Received: 24 April 2021 | Revised: 8 June 2021 | Accepted: 9 June 2021

DOI: 10.1111/cas.15022

REPORT

Cancer Science WILEY

Appropriate use of cancer comprehensive genome profiling assay using circulating tumor DNA

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Abstract

Comprehensive genomic profiling (CGP) is being increasingly used for the routine clinical management of solid cancers. In July 2018, the use of tumor tissue-based CGP assays became available for all solid cancers under the universal health insurance system in Japan. Several restrictions presently exist, such as patient eligibility and limitations on the opportunities to perform such assays. The clinical implementation of CGP based on plasma circulating tumor DNA (ctDNA) is also expected to raise

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issues regarding the selection and use of tissue DNA and ctDNA CGP. A Joint Task Force for the Promotion of Cancer Genome Medicine comprised of three Japanese cancer-related societies has formulated a policy proposal for the appropriate use of plasma CGP (in Japanese), available at https://www.jca.gr.jp/researcher/topics/2021/files/20210120.pdf, http://www.jsco.or.jp/jpn/user_data/upload/File/20210120.pdf, and https://www.jsmo.or.jp/file/dl/newsj/2765.pdf. Based on these recommendations, the working group has summarized the respective advantages and cautions regarding the use of tissue DNA CGP and ctDNA CGP with reference to the advice of a multidisciplinary expert panel, the preferred use of plasma specimens over tissue, and multiple ctDNA testing. These recommendations have been prepared to maximize the benefits of performing CGP assays and might be applicable in other countries and regions.

KEYWORDS

cancer comprehensive genome profiling assay, circulating tumor DNA, liquid biopsy, next-generation sequencer, plasma

1 | INTRODUCTION

DNA fragments derived from apoptotic and necrotic tumor cells of solid cancer[1] are influenced by physiological events induced by microenvironmental stress and therapeutic effects.[2] Liquid biopsy provides an opportunity to detect, analyze, and monitor cancer cells in various body fluids, such as blood and urine. These biopsy samples are composed of different biological matrices, such as circulating tumor cells, cell-free nucleic acids, exosomes, and tumor-educated platelets. Liquid biopsies of circulating tumor DNA (ctDNA) have recently been used as a minimally invasive diagnostic tool for detecting tumor-specific genomic alterations. Recent studies have shown that genomic alterations in solid tumors can be characterized by ctDNA sequencing.[3-5] In addition, the analysis of ctDNA, the results of which are not affected by heterogeneity, may be useful for designing effective treatment strategies.[6]

Highly sensitive next-generation sequencing (NGS)-based technology allows us to analyze comprehensive genomic alterations of ctDNA, providing a better view of tumor heterogeneity and allowing real-time monitoring of cancer evolution. NGS-based assays for ctDNA comprehensive genome profiling (ctDNA CGP) are clinically available in the USA and other countries and regions. In the USA, the Guardant360 CDx (Guardant Health) and the FoundationOne Liquid CDx (Foundation Medicine) were approved in August 2020 as companion diagnostics ("CDx") and CGP assays for the detection of genetic abnormalities in ctDNA using plasma samples.

Two kinds of tissue CGP panels were approved in Japan in December 2018 and became available under universal health insurance coverage in June 2019. Prior to this, in November 2017, the Japanese Society of Medical Oncology, the Japanese Society for Clinical Oncology, and the Japanese Cancer Association published a "Guidance for Cancer Treatment Based on Gene Panel Assay Using Next-Generation Sequencers" (hereinafter referred to as the "Three Society Guidance") to promote the appropriate use of CGP under Japan's universal health insurance system.[7,8]

Currently, the *EGFR* gene mutation, the *MET* exon 14 skipping assay for non–small cell lung cancer, and the *RAS* gene mutation assay for colorectal cancer have been approved as CDx assays for detecting genetic alterations in plasma ctDNA in Japan. Plasma CGP assays that detect genetic abnormalities in ctDNA using plasma samples (hereinafter referred to as "plasma CGP assays") were also approved in Japan on 13 March 2021. The current version of the Three Society Guidance is focused on CGP assays using tissue specimens ("tissue CGP assay").[7,8] For the appropriate use of plasma CGP, especially under the universal health insurance system, further policy recommendations are needed.

The use of the tissue CGP assay under the universal health insurance system in Japan has currently been limited by restrictions on eligible patients and the frequency of assay use.[9] A Joint Task Force on Genome Promotion formed by three academic societies decided to produce policy recommendations for the appropriate clinical implementation of plasma CGP assays in Japan without delay. These recommendations may be useful not only for Japanese cancer patients, who are treated under Japan's universal health insurance system, but also for patients in countries that have unlimited access to plasma CGP panels. This paper summarizes the contents of the recommendations by discussing the advantages and disadvantages of plasma CGP assay.

2 | CURRENT STATUS OF PLASMA COMPREHENSIVE GENOMIC PROFILING ASSAY

Compared with tissue CGP assays, evidence for plasma CGP assays remains insufficient in some areas. In this section, we will introduce

the major findings that will be useful for the application of plasma CGP assays.

2.1 | Handling of plasma samples

Plasma samples are easier to collect than tissue samples. Plasma samples can be affected by a variety of patient factors, including inflammatory diseases, autoimmune diseases, smoking, pregnancy, and exercise.[3,5,10] Because ctDNA does not undergo formalin fixation, which is used for tissue specimens, it is not affected by the degradation associated with formalin fixation. The amount of tumor-derived ctDNA obtained from the plasma is generally lower than the amount of normal cell-derived cell-free DNA, and there are specific cancer types and conditions that affect the detection rates of gene alterations.[7]

2.2 | Technical aspects of plasma comprehensive genomic profiling assay

The increased mixture of normal cell-derived cell-free DNA as a result of leukocyte lysis during blood coagulation affects the detection sensitivity of tumor-derived ctDNA.[5] Because inter-tumor heterogeneity exists, the presence of genetic abnormalities throughout the entire tumor is difficult to evaluate using tissue samples, but not plasma samples.[11,12] The tumor mutation profile can change over time, particularly between the time of tumor tissue collection and after therapeutic intervention, but plasma samples can provide information on genetic alterations that reflect the real-time biological characteristics of the tumor at any given collection point.[1,12] Plasma CGP assays reportedly have higher detection rates than tissue CGP assays for re–biopsy samples with regard to the detection of molecular changes associated with resistance to molecular targeted therapy.[12] Tumors with a slow clinical course, that are slow growing, or that are in an early disease stage have a higher frequency of false negative results.[2,13,14]

In lung cancer, plasma specimens generally have a higher false-negative rate than tissue specimens. Therefore, prioritizing the tissue CGP assay may be reasonable.[4,6,15] The detection rate of fusion genes and other gene alterations is known to be lower in DNA-based plasma samples.[16,17] ctDNA gene mutations are difficult to distinguish from clonal hematopoiesis-derived gene mutations in normal cells (clonal hematopoiesis of indeterminate potential, "CHIP") using a plasma-based analysis. CHIP is more frequent in older patients, but the majority (approximately 97%) of clonal hematopoiesis-derived genes have a variant allele frequency (VAF) of less than 1%.[18] In colorectal cancer, the VAF of *RAS* mutations has been reported to be low in patients with mucinous carcinoma, lung metastasis cases only, peritoneal dissemination cases only, and cases within 30 days of the completion of chemotherapy.[19,20] In prostate cancer, there are reports showing that plasma CGP performs almost as well as tissue CGP, or even better.[21,22] The detection rate varied from 21.4% for patients with GBM to >95% for patients with SCLC and nasopharyngeal carcinoma.[23]

Tissue CGP assays provide results for microsatellite instability (MSI) assays and tumor mutation burden (TMB). Plasma CGP assays reportedly have a high concordance rate with PCR and NGS assays using tissue specimens for MSI assays.[24,25]

In the plasma CGP assay, the limit of detection (LOD) has been improved by molecular barcoding and error suppression methods. In general, the frequency of somatic mutation alleles is low, and the number of cases where it is difficult to distinguish single nucleotide polymorphism (SNP) or germline mutations is relatively small compared with tissue CGP assays. The LOD reportedly varies by assay, and results can vary according to the assay method.[26] When the ratio of ctDNA to cell-free DNA (tumor fraction [TF]) is low, copy number changes can be difficult to evaluate (using FoundationOne Liquid CDx, the detection limit for copy number changes is considered to be 20% TF).[26] Caution should be exercised with regard to false-negative results for copy number changes.[27]

2.3 | Turnaround time

The plasma CGP assay has a shorter turnaround time (TAT: time from specimen collection until the return of assay results) than the tissue CGP assay because of the simplicity of its collection process and the shorter time from specimen collection until specimen delivery.[28]

In a study examining esophageal squamous cell carcinoma, gastric carcinoma, colorectal carcinoma, pancreatic carcinoma, and biliary tract carcinoma, a median TAT of 7 days was reported for the plasma CGP assay (Guardant 360), compared with a median TAT of 19 days for the tissue CGP assay.[11]

3 | IMPORTANCE OF MULTIPLE ASSAYS

Studies examining hormone receptor-positive breast cancer have reported the increased detection of *ESR1* mutations using plasma CGP assays over time.[29] As a CDx, a *RAS* mutation detection kit (OncoBEAM RAS CRC Kit) has already been approved for use on multiple occasions to determine the need for the re–administration of anti–EGFR antibody drugs as well as in cases requiring re–assay because of the failure of a tissue specimen assay (only one tissue specimen assay is allowed). Acquired *RAS* mutation, which is a major mechanism of resistance to anti–EGFR antibody therapy, is known to occur in minor alleles and to attenuate over time. In cases where mutations are not detected by a ctDNA-based *RAS* mutation assay, the re–administration of anti–EGFR antibody can provide a clinical benefit.[30-32] The use of multiple plasma gene assays for detecting resistance mutations in *EGFR* and *ALK* fusion genes in non–small cell lung cancer and the monitoring of the *RAS* mutation status during treatment in colorectal cancer enable appropriate evaluations and may contribute to the re–selection of appropriate post–treatment therapy.[28]

4 | ASSOCIATION WITH TREATMENT

In a study conducted in Japan on esophageal squamous cell carcinoma, gastric cancer, colorectal cancer, pancreatic cancer, and biliary tract cancer, 4.1% of patients who underwent a tissue CGP assay and 9.5% of patients who underwent a plasma CGP assay (Guardant 360) were subsequently enrolled in clinical trials according to the genetic abnormalities that were found ($P < .0001$).[11] In colorectal cancer, acquired *RAS* mutation, *EGFR* extracellular domain mutation, and the gene amplification of *EGFR*, *MET*, and *ERBB2* have been reported as resistance mechanisms after treatment with anti–EGFR antibody drugs, and a plasma CGP assay might be useful for investigating treatment options in later lines of treatment.[1,33] In a cohort study of non–small cell lung cancer (n = 323), the detection rate of actionable gene alterations (*EGFR*, *ALK*, *MET*, *BRCA1*, *ROS1*, *RET*, *ERBB2*, and *BRAF*) was 33% using the plasma CGP assay only and 20.5% using the tissue CGP assay only. When both tissue and plasma CGP assays were used, the detection rate was 35.8%.[34] In a study of plasma CGP assays performed in 93 patients with undetectable *EGFR*, *ALK*, and *ROS1* gene alterations according to tissue CGP, actionable (OncoKB levels 1 to 4) and level 1 to 2A genetic abnormalities were detected in 53 cases (57%) and 13 cases (14%), respectively. Among them, 20 patients (13%) received corresponding treatments.[35] In non–small cell lung cancer, the detection of secondary mutations in *ALK* fusion gene-positive non–small cell lung cancer was useful for the selection of ALK inhibitors, similar to the detection of *EGFR* mutations. For example, the L1196M or S1206Y mutation is resistant to crizotinib but not to ceritinib, while the I1171T and V1180L mutations are resistant to alectinib and crizotinib but not to ceritinib, and the G1202R, G1123S, and F1174C mutations are known to be resistant to crizotinib; these results support the usefulness of comprehensive genetic analyses. Biopsied tissue samples are obtained at the site of disease progression, but because the analysis of recurrent disease is often difficult, the minimally invasive detection of secondary mutations using a plasma CGP assay is useful. Together, these results suggest that the detection of secondary tyrosine kinase inhibitor-resistance mutations in non–small cell lung cancer that has progressed during treatment with ALK inhibitors is not mandatory at this time, but it could be valuable in determining the optimal choice of ALK inhibitors, which have different activities against different mutations. In a statement made by the International Association for the Study of Lung Cancer,[15] it was announced that a plasma CGP assay is preferred when a re–biopsy of the advanced site is not possible, although a certain percentage of false negatives can occur.

5 | RECOMMENDATIONS

This policy proposal presents the basic concept for the implementation of plasma CGP assays in patients with advanced solid cancers under Japanese insurance reimbursement. This policy recommendation covers only approved plasma CGP assays and not the CDx assay. It does not recommend the use of any specific plasma CGP assays.

In general, the false-negative rate of plasma samples is higher than that of tissue samples.[4,6,15] The continued use of tissue CGP assays to reduce the false-negative rate is reasonable. However, it is important to select appropriate specimens comprehensively according to each patient's condition and specific cancer type. Because the detection rate varies depending on the type of cancer,[23] the decision to use plasma CGP testing should be based on the literature for each cancer type.

5.1 | Expert panel (Molecular Tumor Board)

1. The plasma CGP test will be reviewed by an expert panel, which is currently playing a role in reviewing tissue CGP tests performed under national health insurance coverage.
2. If potential germline variants are identified, it is recommended that patients receive genetic counseling at the corresponding facility.

5.2 | Advantages and cautions of comprehensive genomic profiling assays using plasma and tissue samples

The characteristics of plasma and tissue CGP assays are shown in Table 1.

TABLE 1 Advantages and caveats of comprehensive genomic profiling (CGP) assay using plasma and tissue specimens\

	Advantages	Important points
Plasma CGP	- Easy to collect specimens and to obtain information on genetic abnormalities of the tumor at the time of collection - Short time to obtain results	- If the tumor volume is insufficient, alterations might not be detectable - False-negative rates for plasma CGP are often higher than those for tissue CGP - Increased false-positives because of CHIP in elderly patients - Copy number changes and gene fusions may be difficult to assess
Tissue CGP	- Direct assessment of genetic abnormalities in tumor cells	- Burden to patient and risk of complications at biopsy site - Longer turnaround time - Higher false-negative rate when tumor cell percentage is low - Past samples may not reflect the genetic abnormalities in the tumor cells at present - Specimens obtained more than 3-5 years previously will have deteriorated and will not be suitable for CGP assa

5.2.1 | Situations in which plasma specimens are preferred over tissue comprehensive genomic profiling

1. Information on the current condition, rather than the condition at the time of tissue sample collection, is needed:
   a. Specific cancer types for which tissue collection is difficult or for which samples tend to have a low tumor content.
   b. Presence of multiple lesions or cases in which a single tissue specimen is incapable of reflecting the overall condition.
   c. Tissue specimens have been stored for more than 3-5 years.[36]
   d. Tissue specimens with formalin overfixation and demineralization.
   e. Inadequate tumor content or tumor cell count in tissue samples (eg, after chemotherapy and radiotherapy).
   f. Inadequate quality of nucleic acid derived from tissue samples.
2. Immediate CGP assay results are required.
   a. Aggressive, progressing disease.
   b. CGP assay results are needed for decision-making purposes prior to first-line treatment for patients with certain cancers without a standard therapy (eg, cancer of unknown primary disease with insufficient tissue specimen).

5.2.2 | Situations in which tissue samples are preferred over plasma samples

1. When interpreting the results of a plasma CGP assay alone, the following situations may require additional consideration:
   a. Cases with brain tumors, bladder cancer, and pancreatic cancer.[13,23] In a study examining patients with pancreatic cancer, the success rate of an NGS assay using tissue specimens collected by EUS-FNA was reported to be 57.4%.[37]
   b. Colorectal cancer with lung metastasis only or peritoneal metastasis only.[19,20]
   c. Tumors with a slow clinical course and slow growth.
2. Cases with a high risk of false-positives because of the detection of non–tumor-derived genetic mutations, including CHIP.
3. Types of genetic abnormalities with high false-negative rates, such as fusion genes and *MET* exon 14 skipping in non–small cell lung cancer.

5.2.3 | View for plasma comprehensive genomic profiling assays performed in multiple sequential fashion

Because the collection of plasma samples is minimally invasive, performing multiple plasma CGP assays at different time points is possible. A re–assay is particularly important under the following clinical situations:

1 If the previous tissue CGP assay has failed.

A certain percentage of tissue CGP assays fail. Re–assays using a plasma specimen can be considered, especially if the results of the CGP assay are extremely important for treatment decision-making. Similarly, if a plasma CGP assay fails and the subsequent collection of tissue samples is possible, a re–assay using tissue samples is recommended.

2 Multiple assays are required to determine the treatment plan.

A limited number of CGP assays (tissue CGP assays are available only once per individual) can be performed under the universal health insurance system in Japan.[38] Plasma CGP assays, similar to tissue CGP assays, are intended to reveal genetic changes in cancers in individual patients and to provide opportunities for optimal cancer treatment. The plasma CGP assay reflects the overall tumor status at the time of the assay. Although a single assay is often sufficient for many patients, multiple CGP assays may be necessary if acquired resistance or secondary mutations are suspected during the course of treatment.

3 Multiple assays using multiple plasma specimens are useful for monitoring disease activity.[39-43] However, plasma CGP assays for patients with solid tumors are not suitable for use in therapy or disease monitoring under present insurance coverage in Japan because the purpose of a CGP assay is to identify the need for a specific therapy. There remains substantial room for improvement, and further investigations are needed.

6 | CONCLUSION

We have summarized the policy recommendations on the appropriate use of plasma CGP assays, which were approved in Japan in 2021. As genetic analysis technologies, including CGP assays, are rapidly developing as a result of advances in science and technology, we hope that this report may provide a reference point. The present recommendations may change significantly as further evidence accumulates.

ACKNOWLEDGMENTS

This paper summarizes the policy recommendation “Cancer genome profiling assay using circulating tumor DNA in blood” (in Japanese) established by the Joint Task Force for the Promotion of Genome Medicine created by the Japanese Society of Medical Oncology, the Japanese Society for Clinical Oncology, and the Japanese Cancer Association. We thank the following task force members: Dr Hiroyuki Aburatani, Dr Takashi Khono, Dr Hiroyuki Mano, Dr Tetsuo Noda, Dr Daisuke Aoki, Dr Yuko Kitagawa, Dr Masaki Mori, Dr Gaku Muto, Dr Tetsu Hayashida, Dr Hirotoshi Akita, Dr Chikashi Ishioka, Dr Issei Imoto, Dr Hidehiko Miyake, and Dr Tomosho Nakayama. We also thank Dr Itaru Matsumura, and Dr Manabu Muto for their advice.

DISCLOSURE

Hideaki Bando has received lecture fees from Eli Lilly Japan and Taiho Pharmaceutical. Shinji Kohsaka has received research funds (≥1 million yen per year) from AstraZeneca, Boehringer Ingelheim,

Eisai, and Chordia Therapeutics and has received a scholarship endowment from Daiichi-Sankyo. Yasushi Yatabe has received a speaker's honorarium from Chugai Pharma. Yoichi Naito has received lecture fees, honoraria, or other fees (≥500 000 yen per year) from Chugai, Pfizer, Eli Lilly, and Novartis and has received manuscript fees (≥500 000 yen per year) from Daiichi-Sankyo, Taiho, Pfizer, and Boehringer Ingelheim. Kenji Tamura has received research funds (≥1 million yen per year) from Daiichi Sankyo, Pfizer, and Chugai. Hiroyuki Uetake has received lecture fees, honoraria, or other fees (≥500 000 yen per year) from Taiho, Chugai, and Sanofi. Kazuto Nishio has received lecture fees, honoraria, or other fees (≥500 000 yen per year) from Chugai and has received research funds from Ignyta. Noboru Yamamoto has received lecture fees, honoraria, or other fees (≥500 000 yen per year) from Pfizer, AstraZeneca, Eli Lilly, ONO, Chugai, Sysmex, Eisai, and Daiichi-Sankyo and has received research funds from Astellas, Chugai, Eisai, Taiho, BMS, Pfizer, Novartis, Eli Lilly, AbbVie, Daiichi-Sankyo, Bayer, Boehringer Ingelheim, Kyowa-Hakko Kirin, Takeda, ONO, Janssen Pharma, MSD, MERCK, GSK, Sumitomo Dainippon, Chiome Bioscience, and Otsuka. All the remaining authors have no conflicts of interest to declare.

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REFERENCES

1. Strickler JH, Loree JM, Ahronian LG, et al. Genomic landscape of cell-free DNA in patients with colorectal cancer. *Cancer Discov*. 2018;8:164-173.
2. Avanzini S, Kurtz DM, Chabon JJ, et al. A mathematical model of ctDNA shedding predicts tumor detection size. *Sci Adv*. 2020;6:eabc4308.
3. Guibert N, Pradines A, Favre G, Mazieres J. Current and future applications of liquid biopsy in nonsmall cell lung cancer from early to advanced stages. *Eur Respir Rev*. 2020;29:190052.
4. Lindeman NI, Cagle PT, Aisner DL, et al. Updated molecular testing guideline for the selection of lung cancer patients for treatment with targeted tyrosine kinase inhibitors: guideline from the College of American Pathologists, the International association for the study of lung cancer, and the Association for molecular pathology. *J Thorac Oncol*. 2018;13:323-358.
5. Merker JD, Oxnard GR, Compton C, et al. Circulating tumor DNA analysis in patients with cancer: American Society of Clinical Oncology and College of American Pathologists joint review. *J Clin Oncol*. 2018;36:1631-1641.
6. Esagian SM, Grigoriadou G, Nikas IP, et al. Comparison of liquid-based to tissue-based biopsy analysis by targeted next generation sequencing in advanced non-small cell lung cancer: a comprehensive systematic review. *J Cancer Res Clin Oncol*. 2020;146:2051-2066.
7. Naito Y, Aburatani H, Amano T, et al. Clinical practice guidance for next-generation sequencing in cancer diagnosis and treatment (edition 2.1). *Int J Clin Oncol*. 2021;26:233-283.
8. Sunami K, Takahashi H, Tsuchihara K, et al. Clinical practice guidance for next-generation sequencing in cancer diagnosis and treatment (Edition 1.0). *Cancer Sci*. 2018;109:2980-2985.
9. Ebi H, Bando H. Precision oncology and the universal health coverage system in Japan. *JCO Precis Oncol*. 2019;3:PO.19.00291.
10. Siravegna G, Mussolin B, Venesio T, et al. How liquid biopsies can change clinical practice in oncology. *Ann Oncol*. 2019;30:1580-1590.
11. Nakamura Y, Taniguchi H, Ikeda M, et al. Clinical utility of circulating tumor DNA sequencing in advanced gastrointestinal cancer: SCRUM-Japan GI-SCREEN and GOZILA studies. *Nat Med*. 2020;26:1859-1864.
12. Parikh AR, Leshchiner I, Elagina L, et al. Liquid versus tissue biopsy for detecting acquired resistance and tumor heterogeneity in gastrointestinal cancers. *Nat Med*. 2019;25:1415-1421.
13. Bettegowda C, Sausen M, Leary RJ, et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. *Sci Transl Med*. 2014;6:224ra224.
14. Newman AM, Bratman SV, To J, et al. An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage. *Nat Med*. 2014;20:548-554.
15. Rolfo C, Mack PC, Scagliotti GV, et al. Liquid biopsy for advanced Non-Small Cell Lung Cancer (NSCLC): a statement paper from the IASLC. *J Thorac Oncol*. 2018;13:1248-1268.
16. Dagogo-Jack I, Rooney M, Nagy RJ, et al. Molecular analysis of plasma from patients with ROS1-positive NSCLC. *J Thorac Oncol*. 2019;14:816-824.
17. Supplee JG, Milan MSD, Lim LP, et al. Sensitivity of next-generation sequencing assays detecting oncogenic fusions in plasma cell-free DNA. *Lung Cancer*. 2019;134:96-99.
18. Razavi P, Li BT, Brown DN, et al. High-intensity sequencing reveals the sources of plasma circulating cell-free DNA variants. *Nat Med*. 2019;25:1928-1937.
19. Bando H, Kagawa Y, Kato T, et al. A multicentre, prospective study of plasma circulating tumour DNA test for detecting RAS mutation in patients with metastatic colorectal cancer. *Br J Cancer*. 2019;120:982-986.
20. Vidal J, Muinelo L, Dalmases A, et al. Plasma ctDNA RAS mutation analysis for the diagnosis and treatment monitoring of metastatic colorectal cancer patients. *Ann Oncol*. 2017;28:1325-1332.
21. Wyatt AW, Annala M, Aggarwal R, et al. Concordance of circulating tumor DNA and matched metastatic tissue biopsy in prostate cancer. *J Natl Cancer Inst*. 2017;109:djx118.
22. Tukachinsky H, Madison RW, Chung JH, et al. Genomic analysis of circulating tumor DNA in 3,334 patients with advanced prostate cancer identifies targetable BRCA alterations and AR resistance mechanisms. *Clin Cancer Res*. 2021;27:3094-3105.
23. Zhang Q, Luo J, Wu S, et al. Prognostic and predictive impact of circulating tumor DNA in patients with advanced cancers treated with immune checkpoint blockade. *Cancer Discov*. 2020;10:1842-1853.
24. Gandara DR, Paul SM, Kowanetz M, et al. Blood-based tumor mutational burden as a predictor of clinical benefit in non-small-cell lung cancer patients treated with atezolizumab. *Nat Med*. 2018;24:1441-1448.
25. Willis J, Lefterova MI, Artyomenko A, et al. Validation of microsatellite instability detection using a comprehensive plasma-based genotyping panel. *Clin Cancer Res*. 2019;25:7035-7045.
26. Woodhouse R, Li M, Hughes J, et al. Clinical and analytical validation of FoundationOne liquid CDx, a novel 324-Gene cfDNA-based comprehensive genomic profiling assay for cancers of solid tumor origin. *PLoS One*. 2020;15:e0237802.

27. Clark TA, Chung JH, Kennedy M, et al. Analytical validation of a hybrid capture-based next-generation sequencing clinical assay for genomic profiling of cell-free circulating tumor DNA. *J Mol Diagn*. 2018;20:686-702.
28. Leighl NB, Page RD, Raymond VM, et al. Clinical utility of comprehensive cell-free DNA analysis to identify genomic biomarkers in patients with newly diagnosed metastatic non-small cell lung cancer. *Clin Cancer Res*. 2019;25:4691-4700.
29. Jeselsohn R, Yelensky R, Buchwalter G, et al. Emergence of constitutively active estrogen receptor-α mutations in pretreated advanced estrogen receptor-positive breast cancer. *Clin Cancer Res*. 2014;20:1757-1767.
30. Cremolini C, Rossini D, Dell'Aquila E, et al. Rechallenge for patients with RAS and BRAF wild-type metastatic colorectal cancer with acquired resistance to first-line cetuximab and irinotecan: a phase 2 single-arm clinical trial. *JAMA Oncol*. 2019;5:343-350.
31. Parseghian CM, Loree JM, Morris VK, et al. Anti-EGFR-resistant clones decay exponentially after progression: implications for anti-EGFR re-challenge. *Ann Oncol*. 2019;30:243-249.
32. Sunakawa Y, Nakamura M, Ishizaki M, et al. RAS mutations in circulating tumor DNA and clinical outcomes of rechallenge treatment with Anti-EGFR antibodies in patients with metastatic colorectal cancer. *JCO Precis Oncol*. 2020;4:898-911.
33. Siravegna G, Mussolin B, Buscarino M, et al. Clonal evolution and resistance to EGFR blockade in the blood of colorectal cancer patients. *Nat Med*. 2015;21:795-801.
34. Aggarwal C, Thompson JC, Black TA, et al. Clinical implications of plasma-based genotyping with the delivery of personalized therapy in metastatic non-small cell lung cancer. *JAMA Oncol*. 2019;5:173-180.
35. Zugazagoitia J, Ramos I, Trigo JM, et al. Clinical utility of plasma-based digital next-generation sequencing in patients with advance-stage lung adenocarcinomas with insufficient tumor samples for tissue genotyping. *Ann Oncol*. 2019;30:290-296.
36. The Japanese Society of Pathology. "Guidelines on the handling of pathological tissue samples for genomic medicine" (In Japanese); 2018. https://pathology.or.jp/genome_med/pdf/textbook.pdf. Accessed June 30, 2021.
37. Park JK, Lee JH, Noh DH, et al. Factors of endoscopic ultrasound-guided tissue acquisition for successful next-generation sequencing in pancreatic ductal adenocarcinoma. *Gut Liver*. 2020;14:387-394.
38. Health Insurance Bureau, Ministry of Health, Labour and Welfare. "Partial revision of points to consider for implementation in accordance with partial revision of cost calculation method of medical fee points" (Administrative Notification No. 0531-1) (in Japanese); 2019. https://kouseikyoku.mhlw.go.jp/shikoku/iryo_shido/000099548.pdf. Accessed June 30, 2021.
39. Dagogo-Jack I, Rooney M, Lin JJ, et al. Treatment with next-generation ALK inhibitors fuels plasma ALK mutation diversity. *Clin Cancer Res*. 2019;25:6662-6670.
40. Horn L, Whisenant JG, Wakelee H, et al. Monitoring therapeutic response and resistance: analysis of circulating tumor DNA in patients with ALK+ lung cancer. *J Thorac Oncol*. 2019;14:1901-1911.
41. Iwama E, Sakai K, Hidaka N, et al. Longitudinal monitoring of somatic genetic alterations in circulating cell-free DNA during treatment with epidermal growth factor receptor-tyrosine kinase inhibitors. *Cancer*. 2020;126:219-227.
42. Mok T, Wu YL, Lee JS, et al. Detection and dynamic changes of EGFR mutations from circulating tumor DNA as a predictor of survival outcomes in NSCLC patients treated with first-line intercalated erlotinib and chemotherapy. *Clin Cancer Res*. 2015;21:3196-3203.
43. Sun Q, Liu Y, Liu B, Liu Y. Use of liquid biopsy in monitoring colorectal cancer progression shows strong clinical correlation. *Am J Med Sci*. 2018;355:220-227.

How to cite this article: Sunami K, Bando H, Yatabe Y, et al. Appropriate use of cancer comprehensive genome profiling assay using circulating tumor DNA. *Cancer Sci*. 2021;112:3911–3917. https://doi.org/10.1111/cas.15022