Review Article on Selected Highlights of the 2019 Pulmonary Pathology Society Biennial Meeting

Lung cancer biomarker tests: the history and perspective in Japan

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Abstract: Biomarker testing is recognized as being indispensable for selecting patients with advanced lung cancer. EGFR mutation is the first biomarker for therapeutic selection of lung cancer patients since the identification of the correlation between EGFR TKI response and EGFR mutation status. The biomarker testing in Japan mostly follows those corresponding to the US. However, there are some differences due to the national health care program and the medical environment. In this review, we introduce the history and current status of the biomarker testing for lung cancer in Japan and discuss perspectives, focusing on cell-free DNA (cfDNA)-based panel testing.

Keywords: Lung cancer; biomarker test; Companion diagnosis; next-generation sequencing (NGS); cancer gene profiling test

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Introduction

The biomarker testing is recognized as being indispensable for selecting patients with advanced lung cancer (1). EGFR mutation is the first biomarker for therapeutic selection of lung cancer patients since the identification of the correlation between EGFR TKI response and EGFR mutation status. The second gene for target therapy was ALK fusions, then followed by ROS1 fusions, BRAF V600E mutation and NTRK fusions. These biomarker testing used various techniques including PCR for EGFR, FISH and IHC for ALK, reverse transcriptase PCR for ROS1, next-generation sequencing (NGS) for BRAF and NTRK. Concerning the increased number of analyzing genes and the limitation of tissue size, particularly in advanced-stage patients, multiplex panel testing has a great advantage in clinical practice, and indeed, the biomarker testing for genetic alterations is shifting to multiplex gene panel testing. Another essential therapeutic biomarker, PD-L1 IHC for immune checkpoint inhibitor (ICI), has a different concern regarding multiple assays with different clones (2). In this review, we introduce the history and current status of the biomarker testing for lung cancer in Japan and discuss perspectives, focusing on cell-free DNA (cfDNA)-based panel testing.

Current status of biomarker tests in Japan

Japanese public health care is similar to those of the UK, Canada and Australia. All people have to join a health insurance system, which covers most expenses of the individuals. Under this system, medical doctors request to use the medical procedures, equipment and drugs that have been approved by the Pharmaceuticals and Medical Devices Agency (PMDA) for reimbursement. The biomarker testing, including a series of companion diagnostics and *in vitro* diagnostics (IVD), is also included in the regulation. Currently approved biomarker tests for lung cancer treatment are listed in *Table 1*.

The Japan Lung Cancer Society (JLCS), a major academic organization on lung cancer, has been intensively involved in the promotion of research and distribution of knowledge

Table 1 Biomarker tests approved in Japan

Target	Test	Method	Platform	Regulation category	PMDA-approved year
EGFR	Therascreen EGFR mutation detection kit	PCR	Single	CoDx	2011
	Cobas EGFR v. 2.0	PCR	Single	CoDx	2016
	LDTs (LNA-PNA clamping, PCR-invader, Cycleave PCR)	PCR	Single	IVD/CoDx	2017
ALK	ALK FISH (Vysis)	FISH	Single	CoDx	2012
	ALK IHC (Nichirei iAEP)	IHC	Single	CoDx	2014
	ALK IHC (Venatana D5F3)	IHC	Single	CoDx	2017
ROS1	OncoGuide AmoyDx	RT-PCR	Single	CoDx	2017
PD-L1	PD-L1 IHC (22C3) PharmDx Dako	IHC	Single	CoDx/ IVD	2016
	PD-L1 IHC (28-8) PharmDx Dako	IHC	Single	IVD	2016
	PD-L1 IHC Ventana SP142	IHC	Single	IVD	2018
	PD-L1 IHC Ventana SP263	IHC	Single	IVD	2019
BRAF	Oncomine Dx Target test CDx	NGS	Multiplex	CoDx	2018
EGFR, ALK, ROS1	Oncomine Dx Target test multi CDx	NGS	Multiplex	CoDx	2019
CGP	NCC Oncopanel	NGS	Multiplex	CGP	2018
CGP	FoundationOne CDx	NGS	Multiplex	CGP	2018
EGFR, ALK, ROS1, BRAF	FoundationOne CDx	NGS	Multiplex	CoDx	2018
NTRK	FoundationOne CDx	NGS	Multiplex	CoDx	2019
MSI	MSI test kit (FALCO)	PCR	Single	CoDx	2018
BRCA1/2	FoundationOne CDx	NGS	Multiplex	CDx	2019

CGP, comprehensive genomic profiling; NGS, next generation sequencing; CoDx, companion diagnosis; IVD, in vitro diagnostics.

regarding biomarker testing. The activities are exemplified by released five guidance for individual biomarker testing, some of which were released in English (3). The firstest one, the Guidance for EGFR Testing for Lung Cancer Patients, was published in 2009 and was revised four times according to the development of drugs and changes in companion diagnostics (currently, version 4.2 in March 2019). The latest one is the Guidance for multiplex gene testing using NGS, released in December 2019. The guidance recommends a simultaneous biomarker testing of the five targets (EGFR, ALK, ROS1, BRAF, PD-L1), using either multiple standalone or multiplex testing (*Figure 1*), which represents current biomarker testing recommended in Japan.

Standalone biomarker tests

Individual biomarker assays corresponding to particular targeted-agents have been developed, independently. Therefore, treating physicians used to use multiple standalone assays before the approval of multiplex gene testing. We first show historical changes and the current situation of such standalone assays for the five targeted genes.

EGFR

Because EGFR mutation was identified before the approval of EGFR tyrosine kinase inhibitor (EGFR-TKI), the assays of detecting EGFR mutations initially started with laboratory-developed tests (LDT). More than 90 % of samples for molecular testing in Japan are examined in three large commercial companies, but they adapted different techniques, including PCR-invader, LNA-PNA clamping and Sanger sequencing, the last of which was replaced with cycleave PCR for higher sensitivity. This meant that the

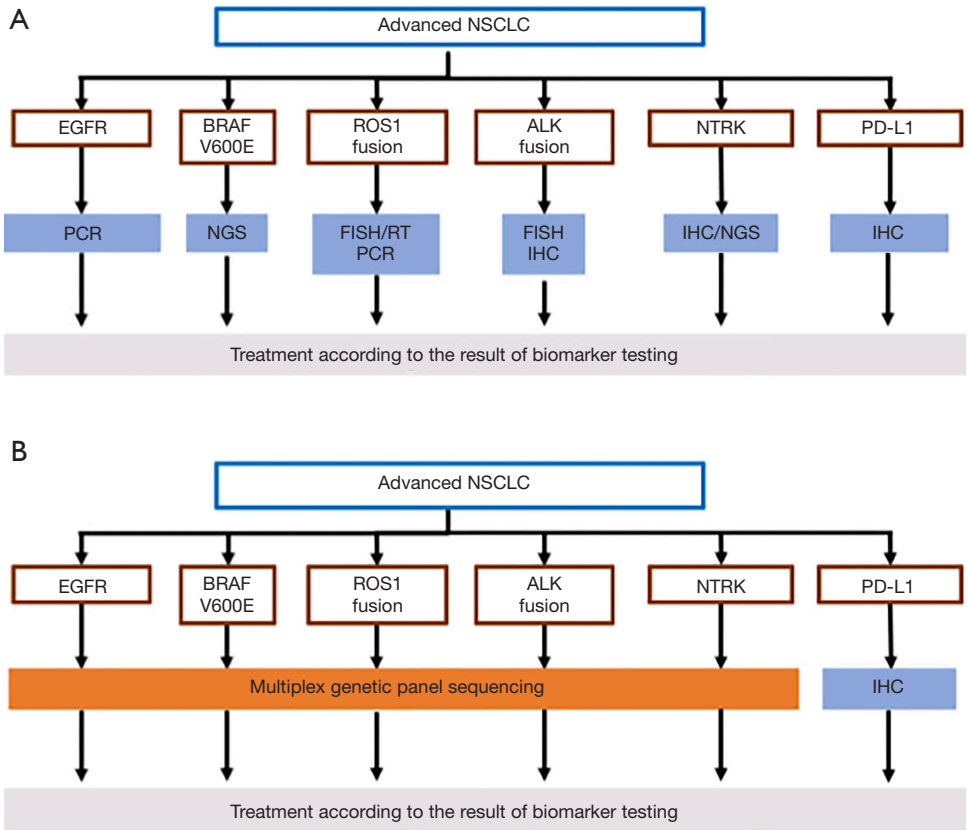

Figure 1 Current biomarker testing recommended by the Japanese Lung Cancer Society, using multiple standalone tests (A) or multiplex genetic testing (B).

treating physicians had to select one of the LDTs, so a study was conducted to examine the concordance among the three assays (4), resulting in almost equal sensitivity and specificity across these assays. Later, Cobas EGFR® and Therascreen EGFR® were approved as a companion diagnosis for gefitinib and erlotinib, respectively, and, thereafter, these assays were used widely. All LDT assays had no regulation for cytology samples, while the companion assays are validated only for tissue specimens. However, most treating physicians do not know the difference in the companion diagnostics, and ignores the limitation. After approval of osimertinib for resistant T790M mutation, Cobas EGFR version 2 was approved for either cell-free DNA (cfDNA) in the plasma and tissue specimens. A blood sample is easy to access, but cfDNA testing is restricted to the patients, any of whom tissue specimens cannot be obtained.

ALK

Currently, several assays including FISH, IHC and NGS, have been approved for detecting ALK fusions. Similar to the US, the Vysis® ALK Break Apart FISH probe kit is the first companion diagnosis for crizotinib. As approval of the agent was so rapid, most laboratories could not prepare to accept lung cancer samples for FISH analysis in a large batch, even though the routine ALK testing was recommended. Together with promoting an efficient screening program, the JLCS released the recommendation to use ALK IHC for screening, followed by FISH confirmation if IHC is positive. In 2014, the Nichirei ALK iAEP IHC kit, using the clone 5A4, was approved for alectinib, but even if the ALK IHC was positive, FISH confirmation was requested to start the treatment with alectinib according to the regulation of the PMDA. Subsequently, the Ventana ALK IHC (D5F3) was approved as a companion diagnostic test for crizotinib, and this IHC test did not need FISH confirmation if positive. Therefore, many treating physicians worried about not having reimbursement when the Nichirei ALK IHC was used for crizotinib because the Nichirei ALK IHC was the

companion diagnostic test for alectinib. In response to such a situation, the JCLS made a statement in 2014 that the diagnostic kits should correspond to driver alternations but not drugs. This statement relieved the treating physicians and the IHC vendors expanded to the designation to other ALK agents by submitting the concordance data to the regulatory authorities. Currently, either IHC assay is used regardless of the selected ALK inhibitors.

PD-L1

Nivolumab is the first approved immune checkpoint inhibitor (ICI) in Japan, and the approval of such an expensive drug caused a general concern that its broad use may result in a huge burden for the budget of the national health care system. Initially, the treatment of a patient cost about 320,000 USD a year, and all expenses were covered by the public health care program. Using a particular regulation rule, the government currently lists the drug price that is now 23.8% of the initial one. Somehow associated with public concerns, PD-L1 IHC was not requested in the beginning to treat a patient with nivolumab. However, at the time of pembrolizumab approval, nivolumab treatment was restricted to patients diagnosed with adenocarcinoma and a TPS ≥1%. For squamous cell carcinoma, PD-L1 IHC is not requested for nivolumab treatment. Of note, the regulation authorities permitted to adapt the TPS of PD-L1 IHC 22 C3 pharmDx, which was a companion diagnostic test of pembrolizumab, instead of PD-L1 28-8 IHC developed for nivolumab. During this period, pembrolizumab was the sole agent eapplied in the first line, so most patients with advanced NSCLC were examined with the PD-L1 22C3 IHC. This regulation rule of PD-L1 IHC was applied to subsequent ICIs despite multiple PD-L1 assays having been developed for each ICI. Currently, PD-L1 IHC is requested for the atezolizumab treatment for squamous cell carcinoma and for durvalumab as post-chemoradiotherapy in stage III patients, and these treatments are reimbursed to the patients with a TPS ≥1% with PD-L1 22C3 IHC, not to those with PD-L1 SP142 and SP263 assays, corresponding to atezolizumab and durvalumab, respectively. This implies that Japan was the first country that adapted several harmonization studies to integrate multiple PD-L1 assays into a representative one. In line with this regulation, Japan does not have a category of complementary diagnosis in contrast to the US.

ROS1

OncoGuide AmoyDx ROS1 fusion gene detection kit, which is a ROS1 detection system based on real-time RT-PCR, is a sole companion diagnostic test before the approval of Oncomine Dx target test (Oncomine Dx TT, described later). This assay can accept either FFPE fresh frozen tumor tissue or cytology samples including pleural fluid and bronchial washing, but FFPE specimens were a major source of this analysis due to difficulty of handling of frozen materials in clinical practice. As expected, the success rate of this assay limited to about 70–80%. Together with a low incidence of ROS1 fusions in NSCLCs, a current testing rate of ROS1 testing was limited to be low. According to the meeting report by Nishino *et al.*, a multicenter retrospective study involving major Japanese cancer center showed that ROS1 testing was conducted in only 67% of the patients, who were treated with some kinds of drugs during Aug 2017 to Dec 2017 (*Table 2*) (5). The testing rate was highly contrasted with those of EGFR (97.5%), ALK (88.1%) and PD-L1 IHC (87.1%). The low testing rate has been greatly improved with the approval of a multiplex genetic test.

BRAF

Similar to the US, Oncomine Dx TT was a first companion diagnostic test using an NGS technique. This NGS test can analyze 46 genes and 21 fusions, but only BRAF V600E status was initially reported to the clinic because the NGS test was approved just for BRAF V600E. After the approval of other driver genes, the treating physicians can access all information obtained with this technique. Cobas BRAF V600 assay has been also approved for melanoma patients, but not for lung cancer patients. Therefore, if a sample was failed with the Oncomine Dx TT test, there are no standalone tests to access BRAF V600 status under the current national healthcare reimbursement program.

Multiplex cancer gene panel tests

In June 2019, the PMDA approved three NGS assays; the Oncomine Dx TT, the FoundationOne CDx (F1CDx) and the OncoGuide NCC OncoPanel (NCC Oncopanel). The characteristics were summarized in *Table 3*. The first two are familiar worldwide, but the NCC-OP is unique to Japan. The NCC Oncopanel was a comprehensive genomic profiling test, developed by a collaboration of the National

Table 2 Real-world data of biomarker testing in the Japanese large cancer center, presented by Nishino *et al.* (5)

	EGFR	ALK	ROS1	PD-L1
Total numbers of patients with advanced NSCLC	202	202	202	202
Number of the patients with the individual test performed	197	178	136	176
Number of the patients missing biomarker test	5 (2.4%)	24 (11%)	66 (33%)	26 (13%)
Reasons for missing biomarker testing				
Insufficient amount of samples	0	3	4	11
The result of the prior biomarker testing was positive	0	11	23	8
Administration of cytotoxic agent was determined before receiving the result	3	3	5	2
Medical history of ILD or active ILD	1	1	1	0
Contraindications for molecular targeted tests or complications	1	0	2	0
Physician or hospital policies	0	6	29	5
Other	0	0	1	0

Table 3 Characteristics of multiplex cancer gene tests approved in Japan

	Oncomine Dx Target Test multi CDx system	FoundationOne® CDx cancer genome profile	OncoGuide™ NCC Oncopanel System
Technology used	Amplicon sequence	Target capture sequence	Target capture sequence
Panel category	Hot-spot panel	Comprehensive genomic profile	Comprehensive genomic profile
Regulation category	*In vitro* diagnostics	Comprehensive genomic profiling	Comprehensive genomic profiling
Number of the genes for companion diagnosis	4	4 (for lung cancer)	None
Genes for CDx	*EGFR*, *ALK*, *ROS1*, *BRAF*	*EGFR*, *ALK*, *ROS1*, *NTRK*, *BRCA1/2*	–
Number of cancer gene	46	324	114
Fusion	21	36	12
TMB estimation	No	Yes	Yes
MSI evaluation	No	Yes	No
Facility requirements	None	The network of the Japan Cancer Genome Medicine	The network of the Japan Cancer Genome Medicine

Cancer Center and Sysmex Corporation (6). The test covers 114 genes and 12 fusions, characterized by a simultaneous sampling of blood, which is utilized to address accurate somatic and germline mutations in addition to tumor mutation burden. The report of the first 230 cases analyzed with this panel (7) was similar to those of the other panels in terms of ratio to lead to the CGP-guided treatment (8,9).

There are three approved panel tests, but not all hospitals in Japan can conduct these tests with the national healthcare reimbursement coverage. The PMDA categorized panel tests into two groups, a conventional IVD and a comprehensive genomic panel (CGP) test, based on the number of genes and necessity of genetic consultation for germline mutations. The F1CDx and NCC Oncopanel are of the CGP test because the panels examine more than 100 genes and have a possibility to suggest or detect germline mutations. For the CGP testing, the Japanese Ministry of Health, Labor, and Welfare (MHLW) organizes a nationwide network for cancer genome medicine, in which 11 core institutes, 34 facilitating and 122 affiliated hospitals were designated (*Figure 2*). CGP testing is limited to these hospitals, but each designation has different roles as will

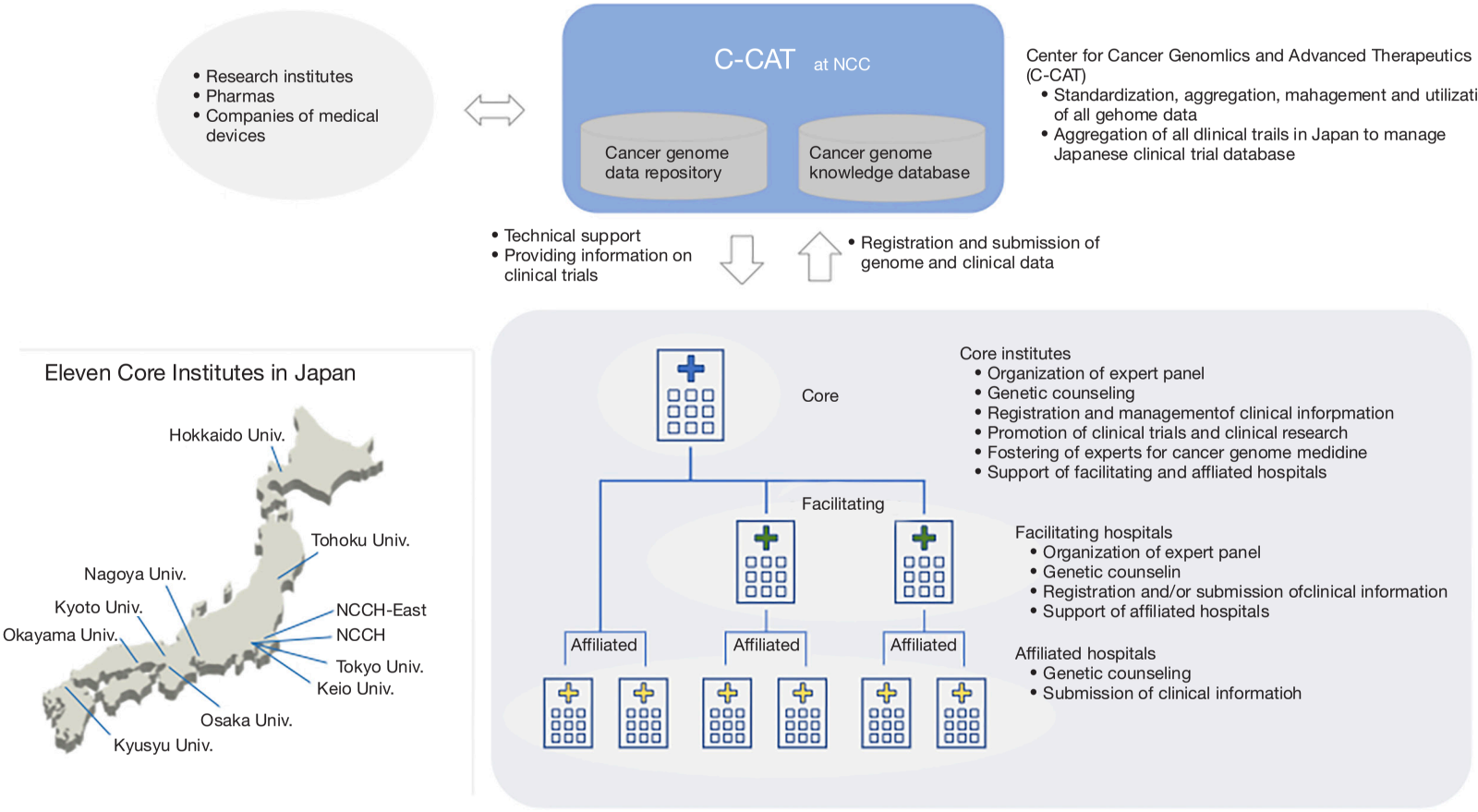

Figure 2 The Cancer Genome Medicine Program in Japan. Eleven institutes were designated as the Core Institutes to promote the program, in addition to 34 facilitating and 122 affiliated hospitals. The Core facilities are tightly connected to the Center for Cancer Genomics and Advanced Therapeutics (C-CAT), which manages all genome data. The C-CAT can cooperate with the research institutes/facilities, pharma and companies of medical devices to promote developing new cancer patient care.

be discussed later. Furthermore, this program is designed for patients with solid tumors that progress after standard therapy and/or rare types of cancer, such as pediatric cancers and sarcomas. The analyzing cost is reimbursed by the national healthcare reimbursement program only when an expert panel discussion is held. The expert panel, which functions like the molecular cancer board in the US, is strongly emphasized in this program, and its active operation with a multidisciplinary team is requested. In addition, the expert panel can be held only in the 11 core and 23 facilitating institutes, so the treating physicians in the affiliated hospitals have to attend the expert panel held at the core or facilitating institutes. This inconvenience is associated with national policy on the cancer genome medicine program. All genome data with clinical information should be submitted to the Center for Cancer Genomics and Advanced Therapeutics (C-CAT) (10), which was established by the Japanese government within the National Cancer Center in June 2018. The C-CAT functions as a hub for aggregating and managing nationwide information on cancer genomic medicine, as well as utilizing this data to enhance the quality of treatment and developing new treatments in collaboration with research facilities and pharma in Japan and worldwide. To establish a special connection to the C-CAT, the facilities were restricted to the 11 core institutes and 51 facilitating hospitals, which cover all over Japan.

Perspectives of biomarker testing in Japan

At the beginning of 2020, panel testing based on cfDNA has not been approved in Japan. As stated, cfDNA-based Cobas EGFR v.2 was commonly used to detect resistant T790M EGFR mutation, whereas a shift to the first line osimertinib is changing the situation. Some institutes conduct the Gardant 360 outside the reimbursement program, mostly under clinical trials, but liquid-based panel testing is under development. In 2019, the SAKIGAKE destination, equivalent to breakthrough therapy of the FDA, includes some anti-cancer agents, which are accompanied by cfDNA-based companion diagnostics. Therefore, panel testing using cfDNA will be approved in 2020 mostly within the category of the IVD. In terms of the cfDNA-based CGP testing, the MHLW and PMDA have an idea of a strong distinction between IVD and CGP, the approval may still need some more time.

Conclusions

Biomarker testing in Japan largely follows those corresponding to the US. However, there are some differences due to the national health care program and the medical environment. We reviewed the history of individual biomarker testing with some perspective in Japan.

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Footnote

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