oncol* 2019[35] Shaw et al., *Lancet Oncol* 2019[36] Drilon et al., *JTO Clin Res Rep* 2022[37]
BRAF	Mutations (p. V600E)	2%	IB	BRAF TKIs + MEK TKIs	Planchard et al., *J Thorac Oncol* 2022[38] Riely et al., *J Clin Oncol* 2023[39]
MET	Mutations exon 14 skipping	3%	IB	MET TKIs	Drilon et al., *Nat Med* 2020[40] Wolf et al., *J Clin Oncol* 2021[41] Lu et al., *Lancet Respir* 2021[42] Thomas et al., *J Thorac Oncol* 2022[43] Wolf et al., *Ann Oncol* 2022[44]
	Focal amplifications	5% as primary 15% as mechanism of acquired resistance on EGFR TKIs	IIB	MET TKIs + third-generation EGFR TKIs EGFR-MET bispecific antibodies + third-generation EGFR TKIs	Yu et al., *Ann Oncol* 2021[45] Bauml et al., *J Clin Oncol* 2021[46] Shu et al., *J Clin Oncol* 2022[47] Marmarelis et al., *J Thorac Oncol* 2022[48] Hartmaier et al., *Cancer Discov* 2023[49] Tan et al., *J Clin Oncol* 2023[50]
ERBB2	Hotspot mutations	3%	IIB	Pan-HER TKIs Anti-HER2 ADCs	Hyman et al., *Nature* 2018[51] Li et al., *N Engl J Med* 2022[52]
NRG1	Fusions	<1%	**IIB**	Anti-HER2/HER3 bispecific antibody	Schram et al., *JCO* 2022[53]

ADC, antibody—drug conjugates; EGFR, epidermal growth factor receptor; ESCAT, ESMO Scale for Clinical Actionability of molecular Targets; HER, human epidermal growth factor receptor; LATAM, Latin America; PD, progressive disease; TKIs, tyrosine kinase inhibitors.

EGFR exon 20 insertions, labelled as level IIB in the previous recommendations, have now been reclassified as level IA. This is supported by the randomised phase III PAPILLON trial, which assessed amivantamab, a bispecific monoclonal antibody targeting both epidermal growth factor receptor (EGFR) and MET receptor, plus chemotherapy as compared to chemotherapy alone in patients with advanced NSCLC with *EGFR* exon 20 insertions. Progression-free survival (PFS) was longer in the amivantamab plus chemotherapy than in the chemotherapy arm [hazard ratio (HR) 0.40; 95% confidence interval (CI) 0.30-0.53; $P < 0.001$].[21] In 2021, amivantamab has obtained an accelerated approval by the Food and Drug Administration (FDA). In 2023, a supplemental biological license application has been submitted to the FDA and a type II extension of indication application to the European Medicines Agency (EMA), seeking expanded approval of amivantamab plus carboplatin/pemetrexed in the frontline treatment of this population. Another alteration that has been subject to reclassification is *KRAS*^{G12C} mutation, which has been reclassified as level IA. This decision was driven by the PFS improvement observed with sotorasib, a specific KRAS^{G12C} tyrosine kinase inhibitor (TKI), versus chemotherapy (HR 0.66; 95% CI 0.51-0.86; $P = 0.0017$) in patients with pre-treated advanced *KRAS*^{G12C}-mutated NSCLC in the randomised phase III study CodeBreaK 200. No improvement in overall survival (OS) was observed (HR 1.01; 95% CI 0.77-1.33).[30] Accelerated approval was

granted by the FDA and conditional authorisation was provided by the EMA for sotorasib. Finally, *RET* fusions have been moved from level IC to IA in accordance with ESCAT, supported by findings from the phase III randomised LIBRETTO-431 trial. In this study, selpercatinib, a selective RET TKI, led to significantly longer PFS than platinum-based chemotherapy with or without pembrolizumab in the first line among patients with advanced *RET* fusion-positive NSCLC (HR 0.46; 95% CI 0.31-0.70; $P < 0.001$).[34] Selpercatinib has received regular approval from the FDA and EMA for this indication. It is noteworthy that the trials resulting in the upgrade of *EGFR* exon 20 insertions,[21] *KRAS*^{G12C} mutations,[30] and *RET* fusions[34] have been reported after the ESMO Clinical Practice Guideline for diagnosis, treatment, and follow-up for patients with advanced NSCLC was published.[54] *NRG1* fusions are classified as level IIB based on the antitumour activity, overall response rate (ORR) 35% (95% CI 21-50), elicited by zenocutuzumab, an anti-human epidermal growth factor receptor 2 (HER2)/HER3 bispecific antibody, in patients with advanced *NRG1*-positive NSCLC.[53] Zenocutuzumab received the breakthrough therapy designation by the FDA for *NRG1* fusion-positive advanced NSCLC.

Summary of recommendations

No changes have been made to the indication of carrying out tumour NGS in patients with advanced non-squamous NSCLC in daily practice, as the working group has already recommended tumour NGS in these patients. However, with the inclusion of new genomic alterations categorised as ESCAT level I, it is crucial to carefully consider the optimal approach for tumour NGS implementation in the clinical management of patients with advanced non-squamous NSCLC.

Genomic alterations according to ESCAT in advanced breast cancer (ABC)

Table 3 provides a list of genomic alterations level I/II according to ESCAT in ABC.

ESR1 mutations have been upgraded to level IA based on the results of the EMERALD trial. In this randomised, phase III study, elacestrant, an oral selective oestrogen receptor degrader (SERD) demonstrated an improvement in PFS among patients with hormone receptor-positive/HER2-negative ABC (HR 0.70; 95% CI 0.55-0.88; $P = 0.02$), with a greater benefit observed in those with detectable *ESR1* mutations (HR 0.55; 95% CI 0.39-0.77; $P = 0.0005$).[64] It is important to emphasise that these data became available after the release of the ESMO Clinical Practice Guideline for the diagnosis, staging, and treatment of patients with metastatic breast cancer.[73] Several data recently reported high performance for tumour NGS in detecting germline *BRCA1/2* mutations; however, around 7% of these alterations were not identified. This suggests that patients presenting a high likelihood of harbouring germline *BRCA1/2* mutations and a negative tumour NGS should undergo dedicated germline testing.[74,75] Capivasertib plus fulvestrant improved median PFS in patients with hormone receptor-positive/HER2-negative ABC (HR 0.60; 95% CI 0.51-0.71; $P < 0.001$), with a slightly greater benefit in patients exhibiting *AKT*-pathway alterations (HR 0.50; 95% CI 0.38-0.65; $P < 0.001$) in a randomised phase III study.[70] Based on these data, the FDA approved this combination for pretreated patients with hormone receptor-positive/HER2-negative ABC with *PIK3CA/AKT1/PTEN* alterations. There is no consensus among experts regarding whether *AKT1/PTEN* mutations should be classified as level I or II in this patient population, given the low prevalence, and the observed

Table 3. List of genomic alterations level I/II according to ESCAT in advanced breast cancer

Gene	Alteration	Estimated prevalence	ESCAT score	Drug class matched	References
ERBB2	Amplifications	15%-20%	IA	Anti-HER2 monoclonal antibodies HER2 TKIs Anti-HER2 ADCs	Baselga et al., *N Engl J Med* 2012[55] Krop et al., *Lancet Oncol* 2014[56] Lin et al., *J Clin Oncol* 2020[57] Saura et al., *J Clin Oncol* 2020[58] Rugo et al., *JAMA Oncol* 2021[59]
	Hotspot mutations	4%	IIB	Pan-HER TKIs Anti-HER2 ADCs	Hyman et al., *Nature* 2018[51] Smyth et al., *Cancer Discov* 2020[60] Li et al., *Ann Oncol* 2023[61]
PIK3CA	Hotspot mutations	30%-40%	IA (ER-positive HER2-negative ABC)	α-specific PI3K inhibitors*	André et al., *N Engl J Med* 2019[62] Rugo et al., *Lancet Oncol* 2021[63] Turner et al, *N Engl J Med* 2023[70]
ESR1	Mutations	30%-40%	IA (ER-positive HER2-negative ABC resistant to AI)	SERDs	Bidard et al., *J Clin Oncol* 2022[64] Bardia et al., *Cancer Res* 2023[65]
BRCA1/2	Germline pathogenic/likely pathogenic variants	4%	IA	PARP inhibitors	Litton et al., *N Engl J Med* 2018[66] Robson et al., *Eur J Cancer* 2023[67]
	Somatic mutations	3%	IIB	PARP inhibitors	Tung et al., *J Clin Oncol* 2020[68]
PTEN	Mutations/deletions	7%	I/II	AKT inhibitors	Schmid et al., *J Clin Oncol* 2020[69] Turner et al., *N Engl J Med* 2023[70]
AKT1	Mutations (p. E17K)	5%	I/II	AKT inhibitors	Kalinsky et al., *JAMA Oncol* 2021[71] Turner et al., *N Engl J Med* 2023[70]
PALB2	Germline pathogenic/likely pathogenic variants	1%	IIB	PARP inhibitors	Tung et al., *J Clin Oncol* 2020[68] Gruber et al., *Nat Cancer* 2022[72]

ABC, advanced breast cancer; ADCs, antibody—drug conjugates; AI, aromatase inhibitors; ER, oestrogen receptor; ESCAT, ESMO Scale for Clinical Actionability of molecular Targets; HER, human epidermal growth factor receptor; PARP, poly (ADP-ribose) polymerase; SERDs, selective oestrogen receptor degrader; TKIs, tyrosine kinase inhibitors.
*AKT inhibitors have shown efficacy in patients with PIK3CA mutated ER-positive HER2-negative ABC

benefit may predominantly arise from *PIK3CA* mutations. Nevertheless, as the determination of *AKT1/PTEN* alterations can provide drug access to these patients, the group recommends carrying out tumour NGS. Finally, poly (ADP-ribose) polymerase inhibitors (PARPi) showed antitumour activity in patients with ABC and either somatic *BRCA1/2* mutations (ORR 50%; 90% CI 28-72) or germline *PALB2* pathogenic/likely pathogenic variants (ORR 82%; 90% CI 53-96), leading to their reclassification as level IIB.[68]

Summary of recommendations

Considering that tumour NGS can substitute germline *BRCA1/2* testing in most of the patients, along with the reclassification of *ESR1* mutations to level IA, it is recommended to carry out tumour NGS of a tumour (or plasma) sample from a patient with hormone receptor-positive/HER2-negative ABC as standard of care. The NGS testing should be done after resistance to endocrine therapy to optimise the likelihood of detecting *ESR1* mutations. Patients with high likelihood of harbouring germline *BRCA1/2* mutations should undergo clinical genetic testing even if these alterations were not detected by tumour NGS.

Genomic alterations according to ESCAT in advanced colorectal cancer (CRC)

Table 4 provides a list of genomic alterations level I/II according to ESCAT in advanced CRC.

In this version of the recommendations, we have integrated *KRAS*^{G12C} mutations in advanced CRC since they became level IA. This decision was grounded on the randomised phase III CodeBreak 300 trial. In this study, sotorasib plus anti-EGFR monoclonal antibody showed an improvement of PFS among patients with pre-treated *KRAS*^{G12C}-mutated advanced CRC (HR 0.49; 95% CI 0.30-0.80; $P = 0.006$).[80] Moreover, hotspot-inactivating missense mutations in the exonuclease domain of the polymerase epsilon *(POLE)* gene in mismatch repair (MMR)-proficient solid tumours were associated with TMB-H and predict high activity from anti-programmed cell death protein 1 (PD-1) therapy, warranting their classification at level IIB.[84] Finally, we have accumulated data on *ERBB2* amplification actionability with novel agents such as trastuzumab plus tucatinib and trastuzumab deruxtecan, which have shown substantial antitumour activity, ORR 30%-40%, in non-randomised studies and are approved/recommended therapies according to different regulatory agencies.[82,83]

Summary of recommendations

ESMO recommends carrying out multigene tumour NGS in daily practice for patients with advanced CRC, if the testing itself does not add extra cost as compared to standard procedures such as immunohistochemistry (IHC), polymerase chain reaction (PCR), or Sanger sequencing.

Genomic alterations according to ESCAT in advanced prostate cancer

Table 5 provides a list of genomic alterations level I/II according to ESCAT in advanced prostate cancer.

Since the prior recommendations, randomised phase III trials have demonstrated that treatment of advanced prostate cancer patients with *BRCA1/2* alterations with PARPi alone or in combination with androgen receptor signalling inhibitors results in prolonged OS, so the panel retained the IA classification for *BRCA1/2* alterations. Subgroup analyses of these studies suggest limited benefit for patients with *ATM* alterations, although these events have been associated with PARPi activity in phase II trials.[85,87,89] Therefore, *ATM* alterations have been ranked as IIB instead of IIA. Additional evidence regarding the predictive value of *PALB2* alterations for PARP inhibition has been included in light of the previous recommendations. Nevertheless, due to their low prevalence, data on *PALB2* alterations mainly come from specific phase II trials, resulting in limited information on survival outcomes.[95,96] Therefore, *PALB2* alterations are still categorised as level IIB. The randomised phase III trial IPATential 150 demonstrated improved radiographic PFS from the addition of ipatasertib to abiraterone in patients with metastatic castration-resistant prostate cancer with *PTEN* loss by NGS with no OS benefit.[91,93] In consequence, the panel decided to retain

Table 4. List of genomic alterations level I/II according to ESCAT in advanced colorectal cancer

Gene/Signature[a]	Alteration	Estimated prevalence	ESCAT score	Drug class matched	References
KRAS, NRAS	Mutations (exon 2, 3 and 4)	53%	NA[b]	Anti-EGFR monoclonal antibodies	Douillard et al., *N Engl J Med* 2013[76] Van Cutsem et al., *J Clin Oncol* 2015[77]
BRAF	Mutations (p. V600E)	8.5%	IA	BRAF inhibitors + EGFR inhibitors	Kopetz et al., *N Eng J Med* 2019[78]
MSI-H/dMMR[a]	MSI-H/dMMR	4.5%	IA	PD-1 checkpoint inhibitors	André et al., *N Engl J Med* 2020[79]
KRAS	Mutations (p. G12C)	4%	**IA**	KRAS ^{G12C} TKIs + anti-EGFR monoclonal antibodies	Fakih et al., *N Engl J Med* 2023[80]
ERBB2	Amplifications	2%	IIB	Anti-HER2 monoclonal antibodies ± anti-HER2 TKIs Anti-HER2 ADCs	Meric-Bernstam et al., *Lancet Oncol* 2019[81] Siena et al., *Lancet Oncol* 2021[82] Strickler et al., *Lancet Oncol* 2023[83]
POLE	Mutations	<1%	**IIB**	PD-1 checkpoint inhibitors	Rousseau et al., *Cancer Discov* 2022[84]

ADCs, antibody—drug conjugates; dMMR, mismatch repair deficient; HER, human epidermal growth factor receptor; EGFR, epidermal growth factor receptor; ESCAT, ESMO Scale for Clinical Actionability of molecular Targets; MSI-H, microsatellite instability-high; NA, not applicable; PD-1, programmed cell death protein 1; TKIs, tyrosine kinase inhibitors.
[a]Signature.
[b]biomarker of resistance.

Table 5. List of genomic alterations level I/II according to ESCAT in advanced prostate cancer

Gene	Alteration	Estimated prevalence	ESCAT score	Drug class matched	References
BRCA1/2	Germline and somatic mutations/deletions	9%-11%	IA	PARP inhibitors	de Bono et al., *N Engl J Med* 2020[85] Hussain et al., *N Engl J Med* 2020[86] Fizazi et al., *N Engl J Med* 2023[87] Chi et al., *Ann Oncol* 2023[88] Fizazi et al., *Nat Med* 2023[89]
PTEN	Deletions/mutations	40%	IIA	AKT inhibitors	Abida et al., *Proc Natl Acad Sci* 2019[90] De Bono et al., *Clin Cancer Res* 2019[91] Sweeney et al., *Lancet* 2021[92] Sweeney et al., *J Clin Oncol* 2022[93]
ATM	Mutations/deletions	6%	IIB	PARP inhibitors	De Bono et al., *N Engl J Med* 2020[85] Fizazi et al., *N Engl J Med* 2023[87] Fizazi et al., *Nat Med* 2024[89]
PALB2	Mutations/deletions	1%	IIB	PARP inhibitors	Mateo et al., *N Engl J Med* 2015[94] de Bono et al., *N Engl J Med* 2020[85] Carreira et al., *Cancer Discov* 2021[95] Abida et al., *Eur Urol* 2023[96]

ESCAT, ESMO Scale for Clinical Actionability of molecular Targets; PARP, poly (ADP-ribose) polymerase.

the IIA classification for *PTEN* loss. Ongoing phase III trials are evaluating other AKT inhibitors in different scenarios of advanced prostate cancer.[97,98]

Summary of recommendations

The earlier version of the manuscript had already recommended conducting tumour NGS in countries where PARPi are available for patients with advanced prostate cancer.

Genomic alterations according to ESCAT in advanced gastric cancer

The ESCAT classification of genomic alterations in advanced gastric cancer remains unmodified to recommendations provided in 2020 (Supplementary Table S2, available at https://doi.org/10.1016/j.annonc.2024.04.005).

Summary of recommendations

In line with its previous recommendations, ESMO does not recommend carrying out tumour NGS in patients with advanced gastric cancer as the standard of care, except in countries where tumour-agnostic targeted therapies are accessible. Cost-effectiveness should be assessed at the local level and the decision to implement NGS should be taken accordingly.

Genomic alterations according to ESCAT in advanced pancreatic ductal adenocarcinoma (PDAC)

Table 6 provides a list of genomic alterations level I/II according to ESCAT in advanced pancreatic ductal adenocarcinoma.

The panel considers *KRAS*^{G12C} mutations as level IB, based on the efficacy of sotorasib in patients with pretreated advanced PDAC and *KRAS*^{G12C} mutation. In a prospective phase II single-arm study, sotorasib achieved an ORR of 21% (95% CI 10-37), a median PFS of 4.0 months (95% CI 2.8-5.6), and a median OS of 6.9 months (95% CI 5.0-9.1).[101] In a phase II study among patients with platinum-sensitive advanced PDAC and harbouring pathogenic germline or somatic variants in *BRCA1/2* or *PALB2,* rucaparib demonstrated a median PFS of 14.5 months (95% CI 0.7-28.3) within the limited subgroup of patients carrying germline *PALB2* variants. These findings led to the categorisation of this alteration as level IIB. Additionally, significant antitumour activity was observed among patients with somatic *BRCA*1/2 mutations, but sample size is too limited to rank them as level II.[103,105]

It is worth mentioning that around 10% of patients with advanced PDAC present with *KRAS* wild-type disease. *KRAS* wild-type pancreatic cancer enriches for (tumour-agnostic) therapeutic targets such as *NRG1* fusions and alternative

Table 6. List of genomic alterations level I/II according to ESCAT in advanced pancreatic ductal adenocarcinoma

Gene	Alteration	Estimated prevalence	ESCAT score	Drug class matched	References
BRCA1/2	Germline pathogenic/likely pathogenic variants	4%-7%	IA	PARP inhibitors	Golan et al., *N Engl J Med* 2019[99] Kindler et al., *J Clin Oncol* 2022[100]
KRAS	Mutations (p.G12C)	1%-2%	IB	KRAS ^{G12C} TKIs	Strickler et al., *N Eng J Med* 2023[101] Bekaii-Saab et al., *J Clin Oncol* 2022[102]
PALB2	Germline pathogenic/likely pathogenic variants	3%-4%	IIB	PARP inhibitors	Reiss et al., *J Clin Oncol* 2021[103]
NRG1	Fusions	7%	IIB	Anti-HER2/HER3 bispecific antibody	Schram et al., *JCO* 2021[104]

ESCAT, ESMO Scale for Clinical Actionability of molecular Targets; HER, human epidermal growth factor receptor; PARP, poly (ADP-ribose) polymerase; TKIs, tyrosine kinase inhibitors.

MAPK drivers such as *BRAF*^{V600E} mutations.[106,107] Zenocutuzumab, an anti-HER2/HER3 bispecific antibody, has been granted breakthrough therapy designation by the FDA for *NRG1* fusion-positive advanced PDAC and dabrafenib plus trametinib obtained the FDA approval for the treatment of *BRAF*^{V600E}-mutated cancers.[108] If multigene sequencing is not readily available, a suitable single-gene test to detect *KRAS* alterations (e.g. allele-specific PCR, PCR plus Sanger or Pyro Sequencing) could be applied to screen for *KRAS* wild-type pancreatic cancer, typically found in young patients. Due to the relevant number of therapeutic targets found in these patients, this specific group could qualify for tumour NGS.

Summary of recommendations

Considering that only germline *BRCA1/2* alterations are ranked ESCAT level I and that *KRAS*^{G12C} mutations can be detected by PCR, there is no evidence that tumour NGS could improve outcome of patients with advanced PDAC in routine practice. Nevertheless, considering the high unmet medical need and the number of alterations ranked ESCAT level II, ESMO recommends that patients get access to tumour NGS in the context of clinical genomics programmes to access PARP and NRG1 inhibitors in clinical trials. Oncologists must verify that *NRG1* fusions are part of the NGS panel. In addition, ESMO recommends using tumour NGS in countries where tumour-agnostic targeted therapies are accessible. Cost-effectiveness should be assessed at the local level and the decision to implement NGS should be taken accordingly.

Genomic alterations according to ESCAT in advanced ovarian cancer

Table 7 provides a list of genomic alterations level I/II according to ESCAT in advanced ovarian cancer.

Considering the benefit of PARPi in patients with advanced ovarian cancer and *BRCA1/2* germline/somatic pathogenic/likely pathogenic variants or homologous recombination deficiency (HRD) signature,[115-117] it is recommended to carry out *BRCA1/2* and HRD testing using a validated assay in patients with advanced ovarian cancer. In a retrospective analysis of a randomised trial, it was reported that *KRAS* mutations could predict benefit of binimetinib in advanced ovarian cancer other than high-grade cancer.[118] Since only one study has been reported so far, this alteration/drug match does not meet criteria of ESCAT level II.

Summary of recommendations

ESMO recommends running tumour NGS for patients with advanced high-grade ovarian cancer combined with an HRD signature. If DNA quality is suboptimal and/or in case of family history, patients who do not exhibit detectable tumour *BRCA1/2* mutations should undergo clinical genetic testing.

Genomic alterations according to ESCAT in advanced hepatocellular carcinoma

Alterations level I/II in advanced hepatocellular carcinoma are the tumour-agnostic biomarkers. Please refer to the section on Tumour-agnostic biomarkers.

Summary of recommendations

It is not currently recommended to carry out tumour NGS in patients with advanced hepatocellular carcinoma in daily practice, except in countries where tumour-agnostic targeted therapies are accessible. Cost-effectiveness should be assessed at the local level and the decision to implement NGS should be taken accordingly. Moreover, it is important to emphasise that immunotherapy is indicated in advanced hepatocellular carcinoma regardless of genomic alterations.[119,120]

Genomic alterations according to ESCAT in rare tumours

The most broadly accepted definition of rare cancer sets a threshold of an annual incidence of $<$6/100 000 people, which encompasses nearly 200 different entities, $\sim$24% of all new cancer cases.[121-123] Large series have consistently

Table 7. List of genomic alterations level I/II according to ESCAT in advanced ovarian cancer

Gene/Signature[a]	Alteration	Estimated prevalence	ESCAT score	Drug class matched	References
BRCA1/2	Germline pathogenic/likely pathogenic variants Somatic pathogenic/likely pathogenic variants	15%-17% 5%-7%	IA	PARP inhibitors	Bell et al., *Nature* 2011[109] Mirza et al., *N Engl J Med* 2016[110] Coleman et al., *Lancet* 2017[111] Pujade-Lauraine et al., *Lancet Oncol* 2017[112] Moore et al., *N Engl J Med* 2018[113] González-Martin et al., *N Engl J Med* 2019[114] Ray-Coquard et al., *N Engl J Med* 2019[115] DiSilvestro et al., *J Clin Oncol* 2023[116] González-Martin et al., *Ann Oncol* 2023[117]
HRD[a]	HRD	50% high-grade serous ovarian cancer	IA	PARP inhibitors	Mirza et al., *N Engl J Med* 2016[110] Coleman et al., *Lancet* 2017[111] González-Martin et al., *N Engl J Med* 2019[114] Ray-Coquard et al., *N Engl J Med* 2019[115] González-Martin et al., *Ann Oncol* 2023[117]

ESCAT, ESMO Scale for Clinical Actionability of molecular Targets; HRD, homologous recombination deficiency; PARP, poly (ADP-ribose) polymerase.
[a]Signature.

demonstrated that rare cancers have impaired 5-year relative survival compared to common cancers.[123] Limited data on molecular profiles and the subsequent paucity of drug approvals are among the most critical leading causes. In addition, the rarity of each particular entity hampers proper drug development, and therefore most of the therapeutic indications for NGS in rare tumours fall on levels II or III. Given the heterogeneity and complexity of diseases and molecular backgrounds, the specific analysis of this subset of tumours is beyond the scope of these recommendations. Thus, a limited number of entities have been reviewed herein, and they were selected based on the recent approval of targeted therapies.

Genomic alterations according to ESCAT in advanced cholangiocarcinoma (CCA)

Table 8 provides a list of genomic alterations level I/II according to ESCAT in advanced CCA.

Trastuzumab plus pertuzumab demonstrated antitumour activity in patients with pre-treated advanced HER2-positive biliary tract cancer (BTC), yielding an ORR of 23% (95% CI 11-39) and a median PFS of 4 months (95% CI 1.8-5.7) in the MyPathway study.[129] This drug combination also displayed efficacy in advanced BTC with *ERBB2* mutations.[133] Additionally, the combination of FOLFOX and trastuzumab showed clinical efficacy in pre-treated HER2-positive BTC in a prospective phase II study.[137] Zanidatamab, a bispecific antibody targeting two distinct HER2 epitopes, achieved a median PFS of 5.5 months (95% CI 3.7-7.2), while median OS data were immature in HER2-positive advanced BTC.[131] Tucatinib and trastuzumab were tested in patients with previously treated HER2-overexpressing or *ERBB2*-amplified BTC. The ORR was 46.7% (90% CI 30.8-63) and the median PFS was 5.5 months (90% CI 3.9-8.1).[132] Furthermore, neratinib, an irreversible pan-HER TKI, was tested in patients with *ERBB2*-mutated BTC. Median PFS for the gallbladder cancer and CCA subsets were 3.7 months (95% CI 0.8-6.4) and 1.4 months (95% CI 0.5-9.1), respectively.[133] Based on these data, the panel assigned *ERBB2* amplifications as level IB, while ranking *ERBB2* mutations as level IIB. At this point, the interplay between *ERBB2* mutations and HER2 overexpression in BTC cannot be assessed because of limited data availability. Accordingly, while some patients with *ERBB2*-mutated BTC and consecutive HER2 overexpression might benefit from HER2-directed therapies such as ADCs, the magnitude of clinical benefit cannot be measured at the moment. Dabrafenib plus trametinib improved clinical outcome in a multicohort trial involving pre-treated $BRAF^{V600E}$-mutated solid tumours, resulting in FDA accelerated approval in 2022 in this population.[136] In the subgroup of 53 patients with advanced BTC, the ORR was 53% (95% CI 37.7-68.6), the median PFS was 9 months (95% CI 5.5-9.4), and the median OS was 13.5 months (95% CI 10.4-17.6).[136] $BRAF^{V600E}$ mutations are ranked as level IB. Finally, adagrasib, a selective irreversible $KRAS^{G12C}$ inhibitor, demonstrated an ORR of 50% (95% CI 15.7-84.3), a median PFS of 11.3 months [95% CI 1.6-not reached (NR)], and a median OS of 15.1 months (95% CI 12.5-NR) in patients with $KRAS^{G12C}$-mutated CCA in the KRYSTAL-01 basket study, leading to the classification of these alterations as level IC.[102]

Summary of recommendations

Based on the number of alterations classified as level I, it is recommended to carry out tumour NGS in patients with advanced CCA.

Genomic alterations according to ESCAT in advanced GIST

Table 9 provides a list of genomic alterations level I/II according to ESCAT in advanced GIST.

GISTs most often harbour oncogenic mutations in the receptor tyrosine kinases KIT or platelet-derived growth factor receptor A (PDGFRA),[143] and therefore GIST therapy is based on TKIs with KIT and PDGFRA inhibitory activity. Imatinib is the first-line treatment for advanced disease after achieving a milestone clinical benefit compared to historical records.[138] Subsequent placebo-controlled randomised clinical trials led to the regulatory approval of

Table 8. List of genomic alterations level I/II according to ESCAT in advanced cholangiocarcinoma

Gene	Alteration	Estimated prevalence	ESCAT score	Drug class matched	References
IDH1	Mutations	8%-18% iCCA	IA	IDH1 inhibitors	Abou-Alfa et al., *Lancet Oncol* 2020[124]
FGFR2	Fusions	5%-15% iCCA	IB	Pan-FGFR TKIs	Javle et al., *J Clin Oncol* 2018[125] Abou-Alfa et al., *Lancet Oncol* 2020[126] Pant et al., *J Clin Oncol* 2023[127] Goyal et al., *N Engl J Med* 2023[128]
ERBB2	Amplifications	10%-20% dCCA, pCCA, GBC	IB	Anti-HER2 monoclonal antibodies Anti-HER2 ADCs Anti-HER2 bispecific antibodies HER2 TKIs	Javle et al., *Lancet Oncol* 2021[129] Meric-Bernstam et al., *JCO* 2023[130] Harding et al., *Lancet Oncol* 2023[131] Nakamura et al., *J Clin Oncol* 2023[132]
	Mutations	3%-5%	IIB	Anti-HER2 monoclonal antibodies Pan-HER TKIs	Hyman et al., *Nature* 2018[51] Cannon et al., *J Clin Oncol* 2023[133] Harding et al., *Nat Comm* 2023[134]
BRAF	Mutations (p. V600E)	50%	IB	BRAF inhibitors + MEK inhibitors	Subbiah et al., *Lancet Oncol* 2020[135] Salama et al., *J Clin Oncol* 2020[8] Subbiah et al., *Nature Med* 2023[136]
KRAS	Mutations (p. G12C)	<1%	IC	KRAS ^{G12C} TKIs	Bekaii-Saab et al., *J Clin Oncol* 2022[102]

ADCs, antibody–drug conjugates; dCCA, distal cholangiocarcinoma; ESCAT, ESMO Scale for Clinical Actionability of molecular Targets; GBC, gallbladder carcinoma; HER, human epidermal growth factor receptor; iCCA, intrahepatic cholangiocarcinoma; IDH1, isocitrate dehydrogenase 1; pCCA, perihilar cholangiocarcinoma; TKIs, tyrosine kinase inhibitors.

Table 9. List of genomic alterations level I/II according to ESCAT in advanced gastrointestinal stromal tumour

Gene	Alteration	Estimated prevalence	ESCAT score	Drug class matched	References
KIT	Mutations/insertions/deletions/indels	85%	IA	KIT/PDGFR TKIs	Demetri et al., *N Engl J Med* 2002[138] Demetri et al., *Lancet* 2006[139] Demetri et al., *Lancet* 2013[140] Blay et al., *Lancet Oncol* 2020[141]
PDGFRA	Mutations/insertions/deletions/indels	10%-15%	IA	KIT/PDGFR TKIs	Demetri et al., *N Engl J Med* 2002[138] Demetri et al., *Lancet* 2006[139] Demetri et al., *Lancet* 2013[140] Blay et al., *Lancet Oncol* 2020[141]
	Exon 18 D842V mutations	5%	IB	KIT/PDGFR TKIs	Heinrich et al., *Lancet Oncol* 2020[142]

ESCAT, ESMO Scale for Clinical Actionability of molecular Targets; PDGFR, platelet-derived growth factor receptor A; TKIs, tyrosine kinase inhibitors.

sunitinib, regorafenib, and ripretinib as the standard second-, third-, and fourth-line treatments,[139-141] displaying improvement of outcomes in patients with GIST and *KIT* or *PDGFRA* mutations. *KIT* and *PDGFRA* alterations are classified as level IA. The *PDGFRA* D842V primary mutation is present in ∼5% of GISTs and leads to resistance to all known TKIs. A prospective non-randomised study showed clinical efficacy of avapritinib in patients with GIST and this specific alteration, but not in the general population.[142,144] Based on this data, the FDA granted approval to avapritinib in this setting.

Summary of recommendations

Based on the number of alterations currently classified as level I, it is recommended to carry out tumour NGS in patients with advanced GIST.

Genomic alterations according to ESCAT in advanced soft-tissue sarcomas

Table 10 provides a list of genomic alterations level I/II according to ESCAT in advanced soft-tissue sarcomas.

Most actionable alterations in soft-tissue sarcomas are histology specific; thus, sarcoma experts recommend carrying out diagnostic procedures in referral institutions.[152] However, as a significant proportion of these patients are not initially seen in such centres, NGS panels looking for actionable targets can aid to reclassify these entities, especially when carried out in the community.[153]

Crizotinib demonstrated effectiveness (ORR 66.7%, 95% CI 34.9-90.1) and improved survival outcomes [median PFS 18 months (95% CI 4.0-not estimable); 3-year OS 83.3% (95% CI 48.2% to 95.6%)] in patients with advanced *ALK* fusion-positive inflammatory myofibroblastic tumours (IMTs),[146] leading to the classification of *ALK* fusions as level IB. Moreover, *COLIA1-PDGFB* fusions are ranked as level IB due to the clinical activity of imatinib in patients with dermatofibrosarcoma protuberans (DFSP) exhibiting these fusions. Imanitib has received regulatory approval for this indication.[148] *INI1/SMARCB1* alterations, frequently observed in epithelioid sarcoma, are categorised as level IB. This decision was based on the outcome improvement [median PFS 5.5 months (95% CI 3.4-5.9) and median OS 19 months (95% CI 11-not estimable)] of tazemetostat, an oral selective EZH2 inhibitor, in a cohort of 62 patients with advanced *INI1/SMARCB1*-altered epithelioid sarcoma from a basket trial.[150] In 2020, the FDA granted accelerated approval to tazemetostat in this subgroup of patients. Finally, mTOR inhibitors displayed antitumour activity in patients with advanced malignant perivascular epithelioid cell tumour (PEComa), achieving a confirmed response rate of 89% and 20% in patients harbouring *TSC2* or *TSC1* mutation, respectively.[151] *TSC1/TSC2* alterations are ranked as level IIA.

Summary of recommendations

NGS is an essential tool for identifying the histological subtype of soft-tissue sarcomas and improving diagnosis. In addition, several alterations are classified as level I according to ESCAT in the metastatic setting, justifying the use of tumour NGS in this disease. Oncologists must ensure that the fusions they are seeking are included in the panel.

Table 10. List of genomic alterations level I/II according to ESCAT in advanced soft-tissue sarcomas

Gene	Alteration	Estimated prevalence	Sarcoma subtype	ESCAT score	Drug class matched	References
ALK	Fusions	66%	Inflammatory myofibroblastic tumour	IB	ALK TKIs	Schöffski et al., *Lancet Respir Med* 2018[145] Schöffski et al., *Eur J Cancer* 2021[146]
COLIA1-PDGFB	Fusions	100%	Dermatofibrosarcoma protuberans	IB	KIT/PDGFR TKIs	Shimizu et al., *Cancer Res* 1999[147] Rutkowski et al., *J Clin Oncol* 2010[148]
INI1/SMARCB1	Mutations/deletions	90%	Epithelioid sarcoma	IB	EZH2 inhibitors	Modena et al., *Cancer Res* 2005[149] Gounder et al., *Lancet Oncol* 2020[150]
TSC1/2	Mutations/deletions	>80%	PEComa	IIA	mTOR inhibitors	Wagner et al., *J Clin Oncol* 2021[151]

ESCAT, ESMO Scale for Clinical Actionability of molecular Targets; EZH2, enhancer of zeste homologue 2; mTOR, mammalian target of rapamycin; PEComa; perivascular epithelioid cell tumour; PDGFR, platelet-derived growth factor receptor; TKIs, tyrosine kinase inhibitors.

Table 11. List of genomic alterations level I/II according to ESCAT in advanced thyroid cancer

Gene	Alteration	Estimated prevalence	ESCAT score	Drug class matched	References
RET	Mutations	60% medullary thyroid cancer	IA	RET inhibitors	Ciampi et al., *iScience* 2019[154] Wirth et al., *N Engl J Med* 2020[155] Kim et al., *Clin Cancer Res* 2021[156] Mansfield et al., *J Clin Oncol* 2022[157] Hadoux et al., *N Engl J Med* 2023[158]
	Fusions	7%	IB	RET inhibitors	Agrawal et al., *Cell* 2014[159] Wirth et al., *N Engl J Med* 2020[155] Kim et al., *Clin Cancer Res* 2021[156] Mansfield et al., *J Clin Oncol* 2022[157]
BRAF	Mutations (p. V600E)	10%-50% anaplastic thyroid cancer 40%-50% papillary thyroid cancer	IB IIB	BRAF inhibitors + MEK inhibitors BRAF inhibitors	Subbiah et al., *J Clin Oncol* 2018[160] Subbiah et al., *Ann Oncol* 2022[161] Brose et al., *Lancet Oncol* 2016[194]

ESCAT, ESMO Scale for Clinical Actionability of molecular Targets.

Genomic alterations according to ESCAT in advanced thyroid cancer

Table 11 provides a list of genomic alterations level I/II according to ESCAT in advanced thyroid cancer.

Selpercatinib as first-line therapy improved median PFS as compared to standard treatment in patients with *RET*-mutated medullary thyroid cancer (HR 0.28; 95% CI 0.16-0.48; $P < 0.001$) in a phase III randomised trial.[158] Consequently, *RET* mutations are classified as level IA. These data have been reported after the publication of the ESMO Clinical Practice Guideline for diagnosis, treatment, and follow-up of patients with advanced thyroid cancer.[162] Furthermore, *RET* fusions are ranked as level IB based on the findings from the ARROW trial. In this prospective, phase II study, pralsetinib showed clinical benefit in patients with previously treated *RET* fusion-positive thyroid cancer, with an ORR of 86% (95% CI 64-97) and a median PFS of 19.4 months (95% CI 13.0-NR).[157] The FDA has granted accelerated approval and the EMA has issued a conditional marketing authorisation for selpercatinib in patients with advanced solid tumours displaying *RET* alterations.[163] Pralsetinib also received FDA accelerated approval for the same indication based on the results of the prospective multicohort ARROW trial.[156] Lastly, the panel designated $BRAF^{V600E}$ mutations as level IB, considering the enhanced clinical outcomes observed in the cohort of pre-treated patients with advanced $BRAF^{V600E}$-mutated anaplastic thyroid cancer in the phase II ROAR basket study.[161] In this trial, dabrafenib plus trametinib displayed a median PFS of 6.7 months (95% CI 4.7-13.8) and a median OS of 14.5 months (95% CI 6.8-23.2). The FDA has granted approval to dabrafenib plus trametinib in this population.

Summary of recommendations

Based on the number of alterations currently classified as level I, ESMO recommends carrying out tumour NGS in patients with advanced thyroid cancer.

Genomic alterations according to ESCAT in unfavourable CUP

Table 12 provides a list of genomic alterations level I/II according to ESCAT in unfavourable CUP.

The use of molecular targeted therapies in CUP is strongly recommended when the respective compound has received cancer type-agnostic approval (*NTRK* fusion-positive, MSI-high, and TMB-high cancers). Likewise, $BRAF^{V600E}$ mutations and *RET* alterations can be considered as cancer type-agnostic targets. In addition, the CUPISCO study displayed an important improvement of PFS (HR 0.72; 95% CI 0.56-0.92; $P = 0.0079$) with molecular-guided therapy compared to chemotherapy as maintenance treatment for patients with unfavourable CUP and actionable genomic alterations. This underscores the crucial role of incorporating comprehensive genomic profiling into the routine practice for these patients.[171] Targeted therapies are also strongly recommended in patients with tumours harbouring a genetic alteration suggestive of a putative primary in which molecular-guided therapies are licensed

Table 12. List of genomic alterations level I/II according to ESCAT in unfavourable cancer of unknown primary

Gene/signature[a]	Alteration	Estimated prevalence	ESCAT score	Drug class matched	References
TMB-H[a]	TMB-H	TMB > 10 (23%) TMB > 16 (12%) TMB > 20 (9%)	IB	PD-1 inhibitors + anti-CTLA4 inhibitors	Ross et al., *Oncologist* 2021[164] Tanizaki et al., *Ann Oncol* 2022[165] Pouyiourou et al., *Ann Oncol* 2022[166] Pouyiourou et al., *Nat Comm* 2023[167]
ALK	Fusions	1%	IIB	ALK TKIs	Ross et al., *JAMA Oncol* 2015[168] Bochtler et al., *Int J Cancer* 2020[169] Ross et al., *Oncologist* 2021[164] Möhrmann et al., *Nat Commun* 2022[170]

CTLA4, cytotoxic T-lymphocyte associated protein 4; ESCAT, ESMO Scale for Clinical Actionability of molecular Targets; PD-1, programmed cell death protein 1; TKIs, tyrosine kinase inhibitors; TMB-H, tumour mutation burden-high.
[a]Signature.

and are the standard of care.[172] In individual cases, NGS might clarify or provide clues regarding the putative primary, although in many cases some ambiguity might remain: NSCLC (*ALK* fusions, *ROS1* fusions), intrahepatic CCA (*FGFR2* fusions), salivary gland carcinoma (*ETV6-NTRK3, MYB*, *MYBL2*, *EWSR1-ATF1*, *MAML2*, *PLAG1*, and *HMG2* fusions, *PRKD1* mutations), NUT carcinoma (*NUTM1* fusions), prostate cancer (*TMPRSS2-ERG* fusions), sarcoma and other mesenchymal tumours (*EWSR1-FLI1, EWSR1-ERG, EWSR1-WT1, EWSR1-POU5F1, HEY1-NCOA2, COL1A1-PDGFB, ETV6-NTRK3, DDIT3, CREB3L2/CREB3L1, TFE3, NAB2-STAT6, SS18(SYT)* and *NR4A3* fusions, *SMARCB1* and *BCOR* alterations, *KIT* mutations), hepatocellular carcinoma (*PRKACA* fusions), renal cell carcinoma (*TFE3* fusions), and breast cancer (*ETV3* fusions). This list is not comprehensive and does not include haematological malignancies. In a prospective, phase II study nivolumab plus ipilimumab showed antitumour activity in patients with CUP relapsed after platinum-based chemotherapy. TMB-H was associated with a higher ORR of 60% (95% CI 15-25) compared to TMB-low, which had an ORR of 7.7% (95% CI 1-25). Moreover, TMB-H exhibited a superior median PFS (HR 0.32; 95% CI 0.09-1.10; $P = 0.056$), as well as a better OS (HR 0.32; 95% CI 0.09-1.09; $P = 0.056$).[167] TMB-H is ranked as level IB.

Summary of recommendations

Given the positive impact on patient outcomes observed with targeted therapy in this population and the capability of NGS to assist in identifying the primary tumour, it is recommended to carry out tumour NGS in patients with unfavourable CUP.

IMPORTANT CONSIDERATIONS WHEN ORDERING AN NGS

Technologies to detect fusion genes

Chromosomal rearrangements can create potent oncogenic fusion genes, which represent important therapeutic targets for precision oncology. Examples of gene fusions for which targeted drugs have been approved are those involving *ALK, ROS1*, *RET*, *FGFR1/2/3,* and *NTRK1/2/3*. In clinical practice, different technologies are employed for the identification of fusion genes. The advantages and limitations of these techniques are described in the following paragraphs. The main point is also to stress the fact that clinicians must assess whether the NGS panel includes the detection of the fusions recommended in a specific disease. NGS panels for gene fusions detection are based on DNA and/or RNA sequencing.[173] DNA sequencing has the advantage that DNA is more stable than RNA. However, DNA sequencing can only identify break points of translocations leading to gene fusions. These breaks may occur in large intronic regions, which may not be fully covered by a gene panel, or in regions comprising homopolymers/segmental duplication/repeated sequences, which can be challenging to sequence. Therefore, precise knowledge of the genomic architecture of a gene fusion of interest is critical, not only for the panel design but also for the interpretation of test results.[174] For example, a test that does not detect a fusion might be false negative if the break point region is not fully covered by the gene panel employed for the diagnostic test. Several limitations of DNA-based fusion gene sequencing are overcome by RNA-based sequencing methods. RNA-based gene panels identify the transcript of the fusion gene resulting from a translocation, and provide data on the 'expression' of the fusion transcript, the fusion partner as well as the potential functionality (e.g. out-of-frame versus in-frame fusions).[175] In contrast to DNA-based assays, it does not provide information on break points. The sensitivity of NGS panels also depends on the technologies used for library preparation: hybrid capture based, amplicon based, or single primer extension (e.g. anchored multiplex PCR).[176] Supplementary Table S3, available at https://doi.org/10.1016/j.annonc.2024.04.005, summarises the pros and cons of the different NGS methods for fusion detection.

Technologies to detect homologous recombination deficiency (HRD)

HRD is indicative that a cell is unable to repair double-strand breaks using the homologous recombination DNA repair (HRR) pathway. To date, this has only been validated for a single clinical indication, namely the use of olaparib and bevacizumab as first-line maintenance in ovarian cancer.[117] The prevalence in ovarian cancer is close to 50%.[177] The clinical utility of this signature as predictor of PARPi response has yet to be demonstrated in breast, prostate, and pancreatic cancers where the prevalence of HRD is comparably high, but under 15%.[178] Supplementary Table S4, available at https://doi.org/10.1016/j.annonc.2024.04.005, summarises the validated methods for detection of HRD.

Incidental results with NGS

The increase in sensitivity in NGS and the extension of the panel size with a large number of genes can expose incidental results from germline variants, secondary cancer, and haematological disease as clonal haematopoiesis.

Incidental germline variants. This question has been addressed by Kuzbari et al. in the ESMO PMWG recommendations.[179]

Secondary cancers. The detection of secondary cancers on the tumour analysis represents the identification of a genetically distinct population of variant in the analysis from the initial solid tumour.[180] The use of large panels and especially on DNA from plasma exposed to the risk of discovering a second cancer (Supplementary Table S5, available at https://doi.org/10.1016/j.annonc.2024.04.005). This should be discussed for any divergence on the initial cancer variants and other dedicated variants to certain cancers. For those cases, a radiological investigation or a serum markers analysis should be proposed to exclude a secondary solid tumour.

Clonal haematopoiesis. Clonal haematopoiesis (CH) on the tumour analysis represents the presence of a genetically distinct population of blood cells in the absence of morphological evidence of haematological malignancies.[181] High-risk CH can be found in patients with solid tumours especially on plasma cell-free DNA sequencing.[182] To be referred as CH of indeterminate potential (CHIP), the somatic variants of haematological malignancy-associated genes should be with a variant allele fraction (VAF) $\geq$2%; however, it can be quite variable depending on the gene. Supplementary Table S6, available at https://doi.org/10.1016/j.annonc.2024.04.005, describes genes involved in CH.

The main driver of CHIP variants is clearly patient age, but other factors can be involved such as chemotherapy, radiation, or smoking.[181,183] The main question is then the risk of transformation to acute myeloid leukaemia or myelodysplastic syndrome. This risk is not well determined except for certain genes as *JAK2*, *MPL,* and *MYD88*.[184] A high burden of variants on myeloid malignancy-associated genes should be followed up—any VAF $>$ 10%.

Health economics evidence

From the payer's point of view, since previous ESMO recommendations, advanced non-squamous NSCLC is the sole indication where the cost-effectiveness of a multigene panel sequencing approach compared with single-marker testing and sequential testing has been demonstrated at acceptable willingness-to-pay thresholds in Europe, Asia, and United States.[185-191] However, uncertainty remains regarding the optimal size of the NGS panel to be used in daily practice. For other indications, although the cost of NGS testing has decreased, relevant economic studies are needed to investigate the cost-effectiveness of a large-scale NGS testing strategy to guide cancer treatment decisions. Moreover, at the country level, the reimbursement/coverage of biomarker-driven therapies is a pre-requisite to the adoption of multigene panel sequencing approaches.

In addition, as shown by a recent survey carried out in Europe by ESMO,[192] organisational and logistics obstacles (e.g. prescription process, access to therapies, molecular tumour board implementation, bioinformatics workflows, trained local personnel and laboratory infrastructures, regulatory and reimbursement environment, etc.) need to be tackled in order to increase the equity of access to biomolecular technologies across and within countries and to deliver results to clinicians in a timely manner.

Finally, the use of genomic scales such as ESCAT is essential to ensure that the adoption of NGS panels in daily practice will lead to an appropriate use of drugs where the level of evidence is high enough (ESCAT I/II). It is a major concern to limit the budget impact of the use of off-label targeted agents where the level of evidence is low and where no or only a small clinical benefit might be expected.[193]

CONCLUSIONS

The ESMO PMWG recommends running tumour NGS in patients with advanced non-squamous NSCLC, breast, colorectal, prostate, and ovarian cancer. In addition, for rare tumours, it is recommended to carry out tumour NGS in patients with advanced CCA, GIST, sarcoma, thyroid cancer, and unfavourable CUP. Finally, ESMO recommends to carry out tumour NGS to detect tumour-agnostic alterations in patients with advanced cancers, in countries where tumour-agnostic targeted therapies are accessible. This recommendation takes into consideration cost-effectiveness and ensures that the sought-after fusions are integrated in the panel.

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