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GUIDELINES

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Expert panel consensus recommendations on the use of circulating tumor DNA assays for patients with advanced solid tumors

Mitsuho Imai[1,2] | Yoshiaki Nakamura[1,3] | Kuniko Sunami[4] | Hidenori Kage[5] | Keigo Komine[6] | Takafumi Koyama[7] | Toraji Amano[8] | Daisuke Ennishi[9] | Masashi Kanai[10] | Hirotsugu Kenmotsu[11] | Takahiro Maeda[12] | Sachi Morita[13] | Daisuke Sakai[14] | Hideaki Bando[1,3] | Akitaka Makiyama[15] | Tatsuya Suzuki[16] | Makoto Hirata[17] | Shinji Kohsaka[18] | Katsuya Tsuchihara[19] | Yoichi Naito[20] | Takayuki Yoshino[1,3]

[1]Translational Research Support Section, National Cancer Center Hospital East, Kashiwa, Japan

[2]Genomics Unit, Keio University School of Medicine, Tokyo, Japan

[3]Department of Gastroenterology and Gastrointestinal Oncology, National Cancer Center Hospital East, Kashiwa, Japan

[4]Department of Laboratory Medicine, National Cancer Center Hospital, Tokyo, Japan

[5]Department of Next-Generation Precision Medicine Development Laboratory, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

[6]Department of Medical Oncology, Tohoku University Hospital, Sendai, Japan

[7]Department of Experimental Therapeutics, National Cancer Center Hospital, Tokyo, Japan

[8]Clinical Research and Medical Innovation Center, Hokkaido University Hospital, Hokkaido, Japan

[9]Center for Comprehensive Genomic Medicine, Okayama University Hospital, Okayama, Japan

[10]Department of Therapeutic Oncology, Graduate School of Medicine, Kyoto University, Kyoto, Japan

[11]Division of Thoracic Oncology, Shizuoka Cancer Center, Suntougun, Japan

[12]Division of Precision Medicine, Kyushu University Graduate School of Medical Sciences, Fukuoka, Japan

[13]Department of Clinical Oncology and Chemotherapy, Nagoya University Hospital, Nagoya, Japan

[14]Center for Cancer Genomics and Personalized Medicine, Osaka University Hospital, Suita, Japan

[15]Cancer Center, Gifu University Hospital, Gifu, Japan

[16]Department of Hematology, National Cancer Center Hospital, Tokyo, Japan

[17]Department of Genetic Medicine and Services, National Cancer Center Hospital, Tokyo, Japan

[18]Division of Cellular Signaling, National Cancer Center Research Institute, Tokyo, Japan

[19]Division of Translational Informatics, Exploratory Oncology Research and Clinical Trial Center, National Cancer Center, Kashiwa, Japan

[20]Department of General Internal Medicine/Experimental Therapeutics/Medical Oncology, National Cancer Center Hospital East, Kashiwa, Japan

Abbreviations: ACMG, American College of Medical Genetics and Genomics; bTMB, blood tumor mutational burden; CGP, comprehensive genomic profiling; CH, clonal hematopoiesis; CI, confidence interval; CNV, copy number variation; CQs, clinical questions; ctDNA, circulating tumor DNA; ECO, expert consensus opinion; EP, expert panel; GI, gastrointestinal; ICIs, checkpoint inhibitors; IHC, immunohistochemistry; LoD, limit of detection; NGS, next-generation sequencing; NR, not Recommended; PPA, positive percent agreement; R, recommended; SR, strongly recommended; TAT, turnaround time; TF, tumor fraction; VAF, Variant allele frequency.

Correspondence
Yoshiaki Nakamura, Translational Research Support Section, National Cancer Center Hospital East, 6-5-1 Kashiwanoha, Kashiwa-shi, Chiba 277-8577, Japan.
Email: yoshinak@east.ncc.go.jp

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Abstract

Comprehensive genomic profiling is increasingly used to facilitate precision oncology based on molecular stratification. In addition to conventional tissue comprehensive genomic profiling, comprehensive genomic profiling of circulating tumor DNA has become widely utilized in cancer care owing on its advantages, including less invasiveness, rapid turnaround time, and capturing heterogeneity. However, circulating tumor DNA comprehensive genomic profiling has some limitations, mainly false negatives due to low levels of plasma circulating tumor deoxyribonucleic acid and false positives caused by clonal hematopoiesis. Nevertheless, no guidelines and recommendations fully address these issues. Here, an expert panel committee involving representatives from 12 Designated Core Hospitals for Cancer Genomic Medicine in Japan was organized to develop expert consensus recommendations for the use of circulating tumor deoxyribonucleic acid-based comprehensive genomic profiling. The aim was to generate guidelines for clinicians and allied healthcare professionals on the optimal use of the circulating tumor DNA assays in advanced solid tumors and to aid the design of future clinical trials that utilize and develop circulating tumor DNA assays to refine precision oncology. Fourteen clinical questions regarding circulating tumor deoxyribonucleic acid comprehensive genomic profiling including the timing of testing and considerations for interpreting results were established by searching and curating associated literatures, and corresponding recommendations were prepared based on the literature for each clinical question. Final consensus recommendations were developed by voting to determine the level of each recommendation by the Committee members.

KEYWORDS
circulating tumor DNA, comprehensive genomic profiling, liquid biopsy, precision oncology, solid tumor

1 | INTRODUCTION

Molecular stratification of cancer, including genomic alteration profiling, provides guidance for selecting the optimal systemic treatments such as molecular targeted agents, ICIs, and cytotoxic agents.[1] Among CGP methods, NGS was the first to be implemented in clinical practice. Plasma CGP test of ctDNA has been recently developed as a less invasive method to detect genomic alterations in tumors from which fragmented DNA is continuously released.[2] ctDNA CGP has clinical advantages including a shorter TAT compared with tissue CGP and detection of heterogeneity,[3,4] that is, genomic alterations from multiple lesions. However, there are some limitations, such as lower rate of detecting mutations, which is affected by the amount of ctDNA,[5,6] and false positives due to CH.[7,8]

In Japan, two tissue CGPs were approved for advanced solid tumors on December 2018: OncoGuide™ NCC Oncopanel System (NCC Oncopanel, Sysmex, Tokyo) and FoundationOne CDx Cancer Genomic Profile (F1CDx, Foundation Medicine; Cambridge, MA). These tests are covered by the Public Health Insurance System and are indicated when (1) no standard treatment exists or the cancer is refractory to standard treatments, and (2) the patient is eligible for treatment based on good performance status according to the CGP results. Two ctDNA CGPs, FoundationOne Liquid CDx (F1LCDx) and Guardant360 CDx were approved with the same indications as those for tissue CGP on March 2020 and March 2021, respectively. Furthermore, unlike other countries, F1LCDx could, at the time of publication, only be used when adequate tissue specimens are unavailable or when tissue CGP failed.

Following the approval of the ctDNA CGP in Japan, the Joint Task Force for the Promotion of Cancer Genome Medicine has formulated policy recommendations for the appropriate use of ctDNA CGP.[9] To refine the use of ctDNA CGP in clinical practice according to accumulative evidence, EP representatives were nominated from all 12 Designated Core Hospitals for Cancer Genomic Medicine and were convened in February 2022 as part of a project (representative: T. Yoshino) funded by the Ministry of Health, Labour, and Welfare. The EP is the molecular tumor board at each core hospital composed of multidisciplinary specialists who review the results of CGP performed under national health insurance coverage and make treatment recommendations.

The ultimate aim of the meeting was to generate EP consensus recommendations for clinicians and allied healthcare professionals on the optimal use of the ctDNA assays in patients with advanced solid tumors based on the results of expert voting on a series of preformulated clinical questions as outlined below.

2 | METHODOLOGY

2.1 | Composition of members and aims

This manuscript represents the opinion of 21 experts in oncology (medical oncologist, genetic oncologist, statistician, and basic researcher), representing EP experts at nominated from all 12 Designated Core Hospitals who participated in an online meeting to discuss CQs regarding the optimal use of the ctDNA assays in patients with advanced solid tumors in the era of precision oncology.

2.2 | Clinical questions and proposed recommendations

In preparation for the meeting, 14 CQs on the optimal use of the ctDNA assays were formulated by Drs. T. Yoshino, M. Imai, Y. Nakamura, Y. Naito, K. Sunami, H. Kage, K. Komine, and T. Koyama (CQ creation members) and reviewed by all the experts (Table 1). The evidence to support the set of recommendations proposed in response to these CQs was provided by the CQ creation members, who searched and curated the associated literatures. After the approval of all the CQs by all the experts, recommendations were proposed by the CQ creation members for each CQ and circulated in advance to all the experts. The responses of the experts were required to represent science-based opinion assuming that all testing modalities were available.

2.3 | Final consensus statements

All the 21 experts discussed all the 14 recommendations prior to voting during the online meeting. We allowed those who wished to abstain for whatever reason prior to voting in each CQ. In voting, whether the contents of medical care (including tests and indications) were approved or covered by public health insurance in Japan was not considered. The 12 EP members (one member in each core hospital) voted following the degrees of recommendation and decision criteria to determine the level of recommendation for each CQ (Table 2). The recommendation level was determined according to the available evidence and considering the potential benefit, harm, cost, patient preference, and other factors. However, relevant information was described in the remarks column as required.

TABLE 1 The 14 clinical questions (CQs) formulated for optimal use of ctDNA CGP in patients with advanced solid tumors

CQ no.	CQ
CQ1	When no gene fusion is detected with a ctDNA CGP, should tissue CGP be recommended?
CQ2	When no copy number variation (CNV) is detected with a ctDNA CGP, should tissue CGP be recommended?
CQ3	Should an immune checkpoint inhibitor (ICI) be recommended for patients with a blood tumor mutational burden (bTMB)-high solid tumor?
CQ4	When microsatellite inhibitor (MSI) is clinically suspected and not detected by ctDNA CGP, is tissue CGP recommended?
CQ5	Should ctDNA CGP be recommended for cancer types with low ctDNA levels?
CQ6	In cases with a low tumor burden, should the NGS tests prioritize tissue CGP over ctDNA CGP?
CQ7	Is ctDNA CGP recommended during a systemic therapy?
CQ8	When should the origin of a variant be suspected of being clonal hematopoiesis CH?
CQ9	When should the origin of a variant be suspected of being germline?
CQ10	Should a variant suspected of being germline be confirmed by a validated method?
CQ11	Should a treatment targeting highly suspected subclonal variants be recommended to the patient?
CQ12	Should a genomically matched therapy be recommended based on an actionable alteration detected by ctDNA CGP that could not be detected by a tissue test?
CQ13	When should ctDNA CGP be prioritized over tissue CGP?
CQ14	Should ctDNA CGP be recommended for all cancer patients with no actionable alterations by tissue CGP that were previously conducted?

Abbreviations: bTMB, blood tumor mutation burden; CGP, comprehensive genomic profiling; CH, clonal hematopoiesis; CNV, copy number variation; ctDNA, circulating tumor DNA; ICI, immune checkpoint inhibitor; MSI, microsatellite instability; NGS, next-generation sequencing.

TABLE 2 Degrees of recommendation and decision criteria

Degree of recommendation	Decision criteria
Strongly recommended (SR)	Strongly recommended based on the available evidence and considering potential benefit, harm, cost, and patient preference
Recommended (R)	Recommended based on the available evidence and considering potential benefit, harm, cost, and patient preference
Expert consensus opinion (ECO)	Should be considered an option based on the available evidence and considering potential benefit, harm, cost, and patient preference
Not recommended (NR)	Not recommended based on the available evidence and considering potential benefit, harm, cost, and patient preference

The Committee's recommendation level was determined in the following manner: (1) strongly recommended (SR): at least 70% of the votes; (2) recommended: if classification (1) was not met, but SR + recommended (R) accounted for at least 70% of the votes; (3) expert consensus opinion (ECO): if (1) and (2) were not met, but SR + R + ECO accounted for at least 70% of the votes; (4) not recommended (NR): at least 50% of the votes; and if none of classifications (1–4) were met, the final consensus was "no recommended level."

3 | RESULTS AND MEETING OUTCOMES

In the pre-meeting survey, eight experts developed and reported 14 CQs on ctDNA assays. Twenty-one experts reviewed the validity of the CQs. After the CQs were validated, 15 recommendations were developed for each CQ. Voting was conducted by the 12 EP members, with each member having one vote for each recommendation (Table 3).

3.1 | Recommendations in response to the CQs

3.1.1 | CQ1: When no gene fusion is detected with a ctDNA CGP, should tissue CGP be recommended?

Recommendation: Tissue CGP is recommended if low ctDNA levels are suspected and the presence of gene fusion is expected.

Recommendation level: R (SR: 8, R: 3, ECO: 1, NR: 0).

Overall, eight experts voted SR; three experts, R; and one expert, ECO. Analyzing 137 tissue CGP in which a fusion gene was detected, 75 paired ctDNA samples had an estimated tumor fraction of ≥1% and the same fusion gene was detected in 64 ctDNA samples (85%).[10] In the remaining 62 samples with an estimated tumor fraction of <1%, the fusion gene in tissue was detected in 32 ctDNA samples (52%), suggesting that low ctDNA content can lead to false-negative fusion results.[10] Given the relatively low frequency of fusion genes in solid tumors,[11,12,13–15] not all patients are candidates for tissue CGP when gene fusions are not detected with a ctDNA CGP. Fusion genes are enriched in certain types of tumors including sarcomas,[16] pediatric central nervous system tumors,[17] *KRAS* wild-type lung invasive mucinous adenocarcinoma,[18,19] and *KRAS* wild-type pancreatic carcinoma.[20,21] In addition, an NGS-based CGP is recommended in the guidelines to detect *NTRK* fusions in tumor types known to harbor them or when the presence of *NTRK* fusion is suspected from other methods such as IHC.[22–24] One expert proposed ECO because of cost. Collectively, tissue CGP is recommended if low ctDNA levels are suspected and the presence of gene fusion is expected.

3.1.2 | CQ2: When no copy number variation (CNV) is detected with a ctDNA CGP, should tissue CGP be recommended?

Recommendation. Tissue CGP should be considered if low ctDNA levels are suspected and the presence of copy number variation (CNV) is expected.

Recommendation level: ECO (SR: 2, R: 4, ECO: 6, NR: 0).

Overall, two experts voted for SR; four experts, R; and six experts, ECO. In a study of 605 patients with solid tumors, the detection rate of CNV was lower than that of other mutation types in the same patients tested for cancer gene alterations using tissue and ctDNA in the blood.[25] Variant allele frequency (VAF) has been reported to be positively correlated with CNV detection rates.[20] This suggests that the ctDNA CGP may give false-negative results for CNVs, especially when the amount of ctDNA is low. Although F1LCDx validation studies have reported a mean analyzable limit of detection (LoD) of 19.8% TF for copy number amplification and 30.4% TF for copy number deletion, the LoD of the ctDNA level to detect CNV varies among different assays. Therefore, the decision to perform tissue CGP should include consideration of LoD. Alternative tests, such as IHC and in situ hybridization, are available to confirm the CNV of one or several specific genes. However, to comprehensively confirm the presence of CNV, tissue CGP should also be considered. Overall, tissue CGP should be considered if low ctDNA levels are suspected and the presence of CNV is expected.

3.1.3 | CQ3: Should an immune checkpoint inhibitor (ICI) be recommended for patients with a blood tumor mutational burden (bTMB)-high solid tumor?

Recommendation: ICIs are strongly recommended for patients with tumor mutation burden (TMB)-high on a tissue CGP because the utility of bTMB for ICI treatment has not been established.

TABLE 3 Summary of consensus recommendations

Consensus recommendations	Degree of recommendation
CQ1. When no gene fusion is detected with a ctDNA CGP, should tissue CGP be recommended?	
Tissue CGP is recommended if low ctDNA levels are suspected and the presence of gene fusion is expected	R
CQ2. When no copy number version (CNV) is detected with a ctDNA CGP, should tissue CGP be recommended?	
Tissue CGP should be considered if low ctDNA levels are suspected and the presence of CNV is expected	ECO
CQ3. Should an immune checkpoint inhibitor (ICI) be recommended for patients with a blood tumor mutational burden (bTMB)-high solid tumor?	
ICIs are strongly recommended for patients with TMB-high on a tissue CGP because the utility of bTMB for ICI treatment has not been established	SR
CQ4. When microsatellite instability (MSI) is clinically suspected and not detected by ctDNA CGP, is tissue CGP recommended?	
For low tumor fraction, tissue CGP, or alternative validated tests are strongly recommended	SR
CQ5. Should ctDNA CGP be recommended for cancer types with low ctDNA levels?	
Tissue CGP is recommended for cancer types with low ctDNA levels	R
CQ6. In cases with a low tumor burden, should the NGS tests prioritize tissue CGP over ctDNA CGP?	
If the tumor volume is small, tissue CGP is recommended	R
CQ7. Is ctDNA CGP recommended during a systemic therapy?	
A1: ctDNA CGP is recommended for patients with tumors that are refractory to systemic therapy	R
A2: ctDNA CGP is not recommended for patients with tumors that are responding to systemic therapy	NR
CQ8. When should the origin of a variant be suspected of being clonal hematopoiesis (CH)?	
Variants may be of CH origin if the gene is commonly implicated in CH and variant allele frequency is low	R
CQ9. When should the origin of a variant be suspected of being germline?	
A variant should be suspected of being a germline in origin if the VAF is ≥30%	R
CQ10. Should a variant suspected of being germline be confirmed by a validated method?	
If a variant is suspected of being germline in origin, a validated method for confirmation should be considered if the variant is (likely) to be pathogenic and the gene is listed in the latest American College of Medical Genetics and Genomics Secondary Findings list	ECO
CQ11. Should a treatment targeting highly suspected subclonal variants be recommended to the patient?	
Subclonal variants may be less likely to benefit from a therapy targeting that variant. However, it is still unclear whether subclonal variants can truly predict a lack of response	R
CQ12. Should a genomically matched therapy be recommended based on an actionable alteration detected by ctDNA CGP that could not be detected by a tissue test?	
When an actionable alteration that could not be identified by a tissue test is detected by ctDNA CGP, a genomically matched therapy is recommended if it is likely to be clonal, and the assay is validated	R
CQ13. When should ctDNA CGP be prioritized over tissue CGP?	
Initial genotyping with ctDNA CGP is recommended over tissue CGP when rapid results are required or when tissue samples are unavailable or inappropriate	R
CQ14. Should ctDNA CGP be recommended for all cancer patients with no actionable alterations by tissue CGP that were previously conducted?	
Longitudinal ctDNA CGP should be considered for certain patients with cancer if actionable alterations are expected	ECO

Abbreviations: ACMG SF v.3.0, American College of Medical Genetics and Genomics Secondary Findings version 3; bTMB, blood tumor mutation burden; CGP, comprehensive genomic profiling; CH, clonal hematopoiesis; CNV, copy number variation; ctDNA, circulating tumor DNA; ECO, expert consensus opinion; ICI, immune checkpoint inhibitor; MSI, microsatellite instability; NGS, next-generation sequencing; NR, not recommended; R, recommended; SR, strongly recommended; TMB, tumor mutation burden; VAF, variant allele frequency.

Recommendation level: SR (SR: 10, R: 2, ECO: 0, NR: 0).

Overall, 10 experts voted SR and two experts voted R. Previous studies have reported that a TMB determined by oncogene panel testing using tumor tissue correlates with that determined by whole exon sequencing.[13,26] The KEYNOTE-158 study demonstrated the efficacy of pembrolizumab in patients with TMB ≥10 muts/Mb assessed by F1CDx.[27] However, in the analysis of TMB using liquid biopsy, attention should be paid to the possibility that TMB values are higher on liquid biopsy analysis than on tumor tissue analysis due to the detection of CH and heterogeneity[28] and the possibility that lower TMB values are obtained by liquid biopsy if ctDNA levels are low.[5] To date, no clear cut-off for bTMB score in patients receiving ICI has been validated. However, the efficacy of atezolizumab for non–small-cell lung cancer with bTMB ≥16 has been suggested.[29] A study comparing F1CDx and F1LCDx in the same patients has reported a high correlation with TMB in patients with a high TF.[30]

Therefore, ICI should be considered for patients with a high TF who are found to have high TMB.

One expert voted R because of the absence of randomized comparative studies of ICI administered to patients with TMB-high. Another expert voted R because of the absence of the cut-off for TMB-high for each cancer type. Regarding tissue TMB, it has been suggested that the cut-off for the efficacy of ICI depends on the drug used and cancer type.[31]

3.1.4 | CQ4: When microsatellite instability is clinically suspected and not detected by ctDNA CGP, is tissue CGP recommended?

Recommendation: For low tumor fraction, tissue CGP, or alternative validated tests are strongly recommended.

Recommendation level: SR (SR: 11, R: 1, ECO: 0, NR: 0).

All the experts agreed with this recommendation and voted SR ($n = 11$) or R ($n = 1$). A low tumor fraction could result in a false-negative result for microsatellite instability (MSI).[32] One study compared the performance of a plasma-based MSI assessment (Guardant360, a next-generation sequencing-based ctDNA assay) to that of a tissue-based MSI assessment (a validated polymerase chain reaction-based method) in patients with advanced GI cancer.[33] In 658 patients with advanced GI cancer who underwent MSI testing with both plasma and tissue, the overall percentage agreement, positive percentage agreement (PPA), and negative percentage agreement were 98.2% (95% CI, 96.8 to 99.1), 71.4% (95% CI, 47.8 to 88.7), and 99.1% (95% CI, 98.0 to 99.7), respectively. In patients whose plasma samples had a tumor fraction ≥1.0%, the PPA was 100.0% (15/15; 95% CI, 78.2 to 100.0). One expert proposed R because tissue-based MSI testing remains the gold standard. However, a case report has suggested the usefulness of ctDNA MSI testing.[33] The patient had cancer of unknown primary origin and urgently needed effective treatment because the tumor was highly aggressive. MSI was detected with ctDNA testing in 5 days, and the patient experienced antitumor effects from the ICI. The efficacy of ICI treatment for patients with MSI-high detected by ctDNA but not by tissue testing remains unclear.

3.1.5 | CQ5: Should ctDNA CGP be recommended for cancer types with low ctDNA levels?

Recommendation: Tissue CGP is recommended for cancer types with low ctDNA levels.

Recommendation level: R (SR: 4, R: 7, ECO: 1, NR: 0).

Overall, four experts voted for SR; seven experts, R; and one expert, ECO. The concentration and detection rate of ctDNA are affected by cancer types. An analysis using digital polymerase chain reaction-based technologies in 640 patients reported that primary brain tumor, kidney cancer, prostate cancer, and thyroid cancer have low ctDNA detection rates.[34] Patients with these cancers have a ctDNA detection rate of <50%.[34] Another study investigated the detectability of ctDNA variants in 978 patients with 16 tumor types in three cohorts (Study 1108, ATLANTIC, and Study 10). Although there are no direct comments on the detectability, the somatic alteration detection rate was low in patients with pancreatic cancer, sarcoma, and glioblastoma.[2,35] That is, liquid biopsy performed on these cancer types may cause false-negative results. One expert proposed ECO, considering it better to make a comprehensive judgment, including other factors such as tumor volume, even for cancer types with a low detection rate of ctDNA. Overall, tissue CGP is recommended for cancer types with low ctDNA levels.

3.1.6 | CQ6: In cases with a low tumor burden, should the NGS tests prioritize tissue CGP over ctDNA CGP?

Recommendation: If the tumor volume is small, tissue CGP is recommended.

Recommendation level: R (SR: 1, R: 9, ECO: 2, NR: 0).

Overall, one expert voted for SR; nine experts, R; and two experts, ECO. The ctDNA level in plasma correlates with tumor volume. [34,36] Data from the TRACERx project, a ctDNA tracking study in early-stage non–small-cell lung cancer, showed that a primary tumor burden of 10 cm^3 would result in a mean clonal plasma VAF of 0.1% (95% CI, 0.06–0.18%).[37] In addition, several clinical factors are associated with ctDNA levels. In colorectal cancer, the VAF of *RAS* mutations has been reported to be low in patients with lung metastasis only or peritoneal dissemination only. To maintain VAF ≥0.2%, which indicates sufficient VAF to detect variants with sufficient sensitivity, the length of the largest legion needs to be ≥20 mm or 20 or more lesions had to be detected in patients with lung-only metastasis and be ≥20 mm in patients with peritoneum-only metastasis.[38] Two experts proposed ECO because this evidence is limited to specific cancer types. In conclusion, tissue CGP is recommended if the tumor volume is small.

3.1.7 | CQ7: Is ctDNA CGP recommended during a systemic therapy?

Recommendation:

- ctDNA CGP is recommended for patients with tumors that are refractory to systemic therapy.

Recommendation level: R (SR: 3, R: 9, ECO: 0, NR: 0).

All the experts agreed with the recommendation voted SR ($n = 3$) or R ($n = 9$). The possible clinical limitations of ctDNA CGP include low sensitivity and the risk of false negatives. ctDNA should be collected when the cancer is progressing, either before treatment or at progression. Blood samples collected when the tumor is

responding to treatment will lead to reduced sensitivity of ctDNA CGP. ctDNA release is considered proportional to tumor growth, with the fastest growing tumor clones shedding the most ctDNA and theoretically having the most clinical relevance.[39]
- ctDNA CGP is not recommended for patients with tumors that are responding to systemic therapy.

Recommendation level: NR (SR: 0, R: 0, ECO: 2, NR: 10).

Overall, two experts voted for ECO, and 10 experts voted for NR. Among the two experts who voted for ECO, one expert considered that ctDNA dynamics assessed through therapy had been shown to correlate with treatment response and identify responses earlier than does clinicoradiological detection.[40–43] The other expert suggested that repeating ctDNA CGP might allow the detection of acquired resistance mutations by selective pressure of kinase inhibitors and the optimal selection of the subsequent treatment. Monitoring the *ESR1* mutation in blood (b*ESR1* mut) expression in advanced breast cancer patients on aromatase inhibitor and CDK4/6 inhibitor therapy may be clinically useful.[44] Targeting increasing b*ESR1* mutations associated with resistance through a change in the endocrine partner of palbociclib was feasible and allowed a doubling in the subsequent median progression-free survival. Although targeted and hormone therapy could lead to substantial evolution in the genetic features, cytotoxic chemotherapy does not seem to significantly alter the genomic landscape.[45]

3.1.8 | CQ8: When should the origin of a variant be suspected of being clonal hematopoiesis (CH)?

Recommendation: Variants may be of CH origin if the gene is commonly implicated in CH and variant allele frequency is low.

Recommendation level: R (SR: 7, R: 5, ECO: 0, NR: 0).

Overall, seven experts voted for SR, and five experts voted for R. Part of the mutations detected as somatic mutations by liquid biopsy are also detected by white blood cell analysis, and the majority of them are mutations of CH origin.[28] The detection rate of CH increases with age.[46] In addition, the detection rates are high for *TP53*, *CHEK2*, and *PPM1D* in patients with a history of chemotherapy.[47] Although it is difficult to identify the origin of a mutation by VAF, the VAF of CH tends to be lower than that of somatic mutations.[48]

3.1.9 | CQ9: When should the origin of a variant be suspected of being germline?

Recommendation: A variant should be suspected of being a germline in origin if the VAF is ≥30%.

Recommendation level: Recommended (SR: 0, R: 12, ECO: 0, NR: 0).

All 12 experts voted for R. Among patients with known germline findings, VAF ranges from 39% to 88% in a ctDNA CGP.[49] In advanced GI cancer, VAF ranges from 26% to 74% for putative germline *BRCA* mutations.[50] Germline mutations may be detected at low VAF due to allelic loss and/or mosaicism. In addition, confirmatory germline testing was performed for patients whose samples had mutations with a VAF of 40%–60% on ctDNA CGP, and 111/160 (69%) patients were confirmed to have germline origin.[49] The Japanese national guideline suggests using a VAF cut-off of 30%,[51] although the validity of the cut-off needs to be evaluated further because germline variants with ctDNA VAFs of <30% were reported. [52] In summary, a variant should be suspected of being germline in origin if the VAF is ≥30%.

3.1.10 | CQ10: Should a variant suspected of being germline be confirmed by a validated method?

Recommendation: If a variant is suspected of being germline in origin, a validated method for confirmation should be considered if the variant is (likely) to be pathogenic and the gene is listed in the latest American College of Medical Genetics and Genomics (ACMG) Secondary Findings list.

Recommendation level: ECO (SR: 1, R: 4, ECO: 7, NR: 0).

Overall, one expert voted for SR; four experts, R; and seven experts, ECO. When a gene mutation with a VAF of ≥30% is detected and the mutation is (likely) to be pathogenic, the next step would be to determine whether a confirmatory germline test should be proposed to the patient. The ACMG has published a recommended list of genes for reporting presumed germline pathogenic variants with consideration for morbidity and mortality, penetrance, and actionability.[53,54] Relative frequency of somatic versus germline variation (germline conversion rate) and tumor type is also important, as mutations detected in some genes (e.g., *APC*, *NF1*, *PTEN*, *RB1*, *STK11*, *TP53*) are more often somatic in origin.[55,56] In addition, clinical information including age at onset, single or multiple primary cancers, and family history of cancer should be reviewed.[57] Therefore, if a variant is suspected of being germline in origin and a pathogenic or likely to be pathogenic variant of gene listed in the latest ACMG Secondary Findings list, a validated method for confirmation should be considered with an appropriate patient counseling.

3.1.11 | CQ11: Should a treatment targeting highly suspected subclonal variants be recommended to the patient?

Recommendation: Subclonal variants may be less likely to benefit from a therapy targeting that variant. However, it is still unclear whether subclonal variants can truly predict a lack of response.

Recommendation level: R (SR: 1, R: 9, ECO: 2, NR: 0).

Overall, one expert voted for SR; nine experts, R; and two experts, ECO. VAF may provide information on the likely subclonal nature of the variant, and subclonal variants may be less likely to benefit from a therapy targeting that variant. However, there is limited evidence on whether VAF of ctDNA CGP can estimate

clonality accurately, and it is still unclear whether true subclonal variants can predict a lack of response. Two experts proposed ECO because there is a lack of evidence in the clinical utility of VAF of ctDNA CGP.

3.1.12 | CQ12: Should a genomically matched therapy be recommended based on an actionable alteration detected by ctDNA CGP that could not be detected by a tissue test?

Recommendation: When an actionable alteration that could not be identified by a tissue test is detected by ctDNA CGP, a genomically matched therapy is recommended if it is likely to be clonal, and the assay is validated.

Recommendation level: R (SR: 3, R: 8, ECO: 1, NR: 0).

All the experts, except one, agreed with and accepted SR ($n = 3$) or R ($n = 8$). ctDNA CGP potentially captures spatial and/or temporal heterogeneity missed by single-lesion tumor biopsies. Plasma ctDNA may also more accurately assess the current genomic profile of an advanced solid tumor compared with the use of archival tissue specimens, such as when recurrence occurs many years after the resection of an early-stage tumor. Previous studies have reported patients who showed a tumor response to targeted therapy based on an alteration detected by ctDNA CGP that could not be identified by tissue tests.[33,58] One expert proposed ECO because of little evidence for the efficacy of targeted therapy based on an actionable alteration detected only in ctDNA. Therefore, when an actionable alteration that could not be identified by a tissue test is detected by ctDNA CGP, a genomically matched therapy should be considered if it is likely to be clonal and the assay is validated.

3.1.13 | CQ13: When should ctDNA CGP be prioritized over tissue CGP?

Recommendation: Initial genotyping with ctDNA CGP is recommended over tissue CGP when rapid results are required or when tissue samples are unavailable or inappropriate.

Recommendation level: R (SR: 7, R: 4, ECO: 1, NR: 0).

All the experts, except one, agreed with and accepted SR ($n = 7$) or SR ($n = 4$). In a comparison between ctDNA (GOZILA study) and tissue (GI-SCREEN study) profiling studies for advanced GI cancers, the TAT to assay results was significantly shorter in ctDNA testing than in tissue testing (median, 11 days versus 33 days; $p < 0.0001$).[50] A molecular profiling study for metastatic non–small-cell lung cancer also showed a shorter TAT using the ctDNA assay than using tissue assay (median, 9 days versus 15 days; $p < 0.0001$).[59] In addition, adequate archival tumor materials suitable for NGS may not be available for some patients due to the difficulty of biopsy, low tumor content, and the storage period of ≥3 years.[60,61] One expert proposed ECO because other factors, such as cancer types and tumor volume as discussed in CQs above, should also be considered for ctDNA CGP. Therefore, initial genotyping with ctDNA CGP should be considered over initial tissue CGP when rapid results are required or tissue samples are unavailable or inappropriate.

3.1.14 | CQ14: Should ctDNA CGP be recommended for all cancer patients with no actionable alterations by tissue CGP that were previously conducted?

Recommendation: Longitudinal ctDNA CGP should be considered for certain patients with cancer if actionable alterations are expected.

Recommendation level: ECO (SR: 0, R: 1, ECO: 11, NR: 0).

Overall, one expert voted for R, and 11 experts voted for ECO. Liquid biopsy allows for detecting heterogeneous changes within or between tumors that cannot be captured by locally collected tissue biopsy specimens.[62] In addition, unlike histological tests using archived specimens, ctDNA CGP detects gene mutations that exist at the time of specimen collection.

Furthermore, repeated biopsies for testing are often invasive and difficult. A study of droplet digital polymerase chain reaction-based ctDNA analysis in non–small-cell lung cancer reported that serial quantification of plasma genotype allowed noninvasive assessment of response and resistance, including the detection of resistance mutations up to 16 weeks before radiographic progression.[63] In addition, studies in patients with prostate cancer and GI cancer suggest the usefulness of ctDNA CGP for detecting acquired gene mutations.[3,64] However, there is insufficient evidence to use regular monitoring of ctDNA during therapy. One expert proposed R because of the possibility of detecting actionable gene mutations using ctDNA CGP by aggressively searching for them. In summary, longitudinal ctDNA CGP should be considered especially after targeted therapy in which the acquisition of actionable alterations is expected.

4 | CONCLUSION

The Committee's vote on the recommendation for each CQ resulted in unified consensus in some and divergent views in others. There was some consensus that a major potential clinical limitation of ctDNA CGP is the risk of false-negative results, and tissue CGP is recommended or should be considered when ctDNA CGP shows a noninformative result. In addition, the experts had different opinions on the recommendation regarding germline mutations, subclonal variants, and longitudinal tests due to the limited evidence. As mentioned above, each expert's judgment of voting was based on the current available scientific evidence.

New technologies of ctDNA CGP currently under development have the potential to overcome their limitations. In addition, multiple clinical trials using the results of the ctDNA CGP are underway.

This test should play an increasingly important role in the treatment of cancer. The accumulation of new evidence will lead to substantial changes in the descriptions in the text and recommended levels. In conclusion, we have developed consensus recommendations that suggest requirements for the proper implementation of ctDNA CGP. We hope that this recommendation will lead to better decision-making in the treatment selection for patients with solid tumors.

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CONFLICT OF INTEREST

Yoshiaki Nakamura received lecture fees from Chugai Pharmaceutical, Merck Biopharma, Guardant Health AMEA, and research funds from Chugai Pharmaceutical, Taiho Pharmaceutical, Daiichi Sankyo, Genomedia Inc., Guardant Health Inc., Roche Diagnostics, Seagen; Kuniko Sunami received research funds from Sysmex; Hidenori Kage received research funds from Konica Minolta; Takafumi Koyama received Honoria fees from Chugai Pharmaceutical and Sysmex and research funds from PACT Pharma; Daisuke Ennishi received lecture fees from Eisai Pharmaceutical Co., Ltd., Kyowa Kirin Pharmaceutical Co., Ltd., Chugai Pharmaceutical Co., Ltd., SymBio Pharmaceuticals Co., Ltd., Bristol Myers Squibb and research funds from Nipponshinyaku Pharmaceutical Co., Ltd., and Chugai Pharmaceutical Co., Ltd; Masashi Kanai receives annual profit from Therabiopharma Inc., manuscript fees from Chugai Pharmaceutical, and research funds from Molecular Health; Hirotsugu Kenmotsu received research funds from AstraZeneca K.K., Novartis, LOXO Oncology Inc., Ono Pharmaceutical Co., Ltd., and Eli Lilly; Hideaki Bando received lecture fees from Eli Lily Japan K.K., Taiho Pharmaceutical Co. Ltd., Ono pharmaceutical, and research funds from Ono pharmaceutical; Akitaka Makiyama received lecture fees from Eli Lily Japan K.K., Taiho Pharmaceutical Co. Ltd, Ono Pharmaceutical Co. Ltd., and Daiichi Sankyo Co. Ltd; Shinji Kohsaka received research funds from Konica Minolta; Yoichi Naito received lecture fees from Chugai, Pfizer, Eli Lilly, and Novartis and research funds from Eisai, Gilead, Takeda, Chugai, Natera, Daiichi-Sankyo, Taiho, Pfizer, and Boehringer Ingelheim, ABBVIE, and AstraZeneca; Takayuki Yoshino received lecture fees from Taiho Pharmaceutical Co., Ltd., Chugai Pharmaceutical Co., Ltd., Eli Lilly Japan K.K., Merck Biopharma Co., Ltd., Bayer Yakuhin, Ltd., Ono Pharmaceutical Co., Ltd., and MSD K.K. and research funds from Ono Pharmaceutical Co., Ltd., Sanofi K.K., Daiichi Sankyo Co., Ltd., PAREXEL International Inc., Pfizer Japan Inc., Taiho Pharmaceutical Co., Ltd., MSD K.K.K., Amgen K.K., Genomedia Inc., Syam Corporation, Chugai Pharmaceutical Co., Ltd., and Nippon Boehringer Ingelheim Co., Ltd. The other authors have no conflict of interest to declare. Katsuya Tsuchihara is an editorial board member.

ETHICS STATEMENT

Approval of the research protocol by an Institutional Reviewer Board: N/A.

Informed Consent: N/A.

Registry and the Registration No. of the study/trial: N/A.

Animal Studies: N/A.

ORCID

Mitsuho Imai https://orcid.org/0000-0001-8736-0399
Yoshiaki Nakamura https://orcid.org/0000-0002-5241-6855
Kuniko Sunami https://orcid.org/0000-0002-7137-5649
Hidenori Kage https://orcid.org/0000-0003-3658-4442
Takafumi Koyama https://orcid.org/0000-0001-5807-8458
Masashi Kanai https://orcid.org/0000-0002-6954-4474
Hirotsugu Kenmotsu https://orcid.org/0000-0003-0590-9259
Sachi Morita https://orcid.org/0000-0002-3115-2336
Hideaki Bando https://orcid.org/0000-0001-5041-2765
Shinji Kohsaka https://orcid.org/0000-0001-8651-6136
Katsuya Tsuchihara https://orcid.org/0000-0001-7507-2349
Yoichi Naito https://orcid.org/0000-0002-8490-9064
Takayuki Yoshino https://orcid.org/0000-0002-0489-4756

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