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REVIEW ARTICLE

Recommendations for the use of next-generation sequencing (NGS) for patients with metastatic cancers: a report from the ESMO Precision Medicine Working Group

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Next-generation sequencing (NGS) allows sequencing of a high number of nucleotides in a short time frame at an affordable cost. While this technology has been widely implemented, there are no recommendations from scientific societies about its use in oncology practice. The European Society for Medical Oncology (ESMO) is proposing three levels of recommendations for the use of NGS. Based on the current evidence, ESMO recommends routine use of NGS on tumour samples in advanced non-squamous non-small-cell lung cancer (NSCLC), prostate cancers, ovarian cancers and cholangiocarcinoma. In these tumours, large multigene panels could be used if they add acceptable extra cost compared with small panels. In colon cancers, NGS could be an alternative to PCR. In addition, based on the KN158 trial and considering that patients with endometrial and small-cell lung cancers should have broad access to anti-programmed cell death 1 (anti-PD1) antibodies, it is recommended to test tumour mutational burden (TMB) in cervical cancers, well- and moderately-differentiated neuroendocrine tumours, salivary cancers, thyroid cancers and vulvar cancers, as TMB-high predicted response to pembrolizumab in these cancers.

Outside the indications of multigene panels, and considering that the use of large panels of genes could lead to few clinically meaningful responders, ESMO acknowledges that a patient and a doctor could decide together to order a large panel of genes, pending no extra cost for the public health care system and if the patient is informed about the low likelihood of benefit. ESMO recommends that the use of off-label drugs matched to genomics is done only if an access programme and a procedure of decision has been developed at the national or regional level. Finally, ESMO recommends that clinical research centres develop multigene sequencing as a tool to screen patients eligible for clinical trials and to accelerate drug development, and prospectively capture the data that could further inform how to optimise the use of this technology.

Key words: next-generation sequencing (NGS), genomic alterations, metastatic cancers

INTRODUCTION

Next-generation sequencing (NGS) allows sequencing of a high number of nucleotides in a short time frame and at an affordable cost per patient.[1–3] In this document, we will discuss the clinical utility of using NGS as a

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technology, and how this technology should be used (small versus large panels) in frequent diseases. The recommendations will be done at three levels: from a public health perspective, from the perspective of academic clinical research centres and the level of each individual patient. NGS has recently moved into the clinics with the aim of sequencing long and complex genes and/or multiple genes per tumour sample, in order to identify driver and/or targetable alterations. Pioneering studies have shown that NGS presents a good analytical validity to detect clonally dominant alterations.[4] Based on this observation, several companies and academic centres have implemented NGS assays to guide treatment decisions. While this technology has been widely implemented, there are no recommendations from scientific societies about their use in daily clinical practice. Several prospective trials have reported outcomes associated with the use of multigene sequencing. In the SHIVA trial, the use of multigene sequencing did not improve outcome in patients with metastatic hard-to-treat cancers in comparison with unmatched therapies.[5] In the single-arm MOSCATO trial, the use of multigene sequencing and comparative genomic hybridisation (CGH) arrays was associated with an improved progression-free survival (PFS) in 30% of patients and an objective response rate (ORR) of 11%.[6] Several other studies have consistently reported that ORRs ranged between 10% and 30% in patients whose tumours harboured actionable alterations.[7–10] One of the major issues with most of the prospective trials testing multigene sequencing is the exclusion of patients whose tumours present a genomic alteration that matches an approved drug. Aside from large prospective trials, several cases have been reported to present an outlier sensitivity to a drug given based on an unforeseen, non-recurrent, somatic genomic alteration.[11,12] In the present article, we present the European Society for Medical Oncology (ESMO) recommendations about whether and how tumour multigene NGS could be used to profile metastatic cancers.

METHOD

The ESMO Precision Medicine Working Group has set up a group of experts in the field of clinical cancer genomics in order to address the following questions:

Should NGS be used in daily practice?

If so, should large panels of genes be used?

These questions should be addressed from the perspective of public health, academic clinical research centres and from the perspective of the individual patients.

In order to address these questions, the group developed the method summarised in Figure 1. The general strategy was to determine whether NGS can substitute complex or multiple testings. First, all recurrent genomic alterations were identified in the eight cancers that are associated with highest number of deaths in the world.[13] The ESMO Scale for Clinical Actionability of molecular Targets (ESCAT) ranking was then determined for each

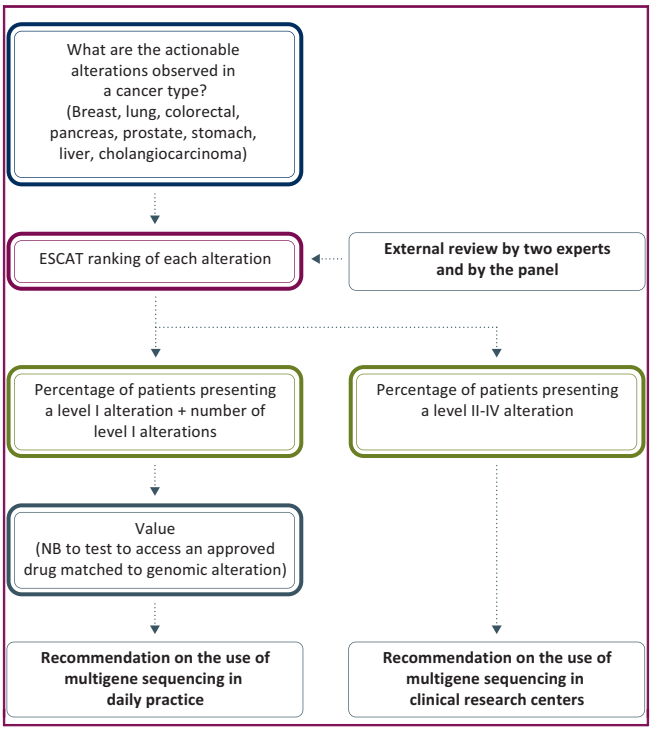

Figure 1. Method to develop recommendation about NGS in daily practice.
ESCAT, ESMO Scale for Clinical Actionability of molecular Targets.

alteration. ESCAT is a framework that ranks a match between drug and genomic alterations, according to their actionability.[14] ESCAT level I means that the match of an alteration and a drug has been validated in clinical trials, and should drive treatment decision in daily practice. ESCAT level II means that a drug that matches the alteration has been associated with responses in phase I/II or in retrospective analyses of randomised trials. ESCAT level III includes alterations that are validated in another cancer, but not in the disease-to-treat. ESCAT level IV includes hypothetically targetable alterations based on preclinical data. ESCAT ranking was generated for each alteration by medical oncologists with an expertise in genomics, then validated by two external experts and by the Working Group. From the ESCAT ranking and prevalence of alterations for each tumour type, we calculated the number of patients to test with NGS, to identify one patient that can be matched to an effective drug in daily practice (ESCAT level I). The main document reports these numbers with the hypothesis that NGS has a perfect analytical validity, while Supplementary Tables, available at https://doi.org/10.1016/j.annonc.2020.07.014, report these numbers taking a hypothesis of 99% and 95% sensitivity/specificity.[15] We assume that there is no proven impact in terms of public health of detecting level II—IV actionable alterations. Finally, in addition to ESCAT ranking, the group integrated the results of the KN158 study[16] in the recommendations. The KN158 study evaluated the efficacy of pembrolizumab single agent according to tumour mutational burden (TMB) in 10 different diseases.

MULTIGENE SEQUENCING: PREREQUISITES FROM THE TECHNICAL SIDE

In vitro diagnostic tests, such as NGS assays, can be broadly separated into two main categories. On one hand, there are manufactured products (reagents, instruments, kits) which have been cleared or approved by the respective authorities [e.g. US Food and Drug Administration (FDA)] and are sold to clinical laboratories for subsequent use. There are numerous instances where there are unmet analytical or clinical needs, not uncommonly due to the lack of approved and commercially available assays; in these cases, laboratory-developed tests (LDTs) are being designed by and deployed for clinical decision-making within a single clinical, often academic, laboratory. In the dynamic and fast-moving field of cancer precision medicine and molecular pathology, LDTs play a central role as they are often driving diagnostic innovation at times when no approved options exist. Regardless of the *in vitro* diagnostic category that is being used in a clinical laboratory, an environment that continuously assures and monitors assay quality and performance is critical, as inadequate validation and use of assays could place patients at risk. Whilst the assessment of test characteristics and quality assurance schemes are governed by country-specific legislation and different regulatory models, technical parameters, including modality of sequencing, sequencing depth, fraction of on-target reads, alignment quality, read quality, error rates, types of sources of DNA [ctDNA, frozen, formalin-fixed paraffin-embedded (FFPE)], minimal tumour cell content are essential and combined under the umbrella of 'analytical validity'. Once the analytical validity and the robustness of the assay are ascertained, its clinical validity and clinical utility need to be considered. Professional groups have endeavoured to provide guidelines for the standardisation of the parameters of sequencing, data analysis and interpretation of the findings, and are listed in Table 1.

In fact, a framework that includes standardised validation protocols and reflects the concepts of (i) analytical validity (i.e. the ability of a test to accurately measure the analyte of interest as e.g. defined by the parameters: accuracy, precision, sensitivity, specificity, positive and negative predictive values), (ii) clinical validity (i.e. the accuracy with which a genetic test identifies a particular clinical condition with respect to a diagnostic, prognostic or predictive category) and (iii) clinical utility (i.e. whether the test and any subsequent interventions result in an improved health outcome among people with a positive test result and the risks that occur as a result of the test being carried out) should be universally considered and applied. ESMO recommends that genomic reports include the ranking of the genomic alterations either by ESCAT or OncoKb.[17]

Table 1. Recommendations and guidelines for the standardisation of multigene sequencing

Society guidelines	Author/journal
Joint Recommendation of the Association for Molecular Pathology and the College of American Pathologists	Roy S, et al. *J Mol Diagn.* 2018.[136]
Canadian College of Medical Geneticists	Hume S, et al. *J Med Genet.* 2019.[137]
College of American Pathologists	www.cap.org 2020.[138]
	Szymanski J, et al. *J Pathol Inform.* 2018.[139]
	Burke W, et al. *Curr Protoc Hum Genet.* 2014.[140]
US FDA	Kaul K, et al. *J Mol Diag.* 2001.[141]
IQN Path	Deans Z, et al. *Virchows Arch.* 2017.[142]
	Matthijs G, et al. *Eur J Hum Genet.* 2015.[143]
A Joint Consensus Recommendation of the Association for Molecular Pathology and College of American Pathologists	Jennings L, et al. *J Mol Diagn.* 2017.[144]
College of American Pathologists	Aziz N, et al. *Arch Pathol Lab Med.* 2015.[145]

FDA, Food and Drug Administration; IQN Path, International Quality Network for Pathology.

RECOMMENDATIONS

General frame

Recommendations for NGS (summarised in Table 2) are done at three levels.

1. Recommendations for daily practice (ESCAT level I) aim to reflect the impact of the use of tumour multigene NGS on public health.
2. Recommendations for clinical research centres aim to determine whether performing multigene sequencing could increase access to innovation, accelerate drug development and could therefore be a mission of clinical research centres.
3. Patient-centric recommendations.

Health economics evidence

From a payer perspective, evidence of the cost-effectiveness of the use of multigene sequencing in daily practice is weak.[18–21] We identified two economic studies in non-small-cell lung cancer (NSCLC). The first one has compared the performance of targeted NGS panels with traditional assays in an *EGFR*-mutant predominant population.[22] The second one has studied the cost-effectiveness of multigene panel sequencing compared with single-marker testing.[23] These studies suggest that multigene sequencing in NSCLC is moderately cost-effective. Moreover, implementation of multigene sequencing in daily practice requires investments that have to be considered, especially regarding sequencing and bioinformatics workflows in order to deliver results to clinicians in a timely manner.[24] ***Finally, from a public health perspective, it must also be considered that the results of NGS panels could lead to recommend expensive drugs outside of their approved indications.[25] There is a need to regulate the volumes of NGS procedures at the national level.***

GENOMIC ALTERATIONS IN ADVANCED NON-SQUAMOUS NSCLC CLASSIFIED ACCORDING TO ESCAT

EGFR mutations represent the first driver alterations identified in advanced non-squamous NSCLC.[26] Most of them

Table 2. Summary recommendations

Tumour types	General recommendations for daily practice	Recommendation for clinical research centres	Special considerations for patients
Lung adenocarcinoma	Tumour multigene NGS to assess level I alterations. Larger panels can be used only on the basis of specific agreements with payers taking into account the overall cost of the strategy (drug included[a]) and if they report accurate ranking of alterations. NGS can either be done on RNA or DNA, if it includes level I fusions in the panel.	It is highly recommended that clinical research centres perform multigene sequencing in the context of molecular screening programmes in order to increase access to innovative drugs and to speed up clinical research. This is particularly relevant in breast, pancreatic and hepatocellular cancers where level II–IV alterations are numerous.	Using large panels of genes could lead to few clinically meaningful responders, not detected by small panels or standard testings. In this context and outside the diseases where large panels of genes are recommended, ESMO acknowledges that a patient and a doctor could decide together to order a large panel of genes, pending no extra cost for the public health care system, and if the patient is informed about the low likelihood of benefit.
Squamous cell lung cancers	No current indication for tumour multigene NGS		
Breast cancers	No current indication for tumour multigene NGS		
Colon cancers	Multigene tumour NGS can be an alternative option to PCR if it does not result in additional cost.		
Prostate cancers	Multigene tumour NGS to assess level I alterations. Larger panels can be used only on the basis of specific agreements with payers taking into account the overall cost of the strategy and if they report accurate ranking of alterations.		
Gastric cancers	No current indication for tumour multigene NGS		
Pancreatic cancers	No current indication for tumour multigene NGS		
Hepatocellular carcinoma	No current indication for tumour multigene NGS		
Cholangiocarcinoma	Multigene tumour NGS could be recommended to assess level I alterations. Larger panels can be used only on the basis of specific agreements with payers taking into account the overall cost of the strategy (drug included[a]) and if they report accurate ranking of alterations. RNA-based NGS can be used.		
Others	Tumour multigene NGS can be used in ovarian cancers to determine somatic *BRCA1/2* mutations. In this latter case, larger panels can be used only on the basis of specific agreements with payers taking into account the overall cost of the strategy (drug included[a]) and if they report accurate ranking of alterations. Large panel NGS can be used in carcinoma of unknown primary. It is recommended to determine TMB in cervical cancer, salivary cancer, thyroid cancers, well-to-moderately differentiated neuroendocrine tumours, vulvar cancer, pending drug access (and in TMB-high endometrial and SCL cancers if anti-PD1 antibody is not available otherwise).		

anti-PD1, anti-programmed cell death 1; DRUP, drug rediscovery protocol; ESMO, European Society for Medical Oncology; NGS, next-generation sequencing; SCL, small-cell lung cancer; TMB, tumour mutational burden.

[a] ESMO recommends using off-label drugs matched to genomics only if an access programme and a procedure of decision have been developed at the national or regional level, as illustrated by the DRUP programme.

are in-frame activating deletions in exon 19 and point hotspot activating mutations in exon 21 (*L858R*), followed by acquired resistant mutations in exon 20 (*T790M*). Several randomised, phase III trials have shown that EGFR tyrosine kinase inhibitors (TKIs) improve outcome in patients with *EGFR*-mutated NSCLC.[27–30] Based on these data, these specific *EGFR* mutations reach the highest level in ESCAT. Point mutations or duplications in exons 18–21 (*G719X* in exon 18, *L861Q* in exon 21, *S768I* in exon 20) are unusual *EGFR* mutations. The efficacies of afatinib and osimertinib were assessed in prospective, non-randomised trials, reporting a high ORR and improving PFS.[31,32] In addition, in patients with exon 20 insertions of *EGFR*, poziotinib (a selective TKI) presented a limited therapeutic efficacy, also evaluated in prospective studies.[33,34] Another predictive biomarker that reaches a high position in the ESCAT is *ALK* fusion. In randomised trials, anaplastic lymphoma kinase (ALK) inhibitors confirmed an improvement of clinical outcomes across patients with *ALK*-rearranged NSCLC.[35-39] Some other alterations like *MET* exon 14 skipping, $BRAF^{V600E}$ mutations and *ROS1* fusions have been identified.[40] A significant ORR and clinical meaningful benefit have been shown in phase I/II studies in patients with NSCLC with *METex14* mutations treated with MET TKIs such

as crizotinib, capmatinib or tepotinib, with *BRAF*^{V600E} mutations that received dabrafenib-vemurafenib and with *ROS1* fusions treated with crizotinib, ceritinib or entrectinib.[41–47] No randomised trials were developed for these aberrations. Based on these results, crizotinib obtained the Breakthrough Designation from the FDA for *METex14*-mutated NSCLC, entrectinib for *ROS1*-positive NSCLC by the FDA and dabrafenib-vemurafenib was approved for NSCLC with *BRAF*^{V600E} mutation by both the FDA and the European Medicines Agency (EMA). Fusions involving neurotrophic tyrosine receptor kinase genes (*NTRK1-3*) occur with a low prevalence across different cancer types. Tropomyosin receptor kinase (TRK) inhibitors (larotrectinib, entrectinib) have demonstrated durable responses in *NTRK* fusion-positive tumours including NSCLC,[48–50] leading to agnostic drug approvals by the EMA and FDA. In addition, LOXO-292 showed efficacy in phase I/II studies in patients with *RET* fusion-positive NSCLC, receiving the FDA Breakthrough Designation.[51] Several other drivers with therapeutic potential have been identified including *MET* amplifications, *KRAS*^{G12C} mutations (AMG510) and *ERBB2* mutations and amplifications.[52–57] Although it has been suggested that TMB-high ($\geq$10 mut/Mb) could be a potential predictive biomarker for immune checkpoint inhibitors (ICIs), this data is not mature enough to drive decisions in NSCLC.[58] Finally, some alterations validated in other tumour types can be found in patients with NSCLC, but no evidence for drug efficacy has been reported yet (Table 3A).[59–63] In Table 3B, we have described the main molecular variations classified by ESCAT in advanced squamous NSCLC.

Summary of recommendations. It is recommended that a tumour (or plasma) sample from a patient with advanced non-squamous NSCLC is profiled using NGS technology, in order to detect level I alterations. Considering the high frequency of fusions, RNA-based NGS, or DNA-based NGS designed to capture such fusions, are the preferred options. There is no evidence that panels detecting genes with a lower level of evidence brings additional value from a public health perspective. They could be used only if the report ranks genomic alterations according to valid ranking systems (e.g. ESCAT, OncoKB) and on the basis of specific agreements with payers taking into account the overall cost of the strategy (including off-label use of drugs) as compared with small panels. Regarding this latter point, ESMO does not recommend the use of off-label drugs matched to genomic alterations, except if an access programme and a procedure of decision has been developed at the national or regional level, as illustrated by the drug rediscovery protocol programme.[64] It is recommended that hospitals that run drug

Table 3A. List of genomic alterations level I/II/III according to ESCAT in advanced non-squamous non-small-cell lung cancer (NSCLC)

Gene	Alteration	Prevalence	ESCAT	References
EGFR	Common mutations (*Del19, L858R*)	15% (50%–60% Asian)	IA	Midha A, et al. *Am J Cancer Res.* 2015[26]
	Acquired *T790M* exon 20	60% of *EGFR* mutant	IA	Mok T, et al. *J Clin Oncol.* 2018[27]
	Uncommon *EGFR* mutations (*G719X* in exon 18, *L861Q* in exon 21, *S768I* in exon 20)	NSCLC	IB	Soria J-C, et al. *N Engl J Med.* 2018[28]
		10%	IIB	Ramalingam S, et al. *N Engl J Med.* 2020[29]
	Exon 20 insertions	2%		Mok T, et al. *N Engl J Med.* 2017[30]
				Yang JC-H, et al. *Lancet Oncol.* 2015[31]
				Cho J, et al. *J Thorac Oncol.* 2018[32]
				Cardona A, et al. *Lung Cancer.* 2018[33]
				Heymach J, et al. *J Thorac Oncol.* 2018[34]
ALK	Fusions (mutations as mechanism of resistance)	5%	IA	Solomon B, et al. *J Clin Oncol.* 2018[35]
				Soria J-C, et al. *Lancet.* 2017[36]
				Peters S, et al. *N Engl J Med.* 2017[37]
				Zhou C, et al. *Ann Oncol.* 2018[38]
				Camidge D, et al. *N Engl J Med.* 2018[39]
MET	Mutations *ex 14 skipping*	3%	IB	Tong J, et al. *Clin Cancer Res.* 2016[40]
				Drilon A, et al. *Nat Med.* 2020[41]
	Focal amplifications (acquired resistance on EGFR TKI in *EGFR*-mutant tumours)	3%	IIB	Camidge D, et al. *J Clin Oncol.* 2018[52]
BRAF^{V600E}	Mutations	2%	IB	Planchard D, et al. *Lancet Oncol.* 2016[42]
				Planchard D, et al. *Lancet Oncol.* 2017[43]
				Planchard D, et al. *J Clin Oncol.* 2017[44]
ROS1	Fusions (mutations as mechanism of resistance)	1%–2%	IB	Shaw A, et al. *N Engl J Med.* 2014[45]
				Shaw A, et al. *Ann Oncol.* 2019[46]
				Drilon A, et al. *Lancet Oncol.* 2020[47]
NTRK	Fusions	0.23%–3%	IC	Drilon A, et al. *N Engl J Med.* 2018[48]
				Hong D, et al. *Lancet Oncol.* 2020[49]
				Doebele RC, et al. *Lancet Oncol.* 2020[50]
RET	Fusions	1%–2%	IC	Drilon A, et. *J Thorac Oncol.* 2019[51]
KRAS^{G12C}	Mutations	12%	IIB	Barlesi F, et al. *Lancet.* 2016[53]
				Fakih M, et al. *J Clin Oncol.* 2019[54]
ERBB2	Hotspot mutations	2%–5%	IIB	Hyman D, et al. *Nature.* 2018[55]
	Amplifications			Wang Y, et al. *Ann Oncol.* 2018[56]
				Tsurutani J, et al. *J Thorac Oncol.* 2018[57]
BRCA 1/2	Mutations	1.2%	IIIA	Balasubramaniam S, et al. *Clin Cancer Res.* 2017[63]
PIK3CA	Hotspot mutations	1.2%–7%	IIIA	Cancer Genome Atlas Research Network. *Nature.* 2014[60]
				Vansteenkiste J, et al. *J Thorac Oncol.* 2015[62]
NRG1	Fusions	1.7%	IIIB	Duruisseaux M, et al. *J Clin Oncol.* 2019[59]

Table 3B. List of genomic alterations level I/II/III according to ESCAT in advanced squamous NSCLC

Gene	Alteration	Prevalence	ESCAT	References
NTRK	Fusions	0.23%–3%	IC	Drilon A, et al. *N Engl J Med.* 2018[48] Hong D, et al. *Lancet Oncol.* 2020[49] Doebele RC, et al. *Lancet Oncol.* 2020[50]
PIK3CA	Hotspot mutations	16%	IIIA	Cancer Genome Atlas Research Network, *Nature.* 2012[61] Vansteenkiste J, et al. *J Thorac Oncol.* 2015[62]
BRCA 1/2	Mutations	1.2%	IIIA	Balasubramaniam S, et al. *Clin Cancer Res.* 2017[63]

ESCAT, European Society for Medical Oncology (ESMO) Scale for Clinical Actionability of molecular Targets.

development programmes and clinical trials run multigene sequencing in the context of molecular screening programmes, since lung cancer presents some level II–IV alterations.

GENOMIC ALTERATIONS IN METASTATIC BREAST CANCER CLASSIFIED ACCORDING TO ESCAT

ERBB2 amplifications are predictive of clinical benefit of anti-HER2 therapies, which yield an improvement of overall survival (OS) and PFS,[65–69] while neratinib (an irreversible pan-HER TKI) has been associated with responses in patients with *ERBB2* mutations.[55] Phase III studies reported a significant improvement of PFS with poly ADP ribose polymerase inhibitors (PARPi) in patients with germline *BRCA1/2*-mutated metastatic breast cancer (mBC).[70,71] It is currently estimated that somatic multigene sequencing cannot substitute germline testing for *BRCA1/2* status. Alpelisib, an α-selective phosphatidylinositol 3-kinase (PI3K) inhibitor, improves PFS in patients with HR+/HER2− mBC that harbours *PIK3CA* hotspot mutations, and is approved in this group of patients.[72] Drugs targeting rare alterations found in different solid tumours, like microsatellite instability-high (MSI-H) and *NTRK* fusions, obtained approvals across tumour types.[50,73] Nevertheless, *NTRK* fusions highly correlate with secretory phenotype and MSI-high tumours are enriched in triple-negative breast cancers (TNBCs), where anti-PDL1 antibodies are approved. *ESR1* mutations occur in around 20% of patients previously treated with aromatase inhibitors and are associated with response to selective estrogen receptor degraders.[74] Nevertheless, these data are preliminary and cannot be used in daily practice. Other promising targets in mBC are phosphatase and tensin homologue (*PTEN*) loss of function mutations and/or homozygous deletions (TNBCs) and $AKT1^{E17K}$ mutations, which in retrospective and prospective analyses, respectively, showed a clinical benefit and increased responsiveness to AKT inhibitors. Nevertheless, no results are available from practice changing trials yet.[75,76] In addition, *NF1* mutations were identified as a mechanism of endocrine resistance, but there is no targeted therapy available yet in this genomic segment.[77] Lastly, there are some alterations with no major impact in mBC that are validated in other malignances (Table 4).[55,63,78]

Summary of recommendations. Considering that somatic sequencing cannot fully substitute germline BRCA testing, that PIK3CA status can be determined by PCR on the three hotspots and pending that HER2 testing is accurately done by immunohistochemistry (IHC) in the local centre, there is currently no need to perform tumour multigene NGS for patients with mBC in the context of daily practice. From the perspective of clinical research centres, and considering the high number of level II alterations, it is important to include mBC patients in molecular screening programmes and include them in trials testing targeted therapies matched to genomic alterations ($AKT1^{E17K}$, PTEN, ERBB2 mutations, ESR1 and NF1 mutations).

GENOMIC ALTERATIONS IN METASTATIC COLORECTAL CANCER CLASSIFIED ACCORDING TO ESCAT

Pivotal randomised trials and meta-analysis highlighted that hotspot *RAS* mutations (*K-RAS* and *N-RAS*) predict resistance to EGFR monoclonal antibodies (mAbs) in the metastatic setting.[79–81] https://doi.org/10.1093/annonc/mdw235. The addition of encorafenib (a BRAF inhibitor) to cetuximab was associated with a significant survival benefit in a recent phase III trial in patients presenting a $BRAF^{V600E}$ mutation.[82] Alterations in mismatch repair proteins (MLH1, MSH2, MSH6 and PMS2) can be identified by IHC and MSI-H by PCR to detect smaller length DNA fragments. Testing for MSI-H is of great clinical interest in metastatic colorectal cancer (mCRC) because it predicts the efficacy of pembrolizumab and nivolumab in this setting.[83,84] As mentioned before, TRK inhibitors showed high efficacy in multi-histology trials in *NTRK* fusion-positive tumours[50,85]; and mCRC with *ERBB2* amplifications/overexpression (detected with FISH or IHC) presented significant responses with dual HER2 therapy in prospective studies.[86,87] In Table 5 we mention the main driver alterations categorised according to ESCAT, including those with a lack of clinical data in mCRC, but with impact in other tumours.[76,88–94]

Summary of recommendations. Since most level I alterations are hotspot mutations in KRAS, NRAS and BRAF, and considering that MSI status is determined by IHC or PCR, there is no need to test samples using multigene NGS in the context of daily practice. Nevertheless, multigene NGS can be an alternative to PCR tests only if it does not generate extra cost compared with standard techniques already implemented in routine. This would allow detection of ERBB2 amplifications, and, in some panels, detect MSI status with high accuracy. If large panel NGS is carried out, it should include detection of NTRK fusions. As for mBC patients, patients with mCRC can present oncogenic alterations for which drugs are being developed and it is

Table 4. List of genomic alterations level I/II/III according to ESCAT in metastatic breast cancer (mBC)

Gene	Alteration	Prevalence	ESCAT	References
ERBB2	Amplifications	15%—20%	IA	Slamon D, et al. *N Engl J Med.* 2001[65] Swain S, et al. *N Engl J Med.* 2015[66] Verma S, et al. *N Engl J Med.* 2012[67] Krop I, et al. *Lancet Oncol.* 2014[68] Murthy R, et al. *N Engl J Med.* 2020[69]
	Hotspot mutations	4%	IIB	Hyman D, et al. *Nature.* 2018[55]
PIK3CA	Hotspot mutations	30%—40%	IA	André F, et al. *N Engl J Med.* 2019[72]
BRCA1/2	Germline mutations	4%	IA	Robson M, et al. *N Engl J Med.* 2017[70] Litton J, et al. *N Engl J Med.* 2018[71]
	Somatic mutations	3%	IIIA	Balasubramaniam S, et al. *Clin Cancer Res.* 2017[63]
	MSI-H	1%	IC	Marcus L, et al. *Clin Cancer Res.* 2019[73]
NTRK	Fusions	1%	IC	Doebele RC, et al. *Lancet Oncol.* 2020[50]
ESR1	Mutations (mechanism of resistance)	10%	IIA	Fribbens C, et al. *J Clin Oncol.* 2016[74]
PTEN	Mutations	7%	IIA	Schmid P, et al. *J Clin Oncol.* 2018[75]
AKT1^{E17K}	Mutations	5%	IIB	Hyman D, et al. *J Clin Oncol.* 2017[76]
NF1	Mutations (resistance biomarker)	6%	Not applicable	Pearson A, et al. *Clin Cancer Res.* 2020[77]
MDM2	Amplifications	~1%	IIIA	Dembla V, et al. *Oncotarget.* 2018[78]
ERBB3	Mutations	2%	IIIB	Hyman D, et al. *Nature.* 2018[55]

ESCAT, European Society for Medical Oncology (ESMO) Scale for Clinical Actionability of molecular Targets; MSI-H, microsatellite instability-high.

Table 5. List of genomic alterations level I/II/III according to ESCAT in metastatic colorectal cancer (mCRC)

Gene	Alteration	Prevalence	ESCAT	References
KRAS *NRAS*	Mutations (resistance biomarker)	44% 4%	Not applicable	Van Cutsem E, et al. *J Clin Oncol.* 2015[79] Douillard J-Y, et al. *N Engl J Med.* 2013[80] Sorich M, et al. *Ann Oncol.* 2015[81]
BRAF^{V600E}	Mutations	8.5%	IA	https://doi.org/10.1093/annonc/mdw235 Kopetz S, et al. *N Engl J Med.* 2019[82]
	MSI-H	4%—5%	IA	Overman M, et al. *Lancet Oncol.* 2017[83] Le DT, et al. *J Clin Oncol.* 2020[84]
NTRK1	Fusions	0.5%	IC	Demetri G, et al. *Ann Oncol.* 2018[85] Doebele RC, et al. *Lancet Oncol.* 2020[50]
ERBB2	Amplifications	2%	IIB	Meric-Bernstam F, et al. *Lancet Oncol.* 2019[86] Sartore-Bianchi A, et al. *Lancet Oncol.* 2016[87]
PIK3CA	Hotspot mutations	17%	IIIA	Juric D, et al. *J Clin Oncol.* 2018[90]
ATM	Mutations	5%	IIIA	Wang C, et al. *Transl Oncol.* 2017[92] De Bono J, et al. *N Engl J Med.* 2020[93]
MET	Amplifications	1.7%	IIIA	https://clinicaltrials.gov/ct2/show/NCT03592641[94]
AKT1^{E17K}	Mutations	1%	IIIA	Hyman D, et al. *J Clin Oncol.* 2017[76]
	TMB-high in MSS	1%	IIIA	Fabrizio D, et al. *J Gastrointest Oncol.* 2018[89]
RET	Fusions	0.3%	IIIA	Drilon A, et al. *J Clin Oncol.* 2018[91]
ALK	Fusions	0.2%	IIIA	Yakirevich E, et al. Clin Cancer Res 2016[88]

ESCAT, European Society for Medical Oncology (ESMO) Scale for Clinical Actionability of molecular Targets; MSI-H, microsatellite instability-high; MSS, microsatellite stable.

therefore recommended for clinical research centres to include patients in molecular screening programmes to propose access to innovative agents in clinical trials.

GENOMIC ALTERATIONS IN ADVANCED PROSTATE CANCER CLASSIFIED ACCORDING TO ESCAT

Metastatic castration-resistant prostate cancer (mCRPC) presents aberrations in DNA repair genes with a high frequency (20%—30%). PARPi improved outcomes in patients with different DNA repair gene alterations in a randomised phase III trial; however, exploratory per-gene analysis suggested that most of the benefit was obtained in patients with *BRCA1/2* somatic mutations.[93] This is supported by multiple phase II trials, where patients with *BRCA1/2* alterations achieved the higher response rates. Data about *PALB2, RAD50, RAD51* or *BRIP1* mutations are promising but sparse due to the low frequency of these aberrations.[93,95] Other genes involved in DNA repair, like *MLH1/MSH2/MSH6* lead to MSI-H when mutated. Therapy with ICIs demonstrated effectiveness in multi-histology basket studies, although in advanced prostate cancer have shown minimal activity.[73,96,97] *PTEN* alterations are found very frequently in mCRPC,[98] and AKT inhibitors in combination with abiraterone showed antitumour activity in a retrospective analysis of a randomised phase II trial.[99] Preliminary results of IPATential 150, a phase III randomised trial which evaluated ipatasertib (AKT inhibitor) with abiraterone and prednisone compared with standard therapy, showed an improvement of radiographic PFS (co-primary end point) in patients with PTEN loss and mCRPC, but not in the overall population.[100] Some alterations ranked level I/II in other diseases are observed in prostate cancer, but are not yet validated[101] (see Table 6).

Summary of recommendations. In countries where PARPi are accessible for patients with prostate cancer, it is recommended to perform NGS on tumour samples to assess the mutational status of, at least, BRCA1/2. According to the preliminary results of the phase III trial with AKT inhibitors in patients with PTEN alterations, this gene

Table 6. List of genomic alterations level I/II/III according to ESCAT in advanced prostate cancer

Gene	Alteration	Prevalence	ESCAT	References
BRCA1/2	Somatic mutations/deletions	9%	IA	De Bono J, et al. *N Engl J Med.* 2020[93]
	MSI-H	1%	IC	Cortes-Ciriano I, et al. *Nat Commun.* 2017[96] Abida W, et al. *J Clin Oncol.* 2018[97] Marcus L, et al. *Clin Cancer Res.* 2019[97]
PTEN	Deletions/mutations	40%	IIA[a]	Abida W, et al. *Proc Natl Acad Sci.* 2019[98] De Bono J, et al. *Clin Cancer Res.* 2019[99] NCT03072238[100]
ATM	Mutations/deletions	5%	IIA	De Bono J, et al. *N Engl J Med.* 2020[93]
PALB2	Mutations	1%	IIB	Mateo J, et al. *N Engl J Med.* 2015[95] De Bono J, et al. *N Engl J Med.* 2020[93]
PIK3CA	Hotspot mutations	3%	IIIA	Crumbaker M, et al. *Cancers.* 2017[101]
AKT1^{E17K}	Mutations	1%	IIIA	Crumbaker M, et al. *Cancers.* 2017[101]

ESCAT, European Society for Medical Oncology (ESMO) Scale for Clinical Actionability of molecular Targets; MSI-H, microsatellite instability-high; *PTEN*, phosphatase and tensin homologue.

[a] A press release suggests that AKT inhibitors could work specifically in *PTEN*-mutant prostate cancers. *PTEN* could be upgraded to IA depending on the magnitude of benefit and peer review assessment of the report.

could be added to the panel. Given that they are unlikely to be cost-effective in these cases, larger panels can be used only on the basis of specific agreements with payers taking into account the overall cost of the strategy (including off-label use of drugs) and pending a ranking of additional alterations using a valid ranking system. These panels should include DNA repair genes and MSI signature.

GENOMIC ALTERATIONS IN METASTATIC GASTRIC CANCER CLASSIFIED ACCORDING TO ESCAT

ERBB2 amplifications are observed in around 15% of gastric cancers.[102] In these patients, trastuzumab demonstrated a significant improvement of OS in randomised trials.[103] According to basket trials, patients with MSI-H and *NTRK* fusion-positive tumours treated with ICIs and TRK inhibitors are expected to provide benefit.[48,73] Some limited responses were observed in patients with *EGFR*- and *MET*-amplified metastatic gastric cancer (mGC) treated with cetuximab and crizotinib in prospective analysis.[104,105] These findings require further investigation. In addition, many other level I/II aberrations of other cancer types are observed in gastric cancer, but not validated in this latter disease.[46,55,63,90,106–110] All these alterations are described in Table 7.

Summary of recommendations. There is no current need to perform tumour multigene NGS in patients with mGC in daily practice. Detection of MSI and NTRK fusions should be done using cheap standard methods.

Table 7. List of genomic alterations level I/II/III according to ESCAT in metastatic gastric cancer (mGC)

Gene	Alteration	Prevalence	ESCAT	References
ERBB2	Amplifications	16%	IA	The Cancer Genome Atlas Research Network. *Nature.* 2014[102] Bang Y-J, et al. *Lancet.* 2010[103]
	Hotspot mutations	3%	IIIA	Hyman D, et al. *Nature.* 2018[55]
	MSI-H	8%	IC	The Cancer Genome Atlas Research Network. *Nature.* 2014[102] Marcus L, et al. *Clin Cancer Res.* 2019[97]
NTRK	Fusions	2%	IC	Drilon A, et al. *N Engl J Med.* 2018[48]
EGFR	Amplifications	6%	IIB	Maron S, et al. *Cancer Discov.* 2018[104]
MET	Amplifications	3%	IIB	Lennerz J, et al. *J Clin Oncol.* 2011[105]
	Mutations	1.3%	IIIA	Lee J, et al. *Oncotarget.* 2015[107]
PIK3CA	Hotspot mutations	7%	IIIA	Juric D, et al. *J Clin Oncol.* 2018[90]
FGFR2	Amplifications	4%	IIIA	Van Cutsem E, et al. *Ann Oncol.* 2017[109] Loriot Y, et al. *N Engl J Med.* 2019[110]
ATM	Mutations	3%	IIIA	Bang Y-J, et al. *Lancet Oncol.* 2017[108]
BRCA1/2	Mutations	1%–5%	IIIA	Balasubramaniam S, et al. *Clin Cancer Res.* 2017[63]
ROS1	Fusions	<1%	IIIA	Shaw A, et al. *Ann Oncol.* 2019[46]
RET	Fusions	<1%	IIIA	Oxnard G, et al. *J Thorac Oncol.* 2018[106]
ERBB3	Hotspot mutations	3%	IIIB	Hyman D, et al. *Nature.* 2018[55]

ESCAT, European Society for Medical Oncology (ESMO) Scale for Clinical Actionability of molecular Targets; MSI-H, microsatellite instability-high.

GENOMIC ALTERATIONS IN ADVANCED PANCREATIC DUCTAL ADENOCARCINOMA CLASSIFIED ACCORDING TO ESCAT

Patients with germline *BRCA1/2*-mutated advanced pancreatic ductal adenocarcinoma (PDAC) presented a longer PFS with maintenance olaparib.[111,112] In advanced PDAC with somatic *BRCA1/2* mutations, an increased response with PARPi has been reported in few patients included in a prospective trial.[113] The panel therefore considered that somatic *BRCA1/2* alterations are not yet validated in advanced PDAC. As we mentioned for other tumours, patients with MSI-H and *NTRK* fusion-positive tumours presented meaningful clinical benefit with matched therapies in multi-histology studies.[50,97,114,115] Several additional alterations are classified at high level according to ESCAT in other tumours, but have not yet shown a significant impact in pancreatic cancer like *KRAS*, *PIK3CA*, *BRAF*^{V600E} mutations, *MDM2*, *ERBB2* amplifications and *NRG1*, *ALK*, *RET*, *ROS1* fusions.[55,91,116–125] The main drivers of PDAC and their classification are described in Table 8.

Summary of recommendations. It is not currently recommended to perform tumour multigene NGS in patients with advanced PDAC in daily practice. Considering the unmet medical needs and the high number of alterations ranked as level II–IV, ESMO considers it is the mission of

Table 8. List of genomic alterations level I/II/III according to ESCAT in advanced pancreatic ductal adenocarcinoma (PDAC)

Gene	Alteration	Prevalence	ESCAT	References
BRCA1/2	Germline mutations	1%—4%	IA	The Cancer Genome Atlas Research Network. *Cancer Cell.* 2017[111] Golan T, et al. *N Engl J Med.* 2019[112]
	Somatic mutations	3%	IIIB	Shroff R, et al. *JCO Precis Oncol.* 2018[113]
	MSI-H	1%—3%	IC	Pihlak R, et al. *Cancers.* 2018[115] Marcus L, et al. *Clin Cancer Res.* 2019[97]
NTRK	Fusions	<1%	IC	Cocco E, et al. *Nat Rev Clin Oncol.* 2018[114] Doebele RC, et al. *Lancet Oncol.* 2020[50]
KRAS	Mutations	90%	IIIA	Zeitouni D, et al. *Cancers.* 2016[116]
PIK3CA	Hotspot mutations	3%	IIIA	Heestand G, et al. *Oncotarget.* 2015[117] Payne S, et al. *J Clin Oncol.* 2015[118]
BRAF^{V600E}	Mutations	3%	IIIA	Hyman D, et al. *N Engl J Med.* 2015[119]
MDM2	Amplifications	2%	IIIA	Azmi A, et al. *Eur J Cancer.* 2010[120]
ERBB2	Amplifications/ mutations	1%—2%	IIIA	Waddell N, et al. *Nature.* 2015[121] Harder J, et al. *Br J Cancer.* 2012[122] Hyman D, et al. *Nature.* 2018[55]
NRG1	Fusions	1%	IIIA	Jones M, et al. *Clin Cancer Res.* 2019[123]
ALK	Fusions	<1%	IIIA	Singhi A, et al. *J Natl Compr Canc Netw.* 2017[124]
RET	Fusions	<1%	IIIA	Drilon A, et al. *J Clin Oncol.* 2018[91]
ROS1	Fusions	<1%	IIIA	Pishvaian M, et al. *J Clin Oncol.* 2018[125]

ESCAT, European Society for Medical Oncology (ESMO) Scale for Clinical Actionability of molecular Targets; MSI-H, microsatellite instability-high.

clinical research centres and their networks to propose multigene sequencing to patients with advanced PDAC in the context of molecular screening programmes, in order for patients to get access to innovative drugs. If multigene sequencing is not carried out, detection of MSI and NTRK fusions should be done using cheaper standard methods, pending drugs are approved and reimbursed.

GENOMIC ALTERATIONS IN ADVANCED HEPATOCELLULAR CARCINOMA CLASSIFIED ACCORDING TO ESCAT

While numerous aberrations are being evaluated, very few targets currently have impact on clinical decisions.[126] As we described for the majority of cancers, due to their clinical benefit larotrectinib and ICIs were approved for patients with *NTRK* fusion-positive and MSI-H solid tumours, respectively, who have no alternative treatments.[48,97] There are also other alterations with strong benefit across different tumour types like *PIK3CA*, *RAS* mutations and *MET* amplifications,[72,127,128] and no clinical evidence in this disease (Table 9).

Table 9. List of genomic alterations level I/II/III according to ESCAT in advanced hepatocellular carcinoma (HCC)

Gene	Alteration	Prevalence	ESCAT	References
NTRK	Fusions	1%	IC	The Cancer Genome Atlas Research Network. *Cancer Cell.* 2017[111] Drilon A, et al. *N Engl J Med.* 2018[48]
	MSI-H	1%	IC	Marcus L, et al. *Clin Cancer Res.* 2019[97]
PIK3CA	Hotspot mutations	4%	IIIA	André F, et al. *N Engl J Med.* 2019[72]
MET	Amplifications	2%—6%	IIIA	Rimassa L, et al. *Lancet Oncol.* 2018[127]
RAS	Mutations	2%	IIIA	Lim H, et al. *Clin Cancer Res.* 2018[128]

ESCAT, European Society for Medical Oncology (ESMO) Scale for Clinical Actionability of molecular Targets; MSI-H, microsatellite instability-high.

Summary of recommendations. It is not currently recommended to perform tumour multigene NGS in patients with advanced hepatocellular carcinoma (HCC) in daily practice. Considering the unmet medical needs and the number of alterations ranked as level II—IV, ESMO considers it is the mission of clinical research centres to propose multigene sequencing to patients with advanced HCC in the context of molecular screening programmes. If multigene sequencing is not carried out, detection of MSI and NTRK fusions should be done using cheaper standard methods, pending drugs are approved and reimbursed.

GENOMIC ALTERATIONS IN ADVANCED CHOLANGIOCARCINOMA CLASSIFIED ACCORDING TO ESCAT

IDH1 mutations are ranked level I in ESCAT (IA).[129] In addition, pemigatinib, a selective fibroblast growth factor receptor (FGFR)1,2,3 inhibitor, led to a 35% ORR in patients with advanced *FGFR2* fusion-positive cholangiocarcinoma (CC) in a prospective phase II trial,[130] getting accelerated approval by the FDA. As we mentioned previously, patients with MSI-H and *NTRK* fusion-positive tumours presented clinically meaningful benefit with ICIs and TRK inhibitors in basket studies.[50,131] Finally, rapidly accelerated fibrosarcoma/mitogen-activated protein kinase kinase inhibitors were associated with 42% OR in patients with advanced CC and *BRAF*^{V600E} mutations[132] (Table 10). In Table 10 are also described some alterations with efficacy in other tumours, but not yet validated in this disease.[52,72,93,133]

Summary of recommendations. Tumour multigene NGS could be used to detect level I actionable alterations in cholangiocarcinoma. Given that they are unlikely to be cost-effective in these cases, larger panels can be used only on the basis of specific agreements with payers taking into account the overall cost of the strategy (including off-label use of drugs) and pending a

Table 10. List of genomic alterations level I/II/III according to ESCAT in advanced cholangiocarcinoma (CC)

Gene	Alteration	Prevalence	ESCAT	References
IDH1	Mutations	20%	IA	Abou-Alfa G. K, et al. *Ann Oncol.* 2019[129]
FGFR2	Fusions	15%	IB	Vogel A, et al. *Ann Oncol.* 2019[130]
	MSI-H	2%	IC	Marabelle A, et al. *J Clin Oncol.* 2020[131]
NTRK	Fusions	2%	IC	Doebele RC, et al. *Lancet Oncol.* 2020[50]
BRAF^{V600E}	Mutations	5%	IIB	Wainberg Z, et al. *J Clin Oncol.* 2019[132]
ERBB2	Amplifications Mutations	10% 2%	IIIA	Javle MM, et al. *J Clin Oncol.* 2017[133]
PIK3CA	Hotspot mutations	7%	IIIA	André F, et al. *N Engl J Med.* 2019[72]
BRCA 1/2	Mutations	3%	IIIA	De Bono J, et al. *N Engl J Med.* 2020[93]
MET	Amplifications	2%	IIIA	Camidge D, et al. *J Clin Oncol.* 2018[52]

ESCAT, European Society for Medical Oncology (ESMO) Scale for Clinical Actionability of molecular Targets.

ranking of additional alterations using a valid ranking system.

Other tumour types. While the systematic ranking of genomic alterations was done exclusively for the eight more frequent killers, we also assessed the frequency of level I alterations in other tumour types. In ovarian cancers, where *BRCA1/2* somatic mutations have been associated with increased benefit to PARPi,[134] the use of multigene NGS is justified. Larger panels can be used only on the basis of specific agreements with payers taking into account the overall cost of the strategy (including off-label use of drugs) and pending an appropriate method of reporting. While there is no level I evidence, multigene sequencing could also be used in carcinoma of unknown primary.[135]

Specific situations

Tumour mutational burden and KN158 study. KN158 has evaluated the efficacy of pembrolizumab according to TMB in 10 cancers (anal cancer, cervical cancer, endometrial cancer, small-cell lung cancer (SCLC), salivary cancer, thyroid cancers, well-to-moderately differentiated neuroendocrine tumours (NETs), biliary cancers, vulvar cancer, mesothelioma). Response rates were 27% and 7% in patients with TMB-high (MSI-low) or TMB-low cancers, respectively. There was no TMB-high detected in biliary cancers, and the percentage of response was lower in TMB-high in anal cancer and mesothelioma. We can classify TMB as level IIA according to ESCAT. If we consider that indications of anti-PD(L)1 antibodies are broad in endometrial cancers and SCLC, the ***TMB should be determined only in cervical cancer, NET, salivary cancers, vulvar cancers, thyroid cancers. Considering that the study was not agnostic, but limited to few cancers, the group thinks that additional studies are needed before implementing TMB in all cancers where anti-PD(L)1 antibodies are not approved.***

NTRK fusions. TRK inhibitors have been shown to be effective in a broad range of cancers. *NTRK* fusions occur in $<$1% of cancers. The incidence of *NTRK* fusions is very high in mammary analogue secretory carcinoma of salivary glands and in secretory breast cancers. A high incidence is also observed in sarcoma and thyroid cancers. ***Considering the very low incidence, the group recommends using NGS to detect NTRK fusions only in cancers where this technology is recommended otherwise. In cancers where there is no need for multigene sequencing, it was considered that the detection of NTRK fusion is not an argument per se to recommend NGS since alternative, cheaper, diagnostic methods exist. Such alternative, cheaper methods should be prioritised to screen patients for NTRK fusions, in countries where TRK inhibitors are available.***

CONCLUSION

ESMO recommends using tumour multigene NGS in patients presenting with advanced non-squamous NSCLC, prostate, ovarian cancers and cholangiocarcinoma. Large panels of genes can be used if they generate only an acceptable increase in the overall cost, drugs included. In addition, based on KN158, it is recommended to determine TMB in cervical cancer, salivary cancer, thyroid cancers, well-to-moderately differentiated NETs, vulvar cancer, pending drug access. In colorectal cancers, NGS can be an alternative to PCR-based tests, if it is not associated with extra cost. ESMO strongly recommends that clinical research centres perform multigene sequencing as part of their missions to accelerate cancer research and drug development through clinical trials, provide access to innovation to patients and to collect data. In addition, economic evaluations alongside clinical trials should also be implemented to foster evidence in this field. Outside the indications mentioned before, and considering that the use of large panels of genes could lead to identification of few exceptional responders, ESMO acknowledges that a patient and a doctor could decide together to order a large panel of genes, pending no extra cost for the public health care system, and if the patient is informed about the low likelihood of benefit.

These recommendations will need to be updated on a regular basis as new data emerges for novel therapies across tumour types.

ACKNOWLEDGEMENT

This is a project initiated by the ESMO Translational Research and Precision Medicine Working Group. We would also like to thank ESMO leadership for their support in this manuscript.

FUNDING

This work was supported by the European Society for Medical Oncology (no grant number is applicable).

DISCLOSURE

JR: advisory: Merck Sharp & Dohme (MSD), Boehringer, Bristol-Myers Squibb (BMS), AstraZeneca, Roche; speaker's bureau: Pfizer; travel support: OSE Immunotherapeutics SA, BMS, AstraZeneca, Roche. JM: advisory board: Amgen, AstraZeneca, Clovis Oncology, Janssen, MSD and Roche-Foundation Medicine; research funding: AstraZeneca and Pfizer Oncology; principal investigator of several industry sponsored clinical trials. CBW: personal and speakers' fees, reimbursement for travel and accommodation and honoraria for participance in advisory boards from Bayer, Celgene, Ipsen, MedScape, Rafael Pharmaceuticals, RedHill, Roche, Servier, Shire/Baxalta and Taiho; scientific grant support by Roche. FB: personal fees from AstraZeneca, Bayer, Bristol-Myers Squibb, Boehringer—Ingelheim, Eli Lilly Oncology, F. Hoffmann—La Roche Ltd, Novartis, Merck, MSD, Pierre Fabre, Pfizer and Takeda. MPL: research grants (to hospital): MSD, Astellas, JnJ, Sanofi; advice: Roche, Bayer, Amgen, JnJ, Sanofi, Servier, Pfizer, Incyte. NN: speaker's fee and/or advisory boards: Amgen, AstraZeneca, Bayer, Biocartis, BMS, Boehringer Ingelheim, Eli Lilly, Illumina, Incyte, MERCK, MSD, Qiagen, Roche, Thermofisher, Sanofi; institutional financial interests (financial support to research projects): AstraZeneca, Biocartis, BMS, Illumina, Merck, Qiagen, Roche, Sysmex, Thermofisher; non-financial interests: President of the International Quality Network for Pathology (IQN Path); President of the Italian Cancer Society (SIC). ASc: speakers bureau: Ypsen, Astra Zeneca, Amgen, MSD, GSK; consulting: INCYTE Biosciences. MR: consulting or advisory: AstraZeneca (uncompensated), Change Healthcare, Daiichi-Sankyo (uncompensated), Epic Sciences (uncompensated), Merck (uncompensated), Pfizer (uncompensated); research funding: AbbVie (institution), AstraZeneca (Institution), Invitae (Institution, in-kind), Merck (Institution), Pfizer (institution); travel, accommodation, expenses: AstraZeneca, Merck; editorial services: AstraZeneca, Pfizer. FM-B: consulting: Aduro BioTech Inc., Alkermes, DebioPharm, eFFECTOR Therapeutics, F. Hoffman-La Roche Ltd, Genentech Inc., IBM Watson, Jackson Laboratory, Kolon Life Science, OrigiMed, PACT Pharma, Parexel International, Pfizer Inc., Samsung Bioepis, Seattle Genetics Inc., Tyra Biosciences, Xencor, Zymeworks; advisory committee: Immunomedics, Inflection Biosciences, Mersana Therapeutics, Puma Biotechnology Inc., Seattle Genetics, Silverback Therapeutics, Spectrum Pharmaceuticals, Zentalis; sponsored research: Aileron Therapeutics, Inc., AstraZeneca, Bayer Healthcare Pharmaceutical, Calithera Biosciences Inc., Curis Inc., CytomX Therapeutics Inc., Daiichi Sankyo Co. Ltd, Debiopharm International, eFFECTOR Therapeutics, Genentech Inc., Guardant Health Inc., Millennium Pharmaceuticals Inc., Novartis, Puma Biotechnology Inc., Taiho Pharmaceutical Co.; honoraria: Chugai Biopharmaceuticals, Mayo Clinic, Rutgers Cancer Institute of New Jersey; other (Travel Related): Beth Israel Deaconess Medical Center. NW: research grant from Puma Biotechnology; scientific advisory board and stockholder for Relay Therapeutics; advisor to Eli Lilly. ASt: advisory board/speakers bureau: Astra Zeneca, Eli Lilly, Bayer, BMS, Illumina, Janssen, MSD, Pfizer, Roche, Seattle Genetics, Thermo Fisher; Grants: Bayer, BMS, Chugai. JB: travel support: BMS; consulting fees: BMS, MSD, Astellas. SM: statistical advice: IDDI and Janssen Cilag; Independent Data Monitoring Committee member: Hexal, Steba, IQVIA, Roche, Sensorion, Biophytis, Servier, Yuhan. IB: speaker's fee: AstraZeneca. ER: board participation: AstraZeneca, BMS, Roche; travel funding: AstraZeneca, BMS. JSR-F: paid consultant: Goldman Sachs, REPARE Therapeutics, and Paige.AI; member of the scientific advisory board: REPARE Therapeutics, Paige.AI, and Volition Rx; member of the Board of Directors: Group Oncoclinicas; ad hoc member of the scientific advisory board: Roche Tissue Diagnostics, Roche, Genentech, Novartis, and Invicro; owns shares: REPARE Therapeutics. RD: advisory: Roche, Boehringer Ingelheim; speaker's fee: Roche, Ipsen, Amgen, Servier, Sanofi, Merck Sharp & Dohme; research grants: Merck and Pierre Fabre. FA: research grants and talks/advisory boards compensate to the hospital: Roche, Pfizer, Novartis, AstraZeneca, Daiichi Sankyo, Lilly. All remaining authors have declared no conflicts of interest.

REFERENCES

1. van Nimwegen KJM, van Soest RA, Veltman JA, et al. Is the $1000 genome as near as we think? A cost analysis of next-generation sequencing. *Clin Chem*. 2016;62(11):1458—1464.
2. Marino P, Touzani R, Perrier L, et al. Cost of cancer diagnosis using next-generation sequencing targeted gene panels in routine practice: a nationwide French study. *Eur J Hum Genet*. 2018;26(3):314—323.
3. Pagès A, Foulon S, Zou Z, et al. The cost of molecular-guided therapy in oncology: a prospective cost study alongside the MOSCATO trial. *Genet Med*. 2017;19(6):683—690.
4. Frampton GM, Fichtenholtz A, Otto GA, et al. Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. *Nat Biotechnol*. 2013;31(11):1023—1031.
5. Tourneau CL, Delord J-P, Gonçalves A, et al. Molecularly targeted therapy based on tumour molecular profiling versus conventional therapy for advanced cancer (SHIVA): a multicentre, open-label, proof-of-concept, randomised, controlled phase 2 trial. *Lancet Oncol*. 2015;16(13):1324—1334.
6. Massard C, Michiels S, Ferté C, et al. High-throughput genomics and clinical outcome in hard-to-treat advanced cancers: Results of the MOSCATO 01 trial. *Cancer Discov*. 2017;7(6):586—596.
7. André F, Bachelot T, Commo F, et al. Comparative genomic hybridisation array and DNA sequencing to direct treatment of metastatic breast cancer: a multicentre, prospective trial (SAFIR01/UNICANCER). *Lancet Oncol*. 2014;15(3):267—274.
8. Tsimberidou A-M, Wen S, Hong DS, et al. Personalized medicine for patients with advanced cancer in the phase I program at MD Anderson: validation and landmark analyses. *Clin Cancer Res*. 2014;20(18):4827—4836.
9. Priestley P, Baber J, Lolkema MP, et al. Pan-cancer whole-genome analyses of metastatic solid tumours. *Nature*. 2019;575(7781):210—216.
10. Trédan O, Wang Q, Pissaloux D, et al. Molecular screening program to select molecular-based recommended therapies for metastatic cancer patients: analysis from the ProfiLER trial. *Ann Oncol*. 2019;30(5):757—765.
11. Korphaisarn K, Loree JM, Nguyen V, et al. Genomic analysis of exceptional responder to regorafenib in treatment-refractory metastatic rectal cancer: a case report and review of the literature. *Oncotarget*. 2017;8(34):57882—57888.

12. Espinosa M, Roldán-Romero JM, Duran I, et al. Advanced sporadic renal epithelioid angiomyolipoma: case report of an extraordinary response to sirolimus linked to TSC2 mutation. *BMC Cancer*. 2018;18(1):561.
13. Bray F, Ferlay J, Soerjomataram I. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018;68(6):394—424.
14. Mateo J, Chakravarty D, Dienstmann R, et al. A framework to rank genomic alterations as targets for cancer precision medicine: the ESMO Scale for Clinical Actionability of molecular Targets (ESCAT). *Ann Oncol*. 2018;29(9):1895—1902.
15. Ramsey SD, Shankaran V, Sullivan SD. Basket cases: How real-world testing for drugs approved based on basket trials might lead to false diagnoses, patient risks, and squandered resources. *J Clin Oncol*. 2019;37(36):3472—3474.
16. FDA approves pembrolizumab for adults and children with TMB-H solid tumors - the ASCO Post. Available at: https://www.ascopost.com/news/june-2020/fda-approves-pembrolizumab-for-adults-and-children-with-tmb-h-solid-tumors/. Accessed July 7, 2020.
17. OncoKB. Available at: https://www.oncokb.org/. Accessed March 10, 2020.
18. Veenstra DL, Mandelblatt J, Neumann P, et al. Health economics tools and precision medicine: Opportunities and challenges. *Forum Health Econ Policy*. 2020;23(1). https://doi.org/10.1515/fhep-2019-0013.
19. Weymann D, Pataky R, Regier DA. Economic evaluations of next-generation precision oncology: a critical review. *JCO Precis Oncol*. 2018;2. https://doi.org/10.1200/PO.17.00311.
20. Tan O, Shrestha R, Cunich M, et al. Application of next-generation sequencing to improve cancer management: a review of the clinical effectiveness and cost-effectiveness. *Clin Genet*. 2018;93(3):533—544.
21. Phillips KA, Deverka PA, Deborah A, Marshall, et al. Methodological issues in assessing the economic value of next-generation sequencing tests: many challenges and not enough solutions. *Value Health*. 2018;21(9):1033—1042.
22. Tan AC, Lai GGY, Tan GS, et al. Utility of incorporating next-generation sequencing (NGS) in an Asian non-small cell lung cancer (NSCLC) population: incremental yield of actionable alterations and cost-effectiveness analysis. *Lung Cancer*. 2020;139:207—215.
23. Steuten L, Goulart B, Meropol NJ, et al. Cost effectiveness of multigene panel sequencing for patients with advanced non—small-cell lung cancer. *JCO Clin Cancer Inform*. 2019;3:1—10.
24. Sboner A, Mu XJ, Greenbaum D, et al. The real cost of sequencing: higher than you think!. *Genome Biol*. 2011;12(8):125.
25. Legras A, Barritault M, Tallet A, et al. Validity of targeted next-generation sequencing in routine care for identifying clinically relevant molecular profiles in non-small-cell lung cancer: results of a 2-year experience on 1343 samples. *J Mol Diagn*. 2018;20(4):550—564.
26. Midha A, Dearden S, McCormack R. EGFR mutation incidence in non-small-cell lung cancer of adenocarcinoma histology: a systematic review and global map by ethnicity (mutMapII). *Am J Cancer Res*. 2015;5(9):2892—2911.
27. Mok TS, Cheng Y, Zhou X, et al. Improvement in overall survival in a randomized study that compared dacomitinib with gefitinib in patients with advanced non-small-cell lung cancer and EGFR-activating mutations. *J Clin Oncol*. 2018;36(22):2244—2250.
28. Soria J-C, Ohe Y, Vansteenkiste J, et al. Osimertinib in untreated EGFR-mutated advanced non—small-cell lung cancer. *N Engl J Med*. 2017;378(2):113—125.
29. Ramalingam SS, Vansteenkiste J, Planchard D, et al. Overall survival with osimertinib in untreated, EGFR-mutated advanced NSCLC. *N Engl J Med*. 2020;382(1):41—50.
30. Mok TS, Wu Y-L, Ahn M-J, et al. Osimertinib or platinum—pemetrexed in EGFR T790M—positive lung cancer. *N Engl J Med*. 2017;376(7):629—640.
31. Yang JC-H, Sequist LV, Geater SL, et al. Clinical activity of afatinib in patients with advanced non-small-cell lung cancer harbouring uncommon EGFR mutations: a combined post-hoc analysis of LUX-Lung 2, LUX-Lung 3, and LUX-Lung 6. *Lancet Oncol*. 2015;16(7):830—838.
32. Cho JH, Sun J, Lee S, et al. OA10.05 An open-label, multicenter, phase II single arm trial of osimertinib in NSCLC patients with uncommon EGFR mutation (KCSG-LU15-09). *J Thorac Oncol*. 2018;13(10):S344.
33. Cardona AF, Rojas L, Zatarain-Barrón ZL, et al. EGFR exon 20 insertion in lung adenocarcinomas among Hispanics (geno1.2-CLICaP). *Lung Cancer*. 2018;125:265—272.
34. Heymach J, Negrao M, Robichaux J, et al. OA02.06 A phase II trial of poziotinib in EGFR and HER2 exon 20 mutant non-small cell lung cancer (NSCLC). *J Thorac Oncol*. 2018;13(10):S323—S324.
35. Solomon BJ, Kim D-W, Wu Y-L, et al. Final overall survival analysis from a study comparing first-line crizotinib versus chemotherapy in ALK-mutation-positive non-small-cell lung cancer. *J Clin Oncol*. 2018;36(22):2251—2258.
36. Soria J-C, Tan DSW, Chiari R, et al. First-line ceritinib versus platinum-based chemotherapy in advanced ALK-rearranged non-small-cell lung cancer (ASCEND-4): a randomised, open-label, phase 3 study. *Lancet*. 2017;389(10072):917—929.
37. Peters S, Camidge DR, ALEX Trial Investigators, et al. Alectinib versus crizotinib in untreated ALK-positive non-small-cell lung cancer. *N Engl J Med*. 2017;377(9):829—838.
38. Zhou C, Lee SH, Wang C, et al. Primary results of ALESIA: a randomised, phase III, open-label study of alectinib vs crizotinib in Asian patients with treatment-naïve ALK+ advance NSCLC. *Ann Oncol*. 2018;29(suppl_8):ix173—ix178.
39. Camidge DR, Kim HR, Ahn M-J, et al. Brigatinib versus crizotinib in ALK-positive non—small-cell lung cancer. *N Engl J Med*. 2018;379(21):2027—2039.
40. Tong JH, Yeung SF, Chan AWH, et al. MET Amplification and exon 14 splice site mutation define unique molecular subgroups of non-small cell lung carcinoma with poor prognosis. *Clin Cancer Res*. 2016;22(12):3048—3056.
41. Drilon A, Clark JW, Weiss J, et al. Antitumor activity of crizotinib in lung cancers harboring a MET exon 14 alteration. *Nat Med*. 2020;26(1):47—51.
42. Planchard D, Besse B, Groen HJM, et al. Dabrafenib plus trametinib in patients with previously treated BRAF(V600E)-mutant metastatic non-small cell lung cancer: an open-label, multicentre phase 2 trial. *Lancet Oncol*. 2016;17(7):984—993.
43. Planchard D, Smit EF, Groen HJM, et al. Dabrafenib plus trametinib in patients with previously untreated BRAF(V600E)-mutant metastatic non-small-cell lung cancer: an open-label, phase 2 trial. *Lancet Oncol*. 2017;18(10):1307—1316.
44. Planchard D, Besse B, Kim TM, et al. Updated survival of patients (pts) with previously treated BRAF V600E—mutant advanced non-small cell lung cancer (NSCLC) who received dabrafenib (D) or D + trametinib (T) in the phase II BRF113928 study. *J Clin Oncol*. 2017;35(15_suppl):9075.
45. Shaw AT, Ou S-HI, Bang Y-J, et al. Crizotinib in ROS1-rearranged non-small-cell lung cancer. *N Engl J Med*. 2014;371(21):1963—1971.
46. Shaw AT, Riely GJ, Bang Y-J, et al. Crizotinib in ROS1-rearranged advanced non-small-cell lung cancer (NSCLC): updated results, including overall survival, from PROFILE 1001. *Ann Oncol*. 2019;30(7):1121—1126.
47. Drilon A, Siena S, Dziadziuszko R, et al. Entrectinib in ROS1 fusion-positive non-small-cell lung cancer: integrated analysis of three phase 1-2 trials. *Lancet Oncol*. 2020;21(2):261—270.
48. Drilon A, Laetsch TW, Kummar S, et al. Efficacy of larotrectinib in TRK fusion-positive cancers in adults and children. *N Engl J Med*. 2018;378:731—739.
49. Hong DS, DuBois SG, Kummar S, et al. Larotrectinib in patients with TRK fusion-positive solid tumours: a pooled analysis of three phase 1/2 clinical trials. *Lancet Oncol*. 2020;21(4):531—540.
50. Doebele RC, Drilon A, Paz-Ares L, et al. Entrectinib in patients with advanced or metastatic NTRK fusion-positive solid tumours: integrated analysis of three phase 1-2 trials. *Lancet Oncol*. 2020;21(2):271—282.

51. Drilon A, Oxnard G, Wirth L, et al. PL02.08 registrational results of LIBRETTO-001: a phase 1/2 trial of LOXO-292 in patients with RET fusion-positive lung cancers. *J Thorac Oncol*. 2019;14(10): S6—S7.
52. Camidge DR, Otterson GA, Clark JW, et al. Crizotinib in patients (pts) with MET-amplified non-small cell lung cancer (NSCLC): updated safety and efficacy findings from a phase 1 trial. *J Clin Oncol*. 2018;36: 9062.
53. Barlesi F, Mazieres J, Merlio J-P, et al. Routine molecular profiling of patients with advanced non-small-cell lung cancer: results of a 1-year nationwide programme of the French Cooperative Thoracic Intergroup (IFCT). *Lancet*. 2016;387(10026):1415—1426.
54. Fakih M, O'Neil B, Price TJ, et al. Phase 1 study evaluating the safety, tolerability, pharmacokinetics (PK), and efficacy of AMG 510, a novel small molecule KRASG12C inhibitor, in advanced solid tumors. *J Clin Oncol*. 2019;37(15_suppl):3003.
55. Hyman DM, Piha-Paul SA, Won, et al. HER kinase inhibition in patients with HER2- and HER3-mutant cancers. *Nature*. 2018;554:189—194.
56. Wang Y, Jiang T, Qin Z, et al. HER2 exon 20 insertions in non-small-cell lung cancer are sensitive to the irreversible pan-HER receptor tyrosine kinase inhibitor pyrotinib. *Ann Oncol*. 2019;30(3):447—455.
57. Tsurutani J, Park H, Doi T, et al. OA02.07 Updated results of phase 1 study of DS-8201a in HER2-expressing or —mutated advanced non-small-cell lung cancer. *J Thorac Oncol*. 2018;13(10):S324.
58. Hellmann MD, Paz-Ares L, Bernabe Caro R, et al. Nivolumab plus ipilimumab in advanced non-small-cell lung cancer. *N Engl J Med*. 2019;381(21):2020—2031.
59. Duruisseaux M, Liu SV, Han J-Y, et al. NRG1 fusion-positive lung cancers: Clinicopathologic profile and treatment outcomes from a global multicenter registry. *J Clin Oncol*. 2019;37(15_suppl):9081.
60. The Cancer Genome Atlas Research Network. Comprehensive molecular profiling of lung adenocarcinoma. *Nature*. 2014;511(7511): 543—550.
61. Cancer Genome Atlas Research Network. Comprehensive genomic characterization of squamous cell lung cancers. *Nature*. 2012;489(7417):519—525.
62. Vansteenkiste JF, Canon J-L, De Braud F, et al. Safety and efficacy of buparlisib (BKM120) in patients with PI3K pathway-activated non-small cell lung cancer: results from the phase II BASALT-1 study. *J Thorac Oncol*. 2015;10(9):1319—1327.
63. Balasubramaniam S, Beaver JA, Horton S, et al. FDA approval summary: rucaparib for the treatment of patients with deleterious BRCA mutation-associated advanced ovarian cancer. *Clin Cancer Res*. 2017;23(23):7165—7170.
64. Voest E, van der Velden D, Hoes L, et al. Expanding the use of approved drugs: The CPCT's Drug Rediscovery Protocol (DRUP). *Ann Oncol*. 2017;28(suppl_5):v605—v649.
65. Slamon DJ, Leyland-Jones B, Shak S, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med*. 2001;344(11):783—792.
66. Swain SM, Baselga J, Kim S-B, et al. Pertuzumab, trastuzumab, and docetaxel in HER2-positive metastatic breast cancer. *N Engl J Med*. 2015;372(8):724—734.
67. Verma S, Miles D, Gianni L, et al. Trastuzumab emtansine for HER2-positive advanced breast cancer. *N Engl J Med*. 2012;367(19): 1783—1791.
68. Krop IE, Kim S-B, González-Martín A, et al. Trastuzumab emtansine versus treatment of physician's choice for pretreated HER2-positive advanced breast cancer (TH3RESA): a randomised, open-label, phase 3 trial. *Lancet Oncol*. 2014;15(7):689—699.
69. Murthy RK, Loi S, Okines A, et al. Tucatinib, trastuzumab, and capecitabine for HER2-positive metastatic breast cancer. *N Engl J Med*. 2020;382(7):597—609.
70. Robson M, Im S-A, Senkus E, et al. Olaparib for metastatic breast cancer in patients with a germline *BRCA* mutation. *N Engl J Med*. 2017;377(6):523—533.
71. Litton JK, Rugo HS, Ettl J, et al. Talazoparib in patients with advanced breast cancer and a germline BRCA mutation. *N Engl J Med*. 2018;379(8):753—763.
72. André F, Ciruelos E, Rubovszky G, et al. Alpelisib for PIK3CA-mutated, hormone receptor-positive advanced breast cancer. *N Engl J Med*. 2019;380:1929—1940.
73. Marcus L, Lemery SJ, Keegan P, et al. FDA approval summary: pembrolizumab for the treatment of microsatellite instability-high solid tumors. *Clin Cancer Res*. 2019;25(13):3753—3758.
74. Fribbens C, O'Leary B, Kilburn L, et al. Plasma ESR1 mutations and the treatment of estrogen receptor—positive advanced breast cancer. *J Clin Oncol*. 2016;34(25):2961—2968.
75. Schmid P, Abraham J, Chan S, et al. AZD5363 plus paclitaxel versus placebo plus paclitaxel as first-line therapy for metastatic triple-negative breast cancer (PAKT): a randomised, double-blind, placebo-controlled, phase II trial. *J Clin Oncol*. 2018;36(15_suppl): 1007.
76. Hyman DM, Smyth LM, Donoghue MTA, et al. AKT inhibition in solid tumors with AKT1 mutations. *J Clin Oncol*. 2017;35(20):2251—2259.
77. Pearson A, Proszek P, Ring A, et al. Inactivating NF1 mutations are enriched in advanced breast cancer and contribute to endocrine therapy resistance. *Clin Cancer Res*. 2020;26(3):608—622.
78. Dembla V, Somaiah N, Barata P, et al. Prevalence of MDM2 amplification and coalterations in 523 advanced cancer patients in the MD Anderson phase 1 clinic. *Oncotarget*. 2018;9:33232—33243.
79. Van Cutsem E, Lenz H-J, Köhne C-H, et al. Fluorouracil, leucovorin, and irinotecan plus cetuximab treatment and RAS mutations in colorectal cancer. *J Clin Oncol*. 2015;33(7):692—700.
80. Douillard J-Y, Oliner KS, Siena S, et al. Panitumumab-FOLFOX4 treatment and RAS mutations in colorectal cancer. *N Engl J Med*. 2013;369(11):1023—1034.
81. Sorich MJ, Wiese MD, Rowland A, et al. Extended RAS mutations and anti-EGFR monoclonal antibody survival benefit in metastatic colorectal cancer: a meta-analysis of randomized, controlled trials. *Ann Oncol*. 2015;26(1):13—21.
82. Kopetz S, Grothey A, Yaeger R, et al. Encorafenib, binimetinib, and cetuximab in BRAF V600E-mutated colorectal cancer. *N Engl J Med*. 2019;381(17):1632—1643.
83. Overman MJ, McDermott R, Leach JL, et al. Nivolumab in patients with metastatic DNA mismatch repair-deficient or microsatellite instability-high colorectal cancer (CheckMate 142): an open-label, multicentre, phase 2 study. *Lancet Oncol*. 2017;18(9):1182—1191.
84. Le DT, Kim TW, Van Cutsem E, et al. Phase II open-label study of pembrolizumab in treatment-refractory, microsatellite instability-high/mismatch repair-deficient metastatic colorectal cancer: KEYNOTE-164. *J Clin Oncol*. 2020;38(1):11—19.
85. Demetri GD, Paz-Ares L, Multani PS, et al. Efficacy and safety of entrectinib in patients with NTRK fusion-positive tumours: Pooled analysis of STARTRK-2, STARTRK-1, and ALKA-372-001. *Ann Oncol*. 2018;29(Suppl_8):viii713.
86. Meric-Bernstam F, Hurwitz H, Raghav KPS, et al. Pertuzumab plus trastuzumab for HER2-amplified metastatic colorectal cancer (MyPathway): an updated report from a multicentre, open-label, phase 2a, multiple basket study. *Lancet Oncol*. 2019;20(4): 518—530.
87. Sartore-Bianchi A, Trusolino L, Martino C, et al. Dual-targeted therapy with trastuzumab and lapatinib in treatment-refractory, KRAS codon 12/13 wild-type, HER2-positive metastatic colorectal cancer (HERACLES): a proof-of-concept, multicentre, open-label, phase 2 trial. *Lancet Oncol*. 2016;17(6):738—746.
88. Yakirevich E, Resnick MB, Mangray S, et al. Oncogenic ALK fusion in rare and aggressive subtype of colorectal adenocarcinoma as a potential therapeutic target. *Clin Cancer Res*. 2016;22(15):3831—3840.
89. Fabrizio DA, George TJ, Dunne RF, et al. Beyond microsatellite testing: assessment of tumor mutational burden identifies subsets of colorectal cancer who may respond to immune checkpoint inhibition. *J Gastrointest Oncol*. 2018;9(4):610—617.
90. Juric D, Rodon J, Tabernero J, et al. Phosphatidylinositol 3-kinase α—selective inhibition with alpelisib (BYL719) in PIK3CA-altered solid tumors: results from the first-in-human study. *J Clin Oncol*. 2018;36: 1291—1299.

91. Drilon AE, Subbiah V, Oxnard GR, et al. A phase 1 study of LOXO-292, a potent and highly selective RET inhibitor, in patients with RET-altered cancers. *J Clin Oncol*. 2018;36(suppl). abstr 102.
92. Wang C, Jette N, Moussienko D, et al. ATM-deficient colorectal cancer cells are sensitive to the PARP inhibitor olaparib. *Transl Oncol*. 2017;10(2):190—196.
93. De Bono J, Mateo J, Fizazi K, et al. Olaparib for metastatic castration-resistant prostate cancer. *N Engl J Med*. 2020;382(22):2091—2102.
94. Savolitinib in treating participants with MET amplified metastatic or unresectable colorectal cancer - full text view - ClinicalTrials.gov. Available at: https://clinicaltrials.gov/ct2/show/NCT03592641. Accessed July 7, 2020.
95. Mateo J, Carreira S, Sandhu S, et al. DNA-repair defects and olaparib in metastatic prostate cancer. *N Engl J Med*. 2015;373:1697—1708.
96. Cortes-Ciriano I, Lee S, Park W-Y, Kim T-M, Park PJ. A molecular portrait of microsatellite instability across multiple cancers. *Nat Commun*. 2017;8:15180.
97. Abida W, Cheng ML, Armenia J, et al. Microsatellite instability in prostate cancer and response to immune checkpoint blockade. *J Clin Oncol*. 2018;36(15_suppl):5020.
98. Abida W, Cyrta J, Heller G, et al. Genomic correlates of clinical outcome in advanced prostate cancer. *Proc Natl Acad Sci*. 2019;116(23):11428—11436.
99. de Bono JS, De Giorgi U, Rodrigues DN, et al. Randomized phase II study evaluating akt blockade with ipatasertib, in combination with abiraterone, in patients with metastatic prostate cancer with and without PTEN loss. *Clin Cancer Res*. 2019;25(3):928—936.
100. Ipatasertib plus abiraterone plus prednisone/prednisolone, relative to placebo plus abiraterone plus prednisone/prednisolone in adult male patients with metastatic castrate-resistant prostate cancer (IPATential150). Available at: https://clinicaltrials.gov/ct2/show/NCT03072238. Accessed February 23, 2020.
101. Crumbaker M, Khoja L, Joshua AM. AR signaling and the PI3K pathway in prostate cancer. *Cancers*. 2017;9(4):34.
102. The Cancer Genome Atlas Research Network. Comprehensive molecular characterization of gastric adenocarcinoma. *Nature*. 2014;513(7517):202—209.
103. Bang Y-J, Van Cutsem E, Feyereislova A, et al. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. *Lancet*. 2010;376:687—697.
104. Maron SB, Alpert L, Kwak HA, et al. Targeted therapies for targeted populations: anti-EGFR treatment for EGFR amplified gastroesophageal adenocarcinoma. *Cancer Discov*. 2018;8(6):696—713.
105. Lennerz JK, Kwak EL, Ackerman A, et al. MET amplification identifies a small and aggressive subgroup of esophagogastric adenocarcinoma with evidence of responsiveness to crizotinib. *J Clin Oncol*. 2011;29:4803—4810.
106. Oxnard G, Subbiah V, Park K, et al. Clinical activity of LOXO-292, a highly selective RET inhibitor, in patients with RET fusion+ non-small cell lung cancer. *J Thorac Oncol*. 2018;13(10):S349—S350.
107. Lee J, Ou S-HI, Lee JM, et al. Gastrointestinal malignancies harbor actionable MET exon 14 deletions. *Oncotarget*. 2015;6:28211—28222.
108. Bang Y-J, Xu R-H, Chin K, et al. Olaparib in combination with paclitaxel in patients with advanced gastric cancer who have progressed following first-line therapy (GOLD): a double-blind, randomised, placebo-controlled, phase 3 trial. *Lancet Oncol*. 2017;18(12):1637—1651.
109. Van Cutsem E, Bang Y-J, Mansoor W, et al. A randomized, open-label study of the efficacy and safety of AZD4547 monotherapy versus paclitaxel for the treatment of advanced gastric adenocarcinoma with FGFR2 polysomy or gene amplification. *Ann Oncol*. 2017;28:1316—1324.
110. Loriot Y, Necchi A, Park SH, et al. Erdafitinib in locally advanced or metastatic urothelial carcinoma. *N Engl J Med*. 2019;381:338—348.
111. The Cancer Genome Atlas Research Network. Integrated genomic characterization of pancreatic ductal adenocarcinoma. *Cancer Cell*. 2017;32:185—203.e13.
112. Golan T, Hammel P, Reni M, et al. Maintenance olaparib for germline BRCA-mutated metastatic pancreatic cancer. *N Engl J Med*. 2019;381:317—327.
113. Shroff RT, Hendifar A, McWilliams RR, et al. Rucaparib monotherapy in patients with pancreatic cancer and a known deleterious BRCA mutation. *JCO Precis Oncol*. 2018;2018. https://doi.org/10.1200/PO.17.00316.
114. Cocco E, Scaltriti M, Drilon A. NTRK fusion-positive cancers and TRK inhibitor therapy. *Nat Rev Clin Oncol*. 2018;15:731—747.
115. Pihlak R, Weaver JMJ, Valle JW, et al. Advances in molecular profiling and categorisation of pancreatic adenocarcinoma and the implications for therapy. *Cancers*. 2018;10(1):17.
116. Zeitouni D, Pylayeva-Gupta Y, Der CJ, et al. KRAS mutant pancreatic cancer: no lone path to an effective treatment. *Cancers*. 2016;8(4):45.
117. Heestand GM, Kurzrock R. Molecular landscape of pancreatic cancer: implications for current clinical trials. *Oncotarget*. 2015;6:4553—4561.
118. Payne S, Maher M, Tran N, et al. Mutant PIK3CA-mediated pancreatic tumorigenesis and the response to PI3K pathway inhibition. *J Clin Oncol*. 2015;33:e15273.
119. Hyman DM, Puzanov I, Subbiah V, et al. Vemurafenib in multiple nonmelanoma cancers with BRAF V600 mutations. *N Engl J Med*. 2015;373:726—736.
120. Azmi AS, Aboukameel A, Banerjee S, et al. MDM2 inhibitor MI-319 in combination with cisplatin is an effective treatment for pancreatic cancer independent of p53 function. *Eur J Cancer*. 2010;46(6):1122—1131.
121. Waddell N, Pajic M, Bailey P, et al. Whole genomes redefine the mutational landscape of pancreatic cancer. *Nature*. 2015;518:495.
122. Harder J, Ihorst G, Heinemann V, et al. Multicentre phase II trial of trastuzumab and capecitabine in patients with HER2 over-expressing metastatic pancreatic cancer. *Br J Cancer*. 2012;106(6):1033—1038.
123. Jones MR, Williamson LM, Topham JT, et al. NRG1 gene fusions are recurrent, clinically actionable gene rearrangements in KRAS wild-type pancreatic ductal adenocarcinoma. *Clin Cancer Res*. 2019;25(15):4674—4681.
124. Singhi AD, Ali SM, Lacy J, et al. Identification of targetable ALK rearrangements in pancreatic ductal adenocarcinoma. *J Natl Compr Canc Netw*. 2017;15:555—562.
125. Pishvaian MJ, Rolfo CD, Liu SV, Multani PS, Chow Maneval E, Garrido-Laguna I. Clinical benefit of entrectinib for patients with metastatic pancreatic cancer who harbor NTRK and ROS1 fusions. *J Clin Oncol*. 2018;36(4_suppl):521.
126. Cancer Genome Atlas Research Network. Comprehensive and integrative genomic characterization of hepatocellular carcinoma. *Cell*. 2017;169:1327—1341.
127. Rimassa L, Assenat E, Peck-Radosavljevic M, et al. Tivantinib for second-line treatment of MET-high, advanced hepatocellular carcinoma (METIV-HCC): a final analysis of a phase 3, randomised, placebo-controlled study. *Lancet Oncol*. 2018;19:682—693.
128. Lim HY, Merle P, Weiss KH, et al. Phase II studies with refametinib or refametinib plus sorafenib in patients with RAS-mutated hepatocellular carcinoma. *Clin Cancer Res*. 2018;24:4650—4661.
129. Abou-Alfa GK, Macarulla Mercade T, Javle M, et al. ClarIDHy: a global, phase 3, randomized, double-blind study of ivosidenib (IVO) vs. placebo in patients with advanced cholangiocarcinoma (CC) with an isocitrate dehydrogenase 1 (IDH1) mutation. *Ann Oncol*. 2019;30(suppl_5):v851—v934.
130. Vogel A, Sahai V, Hollebecque A, et al. LBA40 - FIGHT-202: A phase II study of pemigatinib in patients (pts) with previously treated locally advanced or metastatic cholangiocarcinoma (CCA). *Ann Oncol*. 2019;30:v876.
131. Marabelle A, Le DT, Ascierto PA, et al. Efficacy of pembrolizumab in patients with noncolorectal high microsatellite instability/mismatch repair-deficient cancer: Results from the phase II KEYNOTE-158 study. *J Clin Oncol*. 2020;38(1):1—10.
132. Wainberg ZA, Lassen UN, Elez E, et al. Efficacy and safety of dabrafenib (D) and trametinib (T) in patients (pts) with BRAF V600E—

mutated biliary tract cancer (BTC): a cohort of the ROAR basket trial. *J Clin Oncol*. 2019;37(4_suppl):187.

133. Javle MM, Hainsworth JD, Swanton C, et al. Pertuzumab + trastuzumab for HER2-positive metastatic biliary cancer: preliminary data from MyPathway. *J Clin Oncol*. 2017;35(4_suppl):402.
134. Moore K, Colombo N, Scambia G, et al. Maintenance olaparib in patients with newly diagnosed advanced ovarian cancer. *N Engl J Med*. 2018;379(26):2495—2505.
135. Clynick B, Dessauvagie B, Sterrett G, et al. Genetic characterisation of molecular targets in carcinoma of unknown primary. *J Transl Med*. 2018;16(1):185.
136. Roy S, Coldren C, Karunamurthy A, et al. Standards and guidelines for validating next-generation sequencing bioinformatics pipelines: a joint recommendation of the Association for Molecular Pathology and the College of American Pathologists. *J Mol Diagn*. 2018;20(1):4—27.
137. Hume S, Nelson TN, Speevak M, et al. CCMG practice guideline: laboratory guidelines for next-generation sequencing. *J Med Genet*. 2019;56(12):792—800.
138. Next Generation Sequencing (NGS) Worksheets. College of American Pathologists. Available at: https://www.cap.org/member-resources/precision-medicine/next-generation-sequencing-ngs-worksheets. Accessed March 10, 2020.
139. Szymanski J, Duncavage E, Pfeifer J. Next-generation sequencing bioinformatics: Guidance between the sequencing and sign out. *J Pathol Inform*. 2018;9:23.
140. Burke W. Genetic tests: clinical validity and clinical utility. *Curr Protoc Hum Genet*. 2014;81:9.15.1—9.15.8.
141. Kaul KL, Leonard DGB, Gonzalez A, et al. Oversight of genetic testing: an update. *J Mol Diagn*. 2001;3(3):85—91.
142. Deans ZC, Costa JL, Cree I, et al. Integration of next-generation sequencing in clinical diagnostic molecular pathology laboratories for analysis of solid tumours; an expert opinion on behalf of IQN Path ASBL. *Virchows Arch*. 2017;470(1):5—20.
143. Matthijs G, Souche E, Alders M, et al. Guidelines for diagnostic next-generation sequencing. *Eur J Hum Genet*. 2016;24(1):2—5.
144. Jennings LJ, Arcila ME, Corless C, et al. Guidelines for validation of next-generation sequencing-based oncology panels: a joint consensus recommendation of the Association for Molecular Pathology and College of American Pathologists. *J Mol Diagn*. 2017;19(3):341—365.
145. Aziz N, Zhao Q, Bry L, et al. College of American Pathologists' laboratory standards for next-generation sequencing clinical tests. *Arch Pathol Lab Med*. 2015;139(4):481—493.