Pathology International

Received: 24 June 2020 | Accepted: 2 September 2020

DOI: 10.1111/pin.13023

INVITED REVIEW ARTICLE

WILEY Pathology International

Multiplex gene-panel testing for lung cancer patients

Yasushi Yatabe[1] | Kuniko Sunami[2] | Koichi Goto[3] | Kazuto Nishio[4] | Naoko Aragane[5] | Sadakatsu Ikeda[6] | Akira Inoue[7] | Ichiro Kinoshita[8] | Hideharu Kimura[9] | Tomohiro Sakamoto[10] | Miyako Satouchi[11] | Junichi Shimizu[12] | Koji Tsuta[13] | Shinichi Toyooka[14] | Kazumi Nishino[15] | Yutaka Hatanaka[16] | Shingo Matsumoto[3] | Masashi Mikubo[17] | Tomoyuki Yokose[18] | Hirotoshi Dosaka-Akita[19]

[1] Department of Diagnostic Pathology, National Cancer Center, Tokyo, Japan

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

[3] Department of Thoracic Oncology, National Cancer Center Hospital East, Chiba, Japan

[4] Department of Genome Biology, Kindai University, Osaka, Japan

[5] Division of Hematology, Respiratory Medicine and Oncology, Department of Internal Medicine, Saga University, Saga, Japan

[6] Center for Innovative Cancer Treatment, Tokyo Medical and Dental University, Tokyo, Japan

[7] Department of Palliative Medicine, Tohoku University School of Medicine, Miyagi, Japan

[8] Division of Clinical Cancer Genomics, Hokkaido University Hospital, Hokkaido, Japan

[9] Department of Respiratory Medicine, Kanazawa University, Ishikawa, Japan

[10] Division of Respiratory Medicine and Rheumatology, Department of Multidisciplinary Internal Medicine, Tottori University, Tottori, Japan

[11] Department of Thoracic Oncology, Hyogo Cancer Center, Hyogo, Japan

[12] Department of Thoracic Oncology, Aichi Cancer Center Hospital, Aichi, Japan

[13] Department of Otolaryngology, Kansai Medical University, Osaka, Japan

[14] Department of General Thoracic Surgery, Breast and Endocrinological Surgery, Okayama University, Okayama, Japan

[15] Department of Thoracic Oncology, Osaka International Cancer Institute, Osaka, Japan

[16] Research Division of Genome Companion Diagnostics, Hokkaido University Hospital, Hokkaido, Japan

[17] Department of Thoracic Surgery, Kitasato University School of Medicine, Kanagawa, Japan

[18] Department of Pathology, Kanagawa Cancer Center, Kanagawa, Japan

[19] Department of Medical Oncology, Faculty of Medicine and Graduate School of Medicine, Hokkaido University, Hokkaido, Japan

Correspondence
Yasushi Yatabe, MD, PhD, Department of Diagnostic Pathology, National Cancer Center, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan.
Email: yyatabe@ncc.go.jp

The year 2019 was considered to be the first year of cancer genome medicine in Japan, with three gene-panel tests using next-generation sequencing (NGS) techniques being introduced into clinical practice. Among the three tests, the Oncomine CDx Target test was approved under the category of regular molecular testing for lung cancer, which meant that this test could be used to select patients for molecularly targeted drugs. Conversely, the other two tests, NCC OncoPanel and FoundationOne CDx, were assigned to be used under the National Cancer Genome Medicine Network, and implementation was restricted to patients for whom standard treatment was completed or expected to be completed. These NGS tests can detect a series of genetic alterations in individual tumors, which further promotes the development of therapeutic agents and elucidates molecular pathways. The NGS tests require appropriate tissue size and tumor cell content, which can be accessed only by pathologists. In this

report, we review the current reimbursement schema in our national healthcare policy and the requirements of the specimens for NGS testing based on the recently published 'Guidance of Gene-panel Testing Using Next-Generation Sequencers for Lung Cancer', by the Japanese Society of Lung Cancer.

KEYWORDS
gene-panel test, lung cancer, molecular testing, next-generation sequencing

INTRODUCTION

The treatment of cancer has become increasingly personalized based on the precise genomics of individual tumors; therefore, morphological diagnosis alone is no longer sufficient in many cancer types.[1] Pathological reports should also include the results of genetic alterations specific to individual cancers, particularly druggable alterations. Among the various cancers, lung cancer is the leading cancer in which precision medicine has been introduced in clinical practice, and most international guidelines, such as IASLC/CAP/AMP, ESMO, NCCN and ASCO, recommend the implementation of molecular testing. Currently, targeted drugs for *EGFR, ALK, ROS1 BRAF* and *MET* alterations have been approved in Japan and corresponding molecular tests have been introduced individually in clinical practice simultaneous to approval of the drugs. However, each test requires at least five or more unstained slides. Because all targeted therapy is designed to treat advanced stage cancer, the source of the tissues is essentially small biopsy or cytology specimens. Thus, formalin-fixed, paraffin-embedded (FFPE) samples are often not sufficient to cover all individual testing, which means that molecular testing is shifting to multiplex genetic tests.[2]

The year 2019 was considered to be the first year of cancer genomic medicine, because three gene-panel tests using next-generation sequencing (NGS) techniques were approved by the government and have been introduced in clinical practice. The approved gene-panel tests include the Oncomine CDx Target test (Oncomine DxTT), FoundationOne CDx and the NCC OncoPanel. Accordingly, 'the clinical guidance based on gene-panel tests including NGS v2.0' was released from a joint committee of the JCO, JSCO and JCA.[3,4] This guidance focuses on comprehensive genomic profiling tests; therefore, Oncomine DxTT, which targets lung cancer specifically, has not been well documented. Because most pathologists in Japan make diagnoses of all cancer types, detailed information on the usages and differences of the three gene-panel tests is needed. Here, we review the current reimbursement schema in our national healthcare policy and requirement of the specimens for NGS testing based on the recently published 'Guidance of Gene-panel Testing Using Next-Generation Sequencers for Lung Cancer Patients', by the Japanese Society of Lung Cancer. The English version of this document can be provided with supplementary data in this review, and the Japanese version is downloadable from the Internet.[5]

APPROVED GENE-PANEL TESTS USING NEXT-GENERATION SEQUENCING

In April 2020, the approved multiplex gene-panel tests include the following: the Oncomine CDx Target test, FoundationOne CDx and the NCC OncoPanel. The characteristics of these tests are summarized in Table 1.

Oncomine CDx Target test

The Oncomine CDx Target test (Oncomine DxTT) was approved as an extension of regular genetic testing; therefore, it can be used to select patients for molecular targeted drugs for lung cancer. Oncomine DxTT is categorized as a hot spot panel test using the amplicon method and analyzes mutations in 46 genes and fusion in 21 genes, of which *EGFR, ALK, ROS1* and *BRAF* were approved as companion diagnostics. Although this test has the capability to detect *KRAS, NRAS, MET* exon 14 skipping, *FGFR, KIT, RET, PIK3CA* and *NTRK*, the testing cost is reimbursed only for patients with lung cancer. The analysis cost is configured as a sum of those using the single-gene tests of *EGFR, ALK, ROS* and *BRAF*.

FoundationOne CDx Cancer Genome Profile

FoundationOne CDx Cancer Genome Profile (F1CDx) is categorized as a comprehensive genome profile (CGP) test (discussed later) that uses the hybrid capture method. Only DNA from tumor tissues was analyzed. This test covers a total of 324 genes, of which base substitution, insertion, deletion mutation and copy number alteration in 309 genes, gene fusion of 36 genes, microsatellite instability (MSI) and tumor mutation burden (TMB) can be accessed. In addition to CGP testing, one remarkable feature is that this test also

Table 1 Approved genetic panel tests in April 2020

	Oncomine Target Test Multi CDx System	**OncoGuide NCC OncoPanel System**	**FoundationOne CDx Cancer Genome Profile**
Panel type	Hot spot panel	Comprehensive genome profile (CGP)	Comprehensive genome profile (CGP)
Method	Amplicon sequence	Capture sequence	Capture sequence
Function	Companion diagnostics (Nonsmall-cell lung cancer)	Cancer genome profiling	Cancer genome profiling Companion diagnostics (lung cancer, malignant melanoma, breast cancer, colorectal cancer, solid cancer)
Insurance coverage	Somatic gene mutation analysis system (For indication of anticancer drug indication)	Gene mutation analysis program (For cancer genome profile testing)	Somatic gene mutation analysis system (For judging the indication of antineoplastic drugs) Gene mutation analysis program
Number of target genes	46	114	324
Number of fusion genes	21	12	36
Others	TMB: X/MSI-high: X	TMB: ○/MSI-high: X	TMB: ○/MSI-high: ○
Companion Dx	Lung cancer: ○	None	Lung cancer: ○ Malignant melanoma: ○ Breast cancer: ○ Colorectal cancer: ○ Solid cancer: ○
Facility standards	None	Affiliated hospitals for cancer genomic medicine	Affiliated hospitals for cancer genomic medicine

has approval for companion diagnostics across cancer types. However, this feature is not feasible under the current healthcare reimbursement system (discussed later).

OncoGuide NCC OncoPanel

OncoGuide NCC OncoPanel (NCC OncoPanel) is another CGP test that uses the hybrid capture method developed in Japan. This test interrogates base substitutions, insertions/deletions, mutations and gene amplification of 114 genes; fusion in 12 genes; and TMB. Because of the simultaneous analysis of blood DNA, complete exclusion of uncommon polymorphisms and definite determination of germline mutations in 13 genes are characteristics of this test.

CANCER GENOMIC MEDICINE NETWORK AND CGP TESTING

In April 2018, the Japanese Ministry of Health, Labor, and Welfare (MHLW) organized a nationwide network to promote precision medicine or genomic medicine using CGP testing. Initially, 11 nationwide 'core' institutes for cancer genomic medicine (core institutes) and 100 affiliated hospitals for cancer genomic medicine ('cooperative' hospitals) were designated as implementing a framework for genomic medicine. The number of cooperative hospitals has gradually increased, and as of April 2019, 156 facilities have been designated. In September 2019, new 'base' hospitals were established, and 51 hospitals nationwide were designated (Fig. 1). Designation work is also underway with cooperative hospitals. In April 2020, the Cancer Genome Medicine Network is operating with 12 core institutes, 33 base hospitals and 161 cooperative hospitals.

The two CGP tests, F1CDx and the NCC OncoPanel, can be implemented only within the hospitals under this framework, and the core, base and cooperative hospitals have different roles, as shown in Fig. 1. Another characteristic of this program is the collection of all genome data with clinical information to the Center for Cancer Genomics and Advanced Therapeutics (C-CAT). [6] This center functions as a hub for aggregating and managing nationwide efforts in precision cancer medicine, as well as utilizing these data to enhance the quality of treatment and develop new

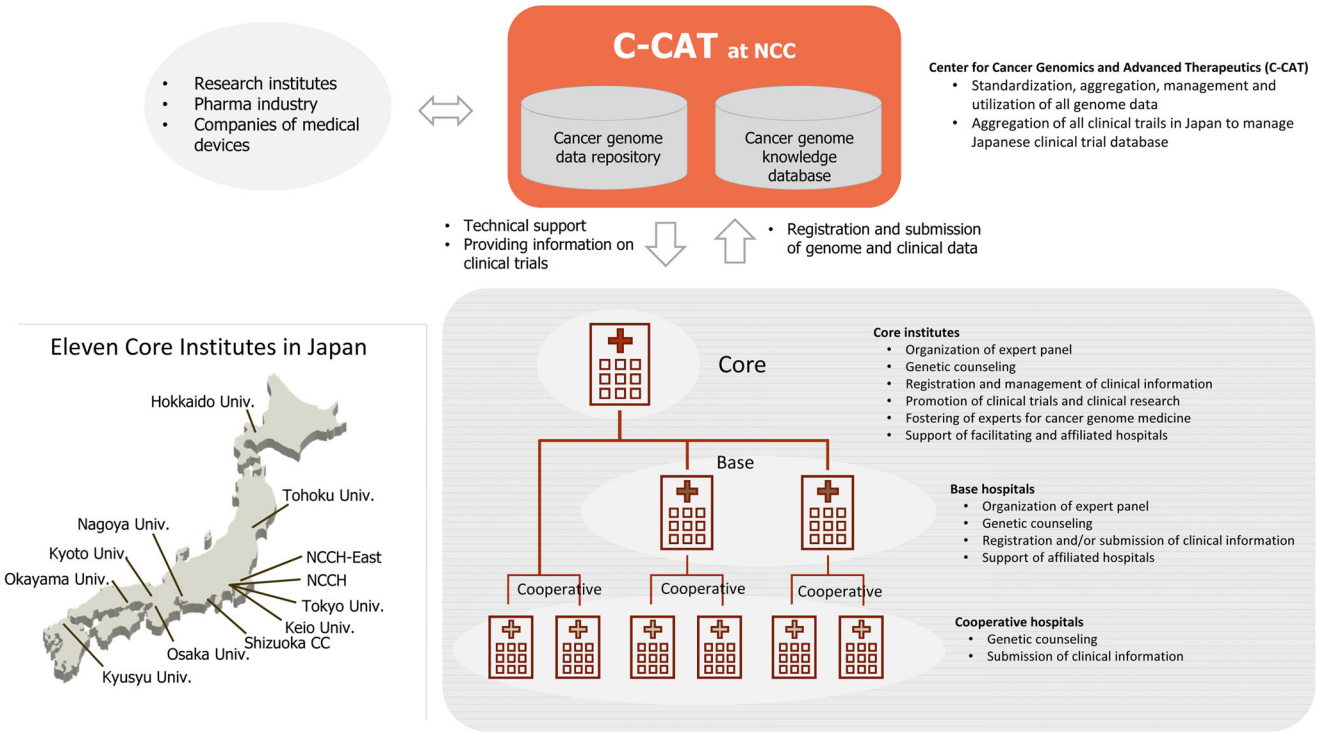

Figure 1 Cancer Genomic Medicine Program in Japan. Eleven institutes were designated the core institutes to promote the program, in addition to 33 base hospitals and 161 cooperative hospitals on April 1, 2020. The core facilities are tightly connected to the Center for Cancer Genomics and Advanced Therapeutics (C-CAT), which manages all genome data. The C-CAT can cooperate with the research institutes/facilities, pharma and medical device companies to promote the development of new cancer patient care. Translated from the document from the Japanese Ministry of Health, Labor, and Welfare.

treatments in collaboration with research facilities and industrial partners.

A major aim of this program is to enable access to selected unapproved drugs based on the genome profile to promote precision cancer medicine or cancer genomic medicine. The patients who completed or are expected to complete standard therapy allow treatment with unapproved drugs according to the advice of the expert panel using the 'Patient-Requested Treatment Program'.

Reimbursement

The reimbursement plan is assigned a total of JPY 560 000 for F1CDx and the NCC OncoPanel. A total of JPY 80 000 is assessed when the specimen is submitted to the testing company, with JPY 480 000 being capable of being assessed after explaining the results to the patient, which happens after the expert panel has discussed the results. Because 4–6 weeks are needed to return the results, the cost for analysis is not reimbursed if the condition of the patient has deteriorated to the point of being unable to consider further treatment; otherwise, each hospital must cover the cost with its own funds.

SPECIMENS FOR GENE-PANEL TESTS USING NGS

Formalin-fixed, paraffin-embedded samples, including surgical resection samples, biopsy specimens, and cell block specimens, are recommended as a source of analyses. Fresh frozen tissue or frozen cytology specimens can be used for analysis with Oncomine DxTT. However, for this type of specimen, it is important to evaluate the tumor cell content ratio, although it is often difficult in practice. In addition to general handling management for molecular testing (proper fixation time with recommended fixatives), specimens for the gene-panel tests are determined to be adequate by two factors: tissue size and tumor cell content ratio (Table 2).

Tissue size/volume

Each assay has different minimum inputs of DNA and RNA to be analyzed, which are correlated with tissue size or volume. FoundationOne CDx requires at least 1 mm^3 of tissue, while 10 unstained slides with a minimal size of 4 mm^2 (16 mm^2 is recommended) are required by the NCC OncoPanel. Although 10 ng is needed for Oncomine DxTT

Table 2 Requirements for samples in each assay

Assay name	Tumor cell content ratio	Tissue size/volume	
		DNA amount	Tissue size (5 μm thickness)
Oncomine DxTT	≥20–30%	10–100 ng	–
FoundationOne CDx	≥20%	50–1000 ng	≥25 mm^2
NCC OncoPanel	≥20%	≥200 ng	≥4 mm^2 (Recommended, ≥16 mm^2)

Abbreviations: Oncomine CDx Target test (Oncomine DxTT), OncoGuide NCC OncoPanel (NCC OncoPanel), or FoundationOne CDx Cancer Genome Profile (F1CDx).

analysis, no practical tissue size is stated. Fig. 2 shows examples of tissue size/volume evaluation for gene-panel testing. Additionally, the average yields of DNA according to the specimen types are summarized in Supplementary Table S1, based on our experience.

Tumor cell content ratio

In the case of low mutation allele frequency, real mutation reads, and reads due to a sequence error, may overlap. Therefore, at least 20% of the tumor cell content ratio is needed to avoid false positive results as a safety margin. Conversely, all analyses are designed and validated for tissue with a 20% or greater tumor cell content ratio. It is well-known that pathologists generally tend to overestimate tumor cell content,[7–9] and it should be noted that this overestimation may cause false negative results. For example, the limit of detection for the BRAF V600E mutation in Oncomine DxTT is 12.4% of the tumor cell content. If the tissue with 10% tumor cell content was overestimated as 20%, the specimen was submitted and analyzed, resulting in false negative results, because the sample actually has 10% tumor cell content, which is below the limit of detection. Proper evaluation of tumor cell content, particularly approximately 20% of tumor cell content, is crucial for gene-panel testing. Figs. 3 and 4 show adequate and inadequate specimens for analyses.

SELECTION OF SINGLE-GENE MOLECULAR OR GENE-PANEL TESTS FOR LUNG CANCER

In contrast to CGP testing, both single-gene molecular tests and Oncomine DxTT can be used to determine therapeutic strategies prior to first-line treatment against lung cancer (Fig. 5). The companion diagnostics of single-gene tests are Cobas and TheraScreen for EGFR mutations; Vysis break-apart FISH, Nichirei iAEP, and Ventana D5F3 IHC for ALK; OncoGuide AmoyROS1 for ROS1; Oncomine BRAF test for BRAF V600E; and ArcherMET for METex14 skipping (Supplementary Table S2). How should we select the molecular test? Both single and multiplex tests have advantages and disadvantages, as listed in Table 3. Furthermore, the following points should be considered with regard to the proper use of the tests.

Turnaround time

While Oncomine DxTT is returned within approximately 2 weeks, the test for EGFR mutations, which accounts for approximately 40–50% of driver mutations of lung adenocarcinoma, takes approximately 1 week, similar to immunohistochemistry, indicating a difference in the time required to obtain the result.

Analysis success rate

As shown in Table 3, the analysis success ratio is different between the single and multiplex molecular tests. Generally, the success rates of single-gene tests are approximately 95–99%, except for the ROS1 test, which is in strong contrast to the 85–90% success rate obtained with Oncomine DxTT. The success rate is highly important, because D004-2 precautions for medical insurance practices stipulate to 'calculate only once per patient'; therefore, the entire cost of a retest will be borne by the hospital. Furthermore, some patients require immediate initiation of therapy, and physicians cannot wait for a retested result.

FREQUENTLY ASKED QUESTIONS AND ANSWERS IN CLINICAL PRACTICE

In addition, the Japanese Society of Lung Cancer raises several FAQs with responses in the guidance. The following questions are those associated with pathologists.

Q. When are multiple single-gene tests recommended rather than Oncomine DxTT?

The guidance recommends multiple single-gene tests for the following cases:

- Only specimens that are not suitable for the multiplex genetic panel test are obtained. These circumstances include when it is determined that the amount of tissue is insufficient, when the tumor cell content ratio is low, or when only a past cytological specimen is available.

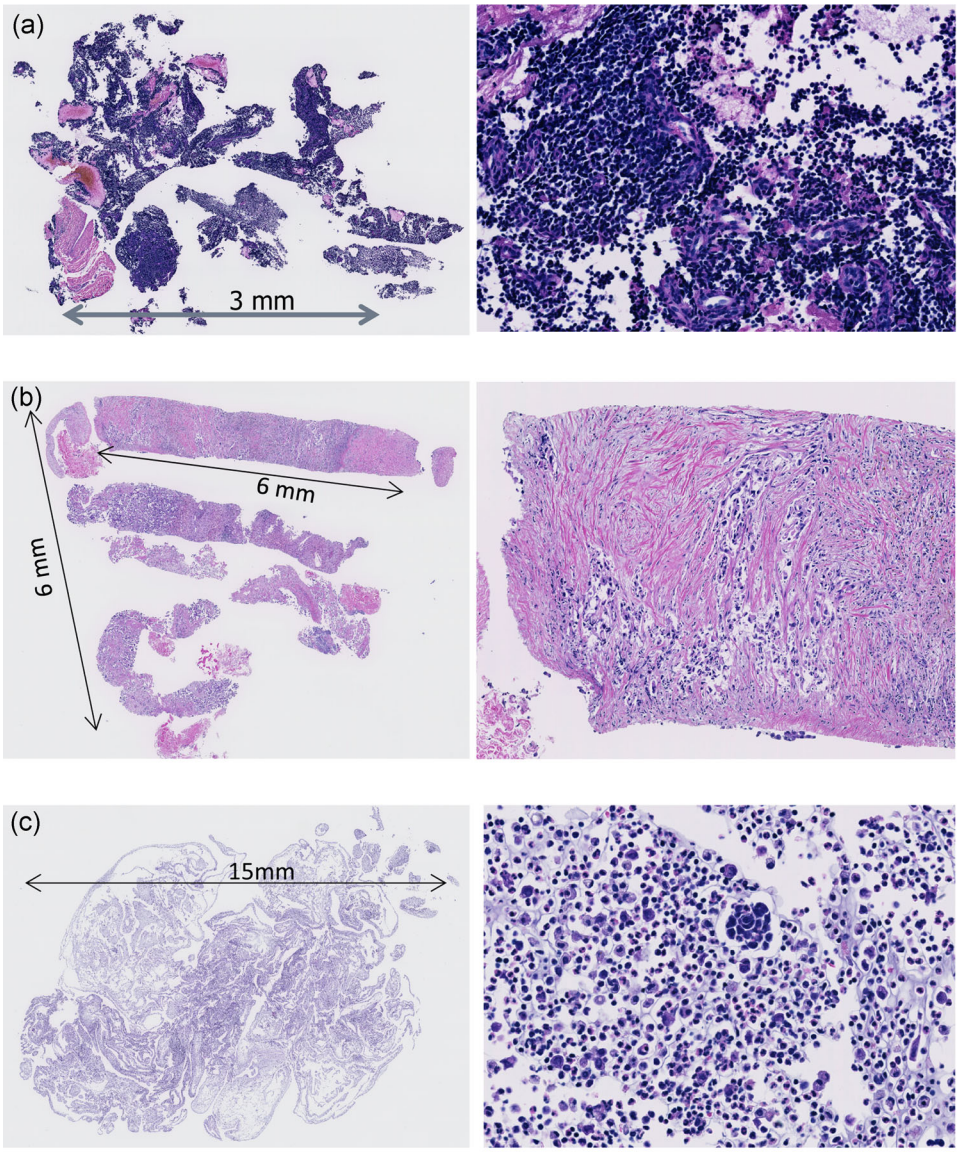

Figure 2 (**a**) Example of soft tissue metastasis with needle biopsy. Although it is a small tissue sample measuring 3 mm and only 6 mm^2, the tumor cells are densely packed, with a high tumor cell content ratio of 80%. Therefore, 1288 ng of DNA can be extracted, and any gene-panel test can be applicable. (**b**) Example of lung tumor tissue with needle biopsy. The tissue volume is approximately 20 mm^2, and the tumor cell content ratio is 30%. Oncomine CDx Target test (Oncomine DxTT), OncoGuide NCC OncoPanel (NCC OncoPanel), and FoundationOne CDx Cancer Genome Profile (F1CDx) can be analyzed. However, the tumor cells exhibited a tendency to degenerate, with only 10.3 ng of DNA extracted, preventing sufficient DNA for analysis from being obtained. (**c**) Example of cell block for malignant pleural effusion of small cell carcinoma. Although the cell volume was sufficient and 408.5 ng of DNA was extracted, the tumor cell content ratio was less than 1%, making it unsuitable for panel testing. Pleural effusions often contain a large number of inflammatory cells and macrophages and cannot be microdissected, making it difficult to obtain an appropriate tumor cell content ratio.

- The patient is in poor general condition and cannot wait for the turnaround time (TAT) for a multiplex genetic panel test.
- When analysis failure of the genetic panel test would seriously interfere with the patient's biomarker testing. For example, clinical re-examination is not possible, or once used, the amount of tissue will be insufficient, making further examination impossible.

Q. Can F1CDx be used as a companion diagnostic test?

Although F1CDx also has a companion diagnostic function and can be ordered for insured medical treatment, the facility still bears a large economic burden. This burden means that, from a practical perspective, it would be difficult to perform this test as a companion diagnostic test.

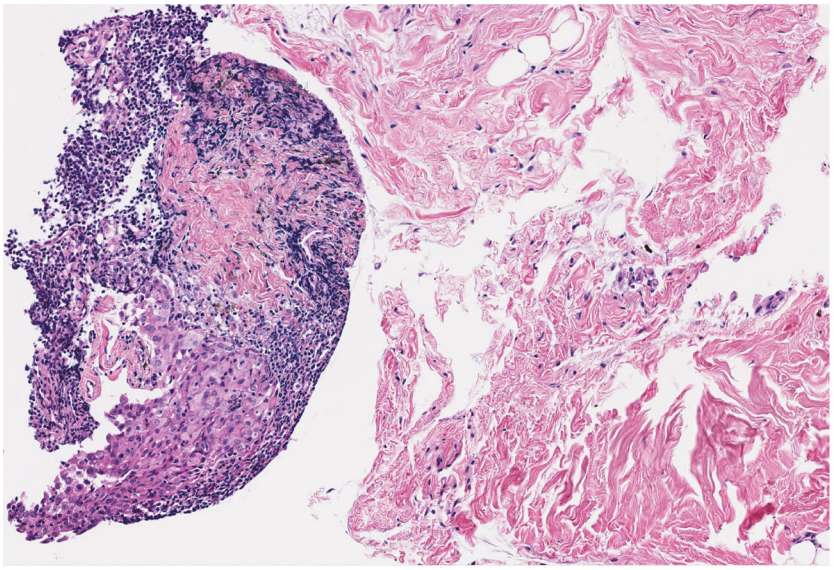

Figure 3 Example of a needle biopsy tissue of squamous cell carcinoma lymph node metastasis. Although fibrous connective tissue accounts for 70% of the area, the number of nucleated cells in the fibrous connective tissue is negligible compared to the left fragment (comprised of lymphocyte infiltration and tumor cells). Furthermore, because lymphocytes have only a small area, three to seven lymphocytes occupy a single unit area per tumor cell. Because the gene-panel test uses DNA for comparison, it is necessary to consider the nucleated cell ratio rather than the occupied area (the nucleated tumor cell content ratio is 15% in this photograph).

Q. What is the appropriate specimen?

As with single-gene testing, close attention must be paid to formalin solutions and fixation times. In addition, the amount of tissue and tumor cell content ratio, which are important for gene-panel testing, vary greatly among the specimen types and differ depending on the characteristics of each tumor; therefore, it cannot be unconditionally determined which specimen type is suitable for analysis. However, in general, the tissue volume tends to be a problem for TBLB and EUS-FNA specimens, while the tumor cell content ratio is often a problem for pleural effusion cell blocks and lymph node specimens. Frozen tissues and frozen pellets from pleural effusions can be used only for Oncomine DxTT, but the tumor cell content ratio of these specimens must be examined to prevent false negative results. Against this issue, it may be a possible solution that sufficient tumor cell content ratio is confirmed with H&E frozen section of OCT-embedded in one of the sliced frozen tissue in two, and the other half is submitted for analysis. For cell pellets, the FFPE cell block is recommended, as the FFPE cell block specimen itself is not of the exclusion criteria for all gene-panel testing; the frequency to meet the required tissue criteria is simply low.

Q. How should the tumor cell content ratio be assessed?

Evaluation by an experienced pathologist is essential. It should be noted that the evaluation is not of the area ratio but the nucleated cell ratio. In general, there is a tendency for the tumor cell content ratio to be overestimated, which can lead to false negatives. Pathologists need to have sufficient experience to count each nucleated cell in the tissue individually and compare the number to their own evaluation.

Q. What do we do if a genetic panel test is a failure?

It is important to clarify why the genetic panel test failed and to take measures to address the cause. If the quality of the DNA is low, there is a high chance that fixation causes the problem; therefore, a step such as rebiopsy can be considered. If the tissue volume or tumor cell content ratio is problematic, it may be useful to examine whether a single-gene test is possible. Of note, for *ROS1* and *BRAF* V600E, the same unsuitable specimen cannot be used for single-gene testing. This problem is observed because the ROS1 AmoyDx test, a companion diagnostic test for ROS1, also requires a tumor cell content ratio of 30%, and there is no single-gene companion diagnostic test for the *BRAF* V600E mutation. For these genes, it is necessary to use different specimens, depending on whether rebiopsy or another method is employed. The use of ROS1 IHC and BRAF V600E IHC for screening also provides clinically useful suggestions,[10,11] although this method is not an approved test.

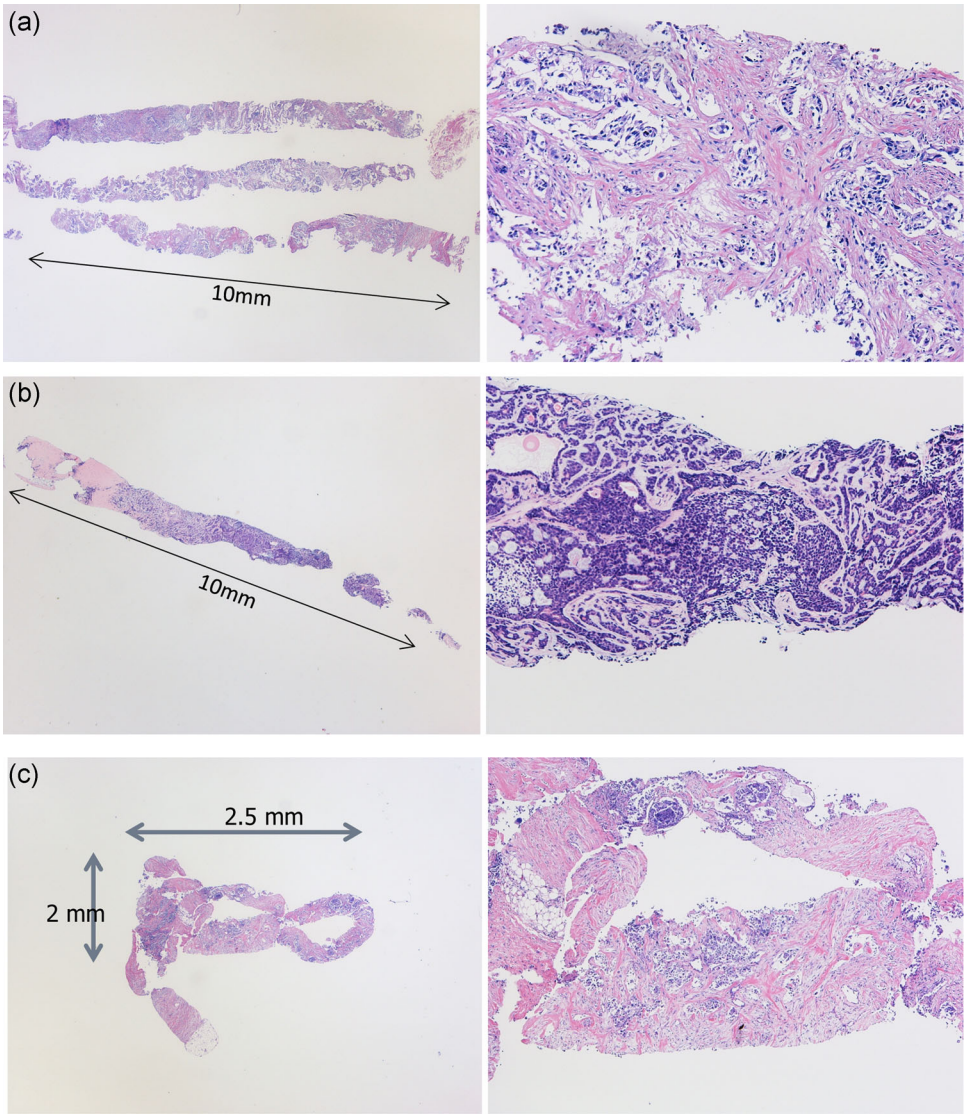

Figure 4 (**a**) Example of lung tumor tissue with needle biopsy. Tumor cells are distributed nearly uniformly as in the right high-power view, with approximately 30% tumor cell content ratio. The tissue volume is also 10 mm × 1 mm × 3 = 30 mm^2, and 10 unstained 5 μm thick specimens can be said to be specimens suitable for analysis with Oncomine CDx Target test (Oncomine DxTT), OncoGuide NCC OncoPanel (NCC OncoPanel), or FoundationOne CDx Cancer Genome Profile (F1CDx). (**b**) Example of lung tumor tissue with needle biopsy. Metastasis of salivary gland adenoid cystic carcinoma. It is a 10 mm long needle biopsy tissue, with an area of 10 mm^2, when considered to be a 1 mm tissue core. Because the stromal component contains few nucleated cells, it is almost entirely composed of the tumor tissue shown in the right figure, with the tumor cell content ratio estimated to be 70%. Although, in these types of examples, Oncomine DxTT and NCC OncoPanel can be analyzed with 10 unstained sections with 5 μm thickness, in the case of F1CDx, 20 unstained specimens are required to satisfy the standard tissue volume of 1 mm^3. (**c**) Example of lung tumor tissue with needle biopsy. Although the tissue volume is 5 mm^2, the tumor cell content ratio is 15%, making none of the techniques (Oncomine DxTT, NCC OncoPanel or F1CDx) suitable for analysis. Although a single-gene test is recommended, the ROS1 AmoyDx fusion gene kit, which also requires a tumor cell content ratio of 30% or more, is also inappropriate for the ROS1 companion diagnosis. As a result, this specimen is evaluated as undeterminable.

Q. What is the practical algorithm for lung cancer?

In addition to the necessity of clinical treatment, it is imperative that a pathologist with sufficient experience evaluate the specimen (tumor cell content ratio, specimen volume, fixation method/time, and storage conditions). Fig. 6 presents an example of a specific algorithm.

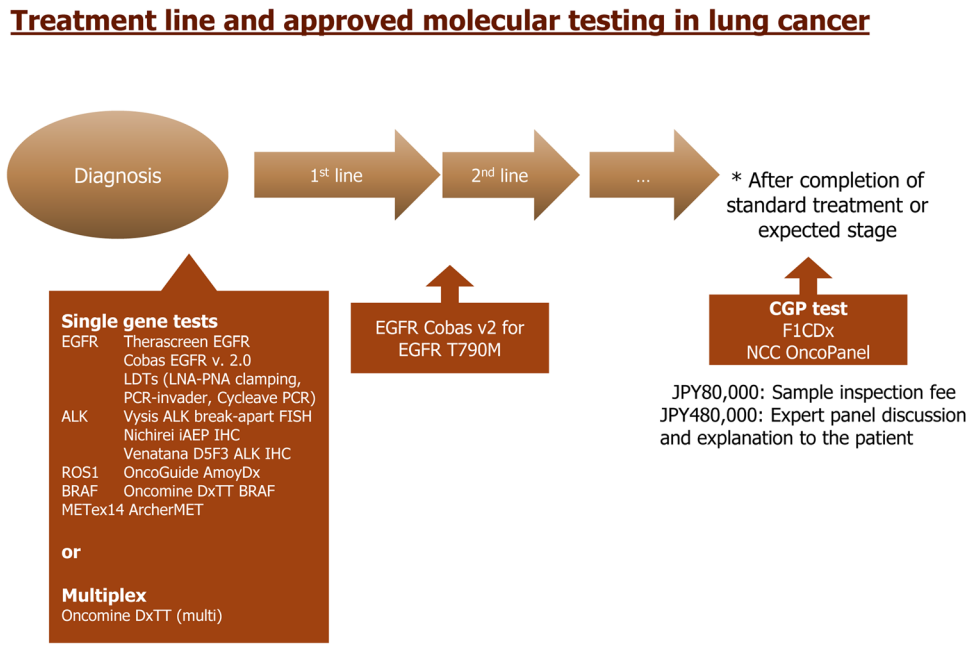

Figure 5 Treatment line and molecular testing for lung cancer patients. After the diagnosis, molecular testing with either multiple single-gene testing or Oncomine CDx Target test (Oncomine DxTT) is recommended. When the tumor is recurrent, molecular testing for EGFR T790M is allowed for selecting the treatment using third-generation EGFR-TKIs if the patient is treated with first- and second-generation EGFR-TKIs. Comprehensive genome profile (CGP) testing is the last option to determine molecular targeted agents based on the results.

Table 3 Comparison of the existing single-gene companion test and Oncomine DxTT (The shaded items are considered to be disadvantages)

Single-gene test	Oncomine DxTT
Can test even small specimens	Requires 20–30% tumor cell content ratio
10 years of experience	BRAF experience only
TAT is short (~1wk)	TAT is slightly longer (~2wks)
Failure rate is low	Failure rate of 10–15%
Only targets single genes	Although genes other than for the companion test are reference information (for research purposes), testing is possible
Numerous unstained specimens are required to examine all biomarkers (20–30 pieces)	10 unstained samples as standard
BRAF does not have a single-gene companion test other than Oncomine DxTT	Includes all companion tests
The order of examination can be arranged in accordance with the case	Results are returned simultaneously

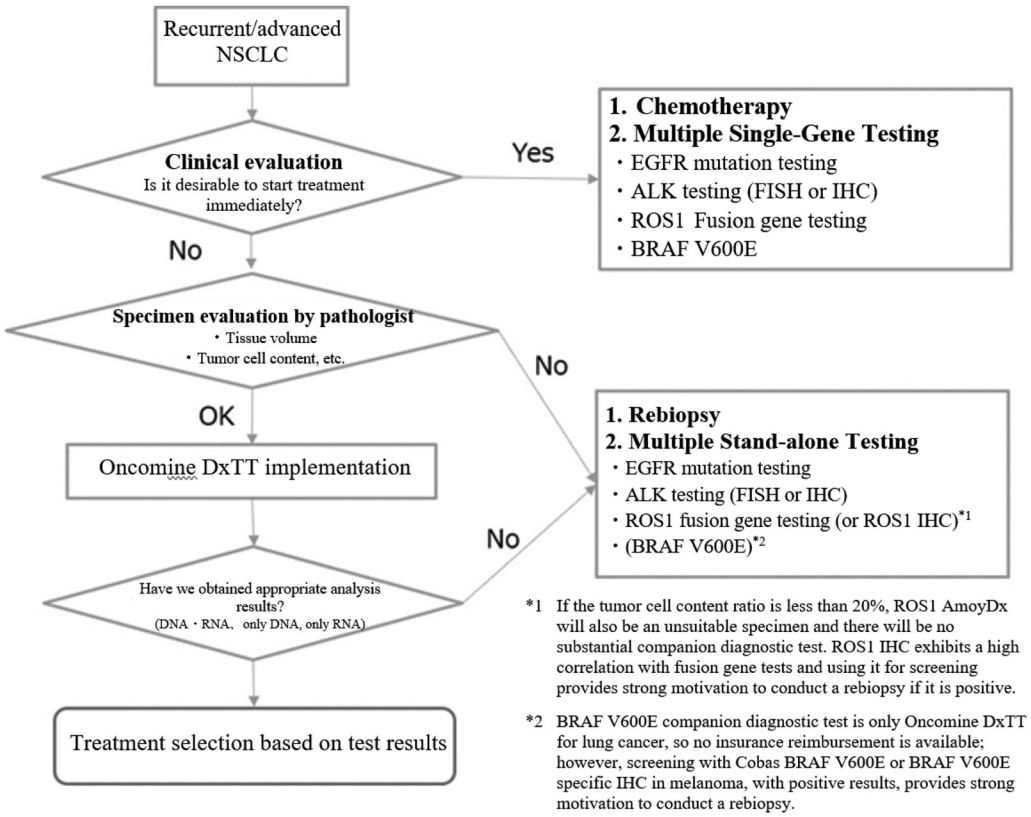

Figure 6 Example of a practical algorithm when using multiplex gene-panel testing.

CONCLUSION

In this review, multiplex gene-panel testing using NGS is reviewed with a practical reference to clinical implementation of lung cancer biomarker testing in Japan based on 'Guidance of Gene-panel Testing Using Next-Generation Sequencers for Lung Cancer', by the Japanese Society of Lung Cancer. Oncomine DxTT is a multiplex hot spot companion diagnostic panel similar to single-gene tests, while F1CDx and the NCC OncoPanel are categorized under CGP testing and performed under the Cancer Genomic Network framework. The purposes of the test and healthcare reimbursement system differ between the multiplex hot spot companion diagnostic panel and CGP testing. For all assays, pathologists are considered to play crucial roles in selecting the specimens for analysis and interpreting the results. It may be useful to refer to the Japanese version of the guidance document, which presents further details.

DISCLOSURE STATEMENT

The authors declare the following financial support or relationships. Dr. Yatabe reports speakers' fees from Thermo Fisher Scientific, Chugai-Pharm and Sysmex; Dr. K. Goto, speakers' fees from Chugai Pharmaceutical Co., Ltd., Astra Zeneka K.K and Novartis Pharma K.K., and research grants from Taiho Pharmaceutical Co., Ltd., Chugai Pharmaceutical Co., Ltd., Ono Pharmaceutical Co., Ltd., AstraZeneca K.K., Sumitomo Dainippon Pharma Co., Ltd., Bristol-Myers Squibb K.K., Eisai Co., Ltd., DAIICHI SANKYO Co., Ltd., Kyowa Hakko Kirin Co.,Ltd., Takeda Pharmaceutical Co., Ltd., Eli Lilly Japan K.K., Merck Biopharma Co., Ltd., Janssen Pharmaceutical K.K., Loxo Oncology, Inc., Amgen Inc., MEDICAL & BIOLOGICAL LABORATORIES CO., LTD., ignyta inc., NEC Corporation., and Amgen Astellas BioPharma K.K; Dr K. Nishio, speakers' fees from Boehringer Ingelheim Japan, AstraZeneca, Chugai and research grants from Chugai, Boehringer Ingelheim Japan and Pfizer; Dr. Tsuta, speakers' fees from AstraZeneca, Chugai Roche, and MSD, and research grants from Ono Pharmaceutical Co., Ltd; Dr. Toyooka, speakers' fees from Chugai-Pharm and Novartis Pharma; Dr. Nishino, speakers' fees from AstraZeneca and Chugai pharmaceutical; Dr. Hatanaka, speakers' fees from AstraZeneca and Novartis, and research grants from Sysmex and Thermo Fisher Scientific; Dr. Matsumoto, speakers' fees from Thermo Fisher Scientific and Chugai-Pharm, and research grant from Chugai-Pharm; Dr. Satouchi, speakers' fee from Chugai-Pharm, Pfizer, AstraZeneca and research grants from Chugai, Novartis and Pfizer; Dr. Dosaka-Akita, speakers'

fees from AstraZeneca and Chugai-Pharm. Drs. Sunami, Shimizu, Ikeda, Aragane, Yokose, Sakamoto, Kinoshita, Kimura, Inoue and Mikubo stated none declared.

AUTHOR CONTRIBUTIONS

The author contributions of this study are as follows. Conception and design; YY and KS. Acquisition and analysis of data; YY and KS. Drafting the manuscript or figures: YY and KS. Final approval of manuscript: All authors.

REFERENCES

1 Vargas AJ, Harris CC. Biomarker development in the precision medicine era: Lung cancer as a case study. *Nat Rev Cancer* 2016; **16**: 525–37.
2 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**: 323–58.
3 *Clinical Guidance of multiplex gene-panle testing using next-generation sequencing* (in Japanese). 2017. Available from: https://www.jca.gr.jp/researcher/topics/2017/files/20171013.pdf
4 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**: 2980–85.
5 *Guidance to multiplex gene-panel testing using next-generation sequencers in lung cancer patients* (in Japanese). 2019. Available from: https://www.haigan.gr.jp/uploads/files/NGS%E3%82%AC%E3%82%A4%E3%83%89%E3%83%A9%E3%82%A4%E3%83%B3%20%E7%90%86%E4%BA%8B%E4%BC%9A%E6%89%BF%E8%AA%8D%20-%201.1%281%29.pdf
6 The Center for Cancer Genomics and Advanced Therapeutics (C-CAT) (homepage on the Internet). Available from: https://www.ncc.go.jp/en/c_cat/
7 Mikubo M, Seto K, Kitamura A *et al*. Calculating the tumor nuclei content for comprehensive cancer panel testing. *J Thorac Oncol* 2020; **15**: 130–37.
8 Smits AJJ, Kummer JA, de Bruin PC *et al*. The estimation of tumor cell percentage for molecular testing by pathologists is not accurate. *Mod Pathol* 2014; **27**: 168–74.
9 Viray H, Li K, Long TA *et al*. A prospective, multi-institutional diagnostic trial to determine pathologist accuracy in estimation of percentage of malignant cells. *Arch Pathol Lab Med* 2013; **137**: 1545–49.
10 Ilie M, Long E, Hofman V *et al*. Diagnostic value of immunohistochemistry for the detection of the BRAFV600E mutation in primary lung adenocarcinoma Caucasian patients. *Ann Oncol* 2013; **24**: 742–48.
11 Conde E, Hernandez S, Martinez R *et al*. Assessment of a new ROS1 immunohistochemistry clone (SP384) for the identification of ROS1 rearrangements in non-small cell lung carcinoma patients: The ROSING study. *J Thorac Oncol* 2019; **14**: 2120–32.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.