The utility of newly emerging DNA and RNA panel in the diagnosis and treatment of soft tissue sarcomas; Real world data based on nation-wide database

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INTRODUCTION:

Comprehensive genomic profiling (CGP) test has enabled simultaneous examination of hundreds of genes to detect clinically relevant genetic alterations. In Japan, data from CGP test are gathered in the Center for Cancer Genomics and Advanced Therapeutics (C-CAT) database. Since June 2019, four CGP tests (FoundationOne® CDx, FoundationOne® Liquid CDx, OncoGuide™ NCC Oncopanel System, and Guardant360 CDx®) have been used under the national health insurance system. Soft tissue sarcomas (STS) are heterogeneous tumors and it is challenging for accurate diagnosis of them as even among expert sarcoma pathologists, diagnostic errors in sarcoma are common, with rates up to 25%. The effective targeted therapies are limited for most sarcomas as the mutational landscape is less studied compared with other malignancies. Recent rapid advances in molecular biology have led the discovery of disease-specific fusion genes in a variety of soft tissue tumors. Detection of these fusion genes can help oncologists with respect to diagnosis and selection of potential treatment regimens involving molecular-targeted therapy. However, traditional DNA-based CGP has limitation to detect these fusion genes. To resolve these problems, a dual DNA-RNA panel had been developed in Japan and also used under the national health insurance system since 2023. We therefore investigated the utility of this new emerging CGP test in the diagnosis and treatment of STS using the nation-wide database.

METHODS:

We retrospectively evaluated the records of patients with STS who received CGP test between November 2019 and May 2024 and registered in C-CAT. Conventional DNA panel include tests that can only examine pathogenic DNA variants. GeneMineTOP® is the new emerging CGP test for all solid tumors approved by the Ministry of Health, Labor, and Welfare in Japan in August 2023. It is a dual DNA-RNA panel as well as a paired tumor–normal matched test. This assay carries 737 genes and determines nucleotide substitutions, insertions/deletions, copy number (CN) alterations and tumor mutation burden (TMB). Furthermore, RNA panel can detect fusions of 455 genes including *ALK, PDGFRB, SSX1,2, CSF1, DDIT3, PAX3, 7*, which cover most of the sarcoma histology. In this study we focused on the utility of this new panel in the detection of fusion gene. We compared the rate of detection of fusion genes, frequently of reclassification by fusion genes, and the rate of detection of tyrosine kinase fusion by traditional DNA panel (DNA panel) and new DNA-RNA panel (DNA-RNA panel).

RESULTS:

From 2019 to 2024, 1922 patients with STS were registered in C-CAT; 1793 patients underwent the DNA panel, while 129 patients underwent the DNA-RNA panel. The histological types included leiomyosarcoma in 331 patients, dedifferentiated liposarcoma in 270 patients, and undifferentiated pleomorphic sarcoma in 142 patients, and the others. The most commonly altered genes included *TP53, CDKN2A, Rb1*, and *CDKN2B*. Fusion genes were observed in 142 (7.9%) and 27 (20.1%) patients in DNA panel and DNA-RNA panel, respectively ($p<0.01$). Twenty-two (1.2%) and 5 (3.8%) patients were re-classified based on the detection of highly histology-specific translocations in DNA panel and DNA-RNA panel, respectively (P=0.01) (table 1). Among them, an initial diagnosis of sarcoma NOS was classified as Ewing sarcoma (*EWSR1-FLI1*), *NTRK*-rearranged spindle cell neoplasm (*NTRK* fusion), *CIC*-rearranged sarcoma (*CIC-DUX4*), and sarcoma with *BCOR* genetic alterations (*BCOR-CCNB3*) as these fusion genes were identified. Of the cases diagnosed as Ewing sarcoma, three were re-classified as *NTRK*-rearranged spindle cell neoplasm (*NTRK* fusion), *CIC*-rearranged sarcoma (*CIC-DUX4*), and sarcoma with *BCOR* genetic alterations (*BCOR-CCNB3*) based on the detection of specific fusion genes rather than *EWSR1-FLI1*. In the DNA–RNA panel, fusion genes of several sarcomas were identified which could not identified in DNA panel including synovial sarcoma (*SS18-SSX1, 2*), solitary fibrous tumor (*NAB2-STAT6*), and alveolar rhabdomyosarcoma (*PAX3-FOXO1*). Two cases of initial diagnosis of fibrosarcoma were re-classified as dermatofibrosarcoma protuberans (DFSP) based on the detection of *COL1A1-PDGFB* by DNA-RNA panel but not by DNA panel. Potentially actionable kinase fusions were identified in 34 patients, including *ALK, BRAF, NTRK1–3, FGFR1, and ROS1* kinase fusions. These kinase fusions were observed in 28 (1.6%) and 6 (4.7%) patients in DNA panel and DNA-RNA panel, respectively (P=0.01). Among them, 6 patients received genotype-matched therapy and complete response and partial response were achieved in 1 and 3 patients, respectively.

DISCUSSION AND CONCLUSION:

In this study, 3.8% of patients were re-classified and the potentially actionable kinase fusions gene mutations were found in 4.7% of the patients with STS by the DNA-RNA panel. On the other hand, DNA panel had limitation in the ability to detect fusion gene compared to DNA-RNA panel. In some cases of initial diagnosis of sarcoma NOS could be identified as Ewing sarcoma and other translocation associated sarcomas by the detection of specific fusion genes. Undifferentiated

small round cell sarcomas are sometimes difficult to identify without molecular diagnosis. In this study, an initial diagnosis of Ewing sarcoma could be identified as sarcoma with *BCOR* genetic alterations and *CIC*-rearranged sarcoma by the detection of *BCOR-CCNB3* and *CIC-DUX4*, respectively. These findings suggest that DNA-RNA panel appears to be an important tool in the diagnosis and treatment of sarcomas. Although it is just introduced to clinical use in short period, accurate and sensitive detection of fusion genes by the DNA-RNA panel would lead to genotype-matched therapy for more patients in the future.

Table 1. Re-classification

DNA panel n=22 (1.2%)

Initial diagnosis	Reclassified diagnosis	Genomic specifisity	No
Ewing sarcoma	Sarcoma with BCOR genetic alterations CIC-rearranged sarcoma NTRK-rearranged spindle cell neoplasm	BCOR-CCNB3 CIC-DUX4 PHF20-NTRK1	1 1 1
Angiomatoid fibrous histiocytoma	NTRK-rearranged spindle cell neoplasm	ETV6-NTRK3	1
Angiosarcoma	CIC-rearranged sarcoma NTRK-rearranged spindle cell neoplasm	CIC-DUX4 PEAR1-NTRK1	1 1
Desmoplastic small round cell tumor	CIC-rearranged sarcoma	CIC-DUX4	1
Spindle Cell/Sclerosing Rhabdomyosarcoma	Extraskeletal myxoid chondrosarcoma	EWSR1-NR4A3	1
Sarcoma, NOS	Sarcoma with BCOR genetic alterations CIC-rearranged sarcoma Ewing sarcoma EWSR1-PATZ1 sarcoma NTRK-rearranged spindle cell neoplasm	BCOR-ZC3H7B CIC-DUX4 EWSR1-FLI1 EWSR1-PATZ1 LMNA-NTRK1 ETV6-NTRK3	1 7 2 2 1 1

DNA-RNA panel n=5 (3.8%)

Initial diagnosis	Reclassified diagnosis	Genomic specifisity	No
Sarcoma, NOS	CIC-rearranged sarcoma	CIC-DUX4	1
Fibrosarcoma	Dermatofibrosarcoma protuberans	COL1A1-PDGFB	2
Spindle Cell/Sclerosing Rhabdomyosarcoma	Alveolar Rhabdomyosarcoma	PAX3-FOXO1	1
Rhabdomyosarcoma NOS	Alveolar Rhabdomyosarcoma	PAX3-FOXO1	1