Chapter 3
Cancer Genomic Profiling of Gynecological Malignancies by Todai OncoPanel, a Twin DNA and RNA Panel

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Abstract Todai OncoPanel (TOP) has been established at The University of Tokyo and consists of DNA (version 3: 464 genes) and RNA panels (version 4: 463 genes).

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The University of Tokyo Hospital started TOP analysis in February 2017 as a research project approximately in 250 patients. Then, clinical sequencing for advanced solid tumors by TOP panel was performed as Advanced Medical Care Category B in 200 patients toward approval from the Ministry of Health, Labor and Welfare (Patient accrual was completed in December 2019). In this study, we performed TOP analysis in 54 gynecological malignancies and found various types of actionable somatic mutations, gene fusions, germline mutations (in *BRCA1*, *BRCA2*, and mismatch repair genes), as well as high tumor mutational burden. We describe the efficacy and the utility of TOP for gynecological malignancies, using our comprehensive analysis of the 54 gynecological malignancy cases. These findings will highlight the usefulness of cancer genomic profiling and shed light on precision medicine in gynecological malignancies.

Keywords Cancer genomic profiling · Gynecological malignancies · Precision medicine · Todai OncoPanel · Twin DNA and RNA panel

3.1 Introduction

Next-generation sequencing (NGS)-based tumor molecular profiling has become a fundamental component of precision medicine for cancer patients, which enables us to identify genetic alterations in genes and pathways for molecular-targeted therapies [1–3]. Several types of cancer genomic profiling (CGP) have proven their utility in cancer precision medicine, and two types of CGP (The OncoGuide™ NCC Oncopanel System and FoundationOne CDx Cancer Genomic Profile) were approved in Japan [4, 5]. However, most CGPs are based on analysis of genomic DNA of target genes, isolated either by polymerase chain reaction (PCR) or probe hybridization, enabling the detection of SNV, small insertions/deletions (indels), and CNV.

At The University of Tokyo, an original CGP assay, named Todai OncoPanel (TOP), was developed, which consists of a DNA panel and an RNA panel [6]. For the analysis, tumor DNA and RNA were prepared from FFPE tissues, and normal-paired DNA was extracted from peripheral blood collected from the same patients as a control to distinguish somatic and germline variants [6]. We analyzed >600 clinical samples since February 2017 under the approval of the institutional ethics committee. This clinical sequence assay consists of DNA and RNA hybridization capture-based next-generation sequencing panels that enable the comprehensive characterization of cancer-related genes. The TOP DNA panel can detect single-nucleotide variant (SNV), indels, and copy number variations (CNV) in 464 genes in version 3 (478 genes in version 4). Tumor mutational burden (TMB) and allele-specific copy number variations can be evaluated, and over 1000 microsatellite probes are included in the TOP DNA panel. The TOP RNA panel covers 463 genes

in version 4 (678 genes in version 5). The current version can detect gene fusions in 504 genes, as well as exon skipping (such as *MET* and *CTNNB1*) and provide gene expression profiling [6]. Fusion genes in the TOP RNA panel include *BCR-ABL1*, *EML4-ALK*, *RET*, *ROS1*, *NTRK1/2/3*, *FGFR1/2/3,* and *NRG1*, all of which can be (or are promising to be) candidates for specific molecular-targeted agents [7, 8]. Here, we describe the efficacy and the utility of TOP for gynecological malignancies, using our comprehensive analysis of 54 gynecological malignancy cases.

3.2 Patient Characteristics

Between February 2017 and April 2018, 54 gynecological cancer patients (with 79 FFPE tumor specimens) were analyzed by the TOP panel at The University of Tokyo Hospital. Tumor specimens were mainly obtained by surgically resected tumors. Under written informed consent, we returned the results to the patients. The number of patients in each tumor type is 6 in cervical cancer, 15 in endometrial cancer, 21 in ovarian cancer (as well as 2 synchronous endometrial and ovarian carcinomas), 9 uterine sarcomas, and 1 choriocarcinoma (Fig. 3.1a). Comprehensive analysis of all tumor types was reported previously [6].

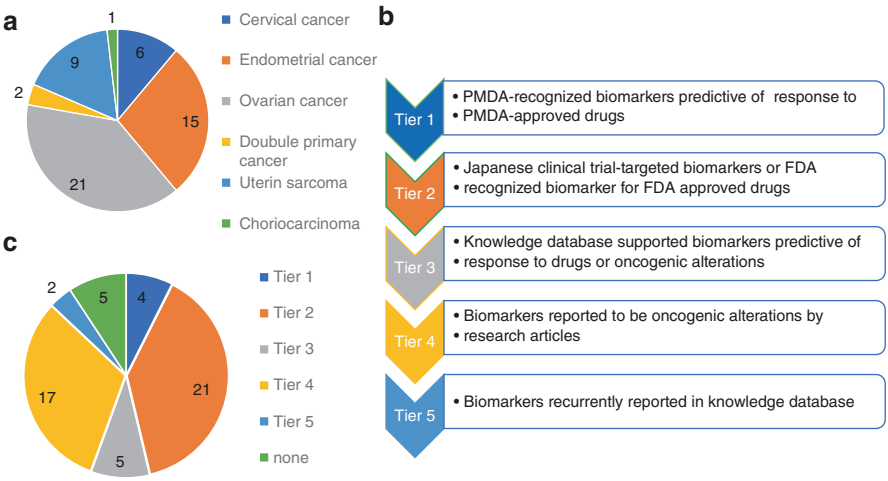

Fig. 3.1 Cancer types and evidence level classification. (**a**) Distribution of tumor types among the 54 gynecological malignancy patients. (**b**) TOP evidence level classification. The evidence level classification was used to annotated gene alterations in TOP between 2017 and 2018. (**c**) Clinical actionability of gene alterations detected in gynecological cohort by the TOP panel. The maximum evidence level for each case is listed

3.3 Clinical Annotations and Recommendation of Clinical Trials

We annotated somatic variants by using various types of public databases, including OncoKB, a curated knowledge database of oncogenic effects and treatment implications of gene alterations (http://oncokb.org); CIViC (Clnical interpretation of Variants in Cancer), a community-based curation database (http://civicdb.org), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), and COSMIC (Catalogue of Somatic Mutations in Cancer, http://cancer.sanger.ac.uk/cosmic) to assess variants frequency in cancer. We additionally used specific databases for annotation of gene variants, including germline variants by BRCA Exchange (http://brcaexchange.org) for *BRCA1/2*, IARC TP53 (http://p53.iarc.fr) for *TP53*, and InSiGHT (https://www.insight-database.org/genes) for mismatch repair genes. We constructed our knowledge database using a website of the Pharmaceuticals and Medical Devices Agency, US Food and Drug Administration (FDA), and National Cancer Institute. We also included clinical trial databases, such as ClinicalTrials.gov and the Japanese clinical trial databases UMIN, JAPIC, and JMACCT.

Annotated variants were classified according to the level of evidence and drug availability [6] (Fig. 3.1b). In summary, Tier 1 was annotated to (pathogenic/likely pathogenic) variants with biomarkers to PMDA-approved drugs in the matched tumor type; Tier 2 was annotated to (pathogenic/likely pathogenic) variants with biomarkers applicable to clinical trials/FDA-approved drugs/PMDA-approved drugs in other tumor types. Tier 3 was for (pathogenic/likely pathogenic) variants, which were supported by knowledge databases for prediction of response to drugs or oncogenic alterations. Tier 4 corresponded to biomarkers, which were to be oncogenic. Tier 5 corresponded to biomarkers, which were recurrently reported in knowledge databases (Fig. 3.1b).

Recently, clinical and/or Experimental Evidence Levels have been standardized in Japan, which were defined by the Center for Cancer Genomics and Advanced Therapeutics (C-CAT) and Equivalent Evidence Levels in Other Guidelines [5].

3.4 Genetic Alterations in Gynecologic Malignancies by the TOP Panel

We evaluated the clinical utility of genetic alterations in each tumor in the TOP panel. All the tumors were subjected to both DNA and RNA panels. Each alteration in each case was discussed and annotated by the molecular tumor board at The University of Tokyo Hospital, which are currently applicable for various types of PMDA-approved CGPs. Therefore, information on clinical trials was confirmed between 2017 and 2018. Overall, 91% (49 out of 54) harbored one or more

clinically annotated alterations (Tier 1 to Tier 5), and 46% (25 out of 54) harbored one or more actionable variants (Tier 1 or Tier 2) in gynecologic cancers (Fig. 3.1c). The proportion of actionable variants in cervical cancer, endometrial cancer, and ovarian cancer was 50%, 73%, and 57%, respectively. In these 54 cancer patients, the most frequently mutated gene with clinical annotation was *TP53*, followed by *PIK3CA*, *PTEN*, *PIK3R1*, *KRAS,* and *ARID1A*.

3.5 Cervical Cancer

Among the six cervical cancer patients, actionable mutations (Tier 2) were identified in three cases (50%) (Fig. 3.2a). Two were somatic oncogenic variants of *PIK3CA*, which matched to the clinical trial for an AKT inhibitor in Japan. Another case harbored *the GOPC-ROS1* fusion gene (Table 3.1). *ROS1* is a proto-oncogene located on chromosome 6q and encodes a receptor tyrosine kinase involved in the regulation of cancer cell growth and differentiation. ROS1 is often involved in genomic rearrangements resulting in constitutively active kinases that stimulate multiple pathways such as JAK-STAT, PI3K-AKT-mTOR, and RAS-RAF-MEK-ERK [9–11]. Fusion products of ROS1 have been observed in a variety of types of cancer, including lung, gastrointestinal tract and hepatobiliary tract, and central nervous system [9]. The *ROS1* fusions are now considered as therapeutic biomarkers of crizotinib and entrectinib [12, 13].

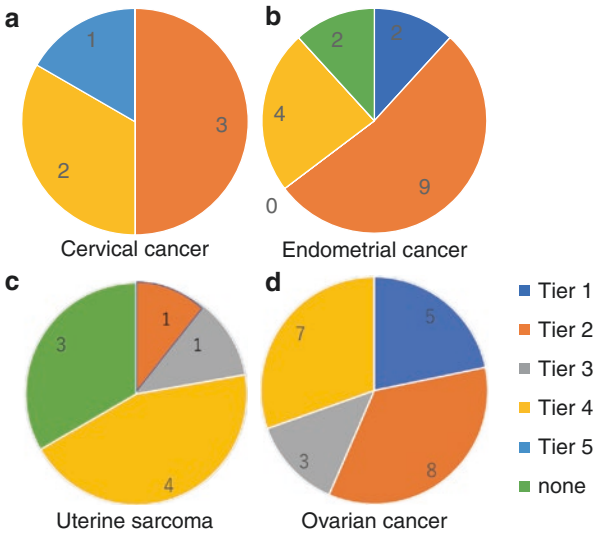

Fig. 3.2 Genetic alterations and clinical actionability by the TOP panel in gynecological cancers. (**a**–**d**) Detected gene alterations were annotated using the TOP classification. All the cases are assigned to the level with the most actionable alterations: (**a**) cervical cancer, (**b**) endometrial cancer, (**c**) uterine sarcoma, and (**d**) ovarian cancer. (**e**) Histological types of ovarian cancers. (**f**) Genetic alterations identified in 23 ovarian cancers

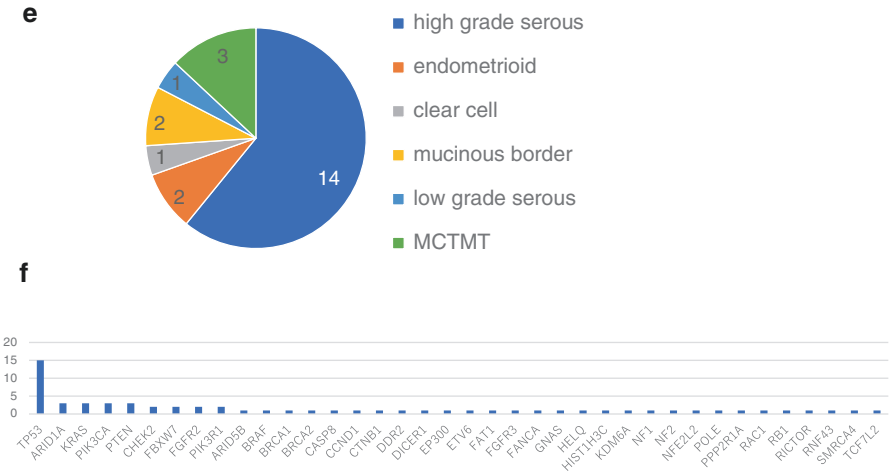

Fig. 3.2 (continued)

3.6 Endometrial Cancer

We analyzed 15 endometrial cancer cases and identified 18 actionable gene alterations in 11 patients (73%) (Fig. 3.2b). Somatic variants of the PI3K-AKT pathway are most frequent, with 6 pathogenic mutations of *PIK3CA*, 7 of *PTEN*, and 2 of *PIK3R1*. These were considered for clinical trials with AKT inhibitors. Pathogenic somatic variants of *BRCA1* were identified in one patient, which may be associated with homologous recombination deficiency (HRD). Thus, poly (ADP-ribose) polymerase (PARP) inhibitors were recommended in this patient, although no PARP inhibitors have been clinically approved in endometrial cancer. Pathogenic germline variants of MMR genes were identified in two patients (*MSH6* (p.F858Sfs*12) and *MSH2* (p.Q170*)) (Table 3.1). In addition to pathogenic variants in MMR genes, the primary tumors of these two patients were TMB-High, which also supports the recommendation of immune checkpoint inhibitors. Pembrolizumab was approved for microsatellite instability-high (MSI-High) solid tumors by FDA in 2017 and by PMDA in 2018 [14, 15].

3.7 Uterine Sarcoma

We analyzed nine uterine sarcomas and found that found no actionable mutations, except for one patient (Fig. 3.2c). No pathogenic alterations were detected in three patients (33%); however, two novel gene fusions were identified by the TOP RNA panel, which may be associated with tumorigenesis of uterine sarcomas. As *NTRK* gene fusions were identified at 4% in uterine leiomyosarcomas [16], the TOP RNA panel would be useful to identify actionable and/or novel gene fusions.

3.8 Ovarian Cancer

We enrolled 23 ovarian cancer cases. Totally, 15 actionable gene alterations were detected in 13 patients (Fig. 3.2d). Histological distribution was as follows: 14 (61%) with high-grade serous carcinomas, 2 with endometrioid carcinomas, 1 with clear cell carcinoma, 2 with mucinous borderline tumors, 1 with low-grade serous carcinoma, and 3 with malignant transformation of mature cystic teratomas (MCTMT) (Fig. 3.2e). The genome profile of ovarian cancers highly depends on histological types. For example, *TP53* is mutated in >90% of high-grade serous carcinomas. Pathogenic somatic variants are listed in Fig. 3.2f. *TP53* is the most frequently mutated gene, followed by *PIK3CA*, *PTEN*, *KRAS,* and *ARID1A*. Fourteen (12 high-grade serous carcinomas and 2 MCTMT) harbored pathogenic somatic variants of *TP53*. Pathogenic germline variants were identified in four patients (*BRCA1* in two and *BRCA2* in two patients, Table 3.1), and pathogenic somatic variant of *BRCA1* was identified in one patient, all of which could be targeted by PARP inhibitors. Pathogenic somatic variants of the PI3K-AKT pathway were identified in five patients, which were the candidate targets of AKT inhibitors. Actionable alterations in *FGF* and *FGFR* were identified in four patients, which led to the enrollment of a clinical trial with an FGFR inhibitor. Pathogenic *BRAF* somatic variant was identified in one case with low-grade serous carcinoma (Table 3.1).

3.9 Choriocarcinoma

We analyzed one choriocarcinoma and detected three clinical annotated genes (Tier 5). However, no actionable gene alterations were identified.

3.10 Germline (Secondary) Findings

In gynecological malignancy, the ratio of germline variants is expected to be high, due to the prevalence of hereditary breast and ovarian cancer syndrome (HBOC) and Lynch syndrome [17, 18]. While the primary purpose of CGP tests has been considered to identify pathogenic somatic variants, germline findings may also guide personalized therapy and may be clinically significant, especially in gynecological malignancies. Indeed, we found pathogenic germline variants of mismatch repair genes and *BRCA1/2* genes in endometrial and ovarian carcinomas, respectively (Table 3.1). The prevalence of pathogenic germline variants can be assessed by the TOP panel, as it analyzes paired-normal DNA from peripheral blood samples. The TOP panel basically includes cancer-related genes, which are recommended to report as germline (secondary) findings in clinical CGP [19, 20], and it is expected to contribute to find germline findings, which would be useful for both patients themselves and their relatives. As described above, TOP analysis of the 54

Table 3.1 Actionable mutations and candidate molecular-targeted drugs in gynecologic cancers

Primary	Drugs	Annotated variants
Cervical cancer	AKT inhibitor (Tier 2)	s*PIK3CA* (p.E545K), s*PIK3CA* (p.G1049A)
	ROS1 inhibitor (Tier 2)	GOPC-ROS1
Endometrial cancer	Immune checkpoint inhibitor (Tier 1)	g*MSH6* (p.F858Sfs*12), g*MSH2* (p.Q170*)
	AKT inhibitor (Tier 2)	s*PIK3CA* (p.E545K), s*PIK3CA* (p.R88Q), s*PIK3CA* (p.E545K), s*PIK3CA* (p.E545K), s*PIK3CA* (p.H1047R), s*PIK3CA* (p.H1047R)
		s*PTEN* (p.Y180fs*3), s*PTEN* (p.R130Q), s*PTEN* (p.T319Nfs*6), s*PTEN* (p.R130Q), s*PTEN* (p.R130Q), s*PTEN* (p.H93Tfs*5), s*PTEN* (p.D92E)
		s*PIK3R1* (p.L581Vfs*19), s*PIK3R1* (p.N564D)
	PARP inhibitor (Tier 2)	s*BRCA1* (p.R1699W)
Ovarian cancer	PARP inhibitor (Tier 1)	g*BRCA2* (p.P3039=), g*BRCA2* (p.Q356*), g*BRCA1* (p.E1257Gf*9), g*BRCA1* (p.Q396*), s*BRCA1* (p.E554*)
	AKT inhibitor (Tier 2)	s*PIK3CA* (p.E545K), s*PIK3CA* (p.G1049A)
		s*PTEN* (p.R130Q)
		s*PIK3R1* (p.V357Gf*7), s*PIK3R1*(p.Y580_M582del)
	FGFR inhibitor (Tier 2)	s*FGFR2* (p.Y375C), s*FGFR2* (p.S252W), s*FGF3* (amplification), s*FGF19* (amplification)
	BRAF inhibitor (Tier 2)	s*BRAF* (p.L597R)

individuals in this study revealed 6 pathogenic germline variants (2 in *BRCA1*, 2 in *BRCA2*, 1 in *MSH2*, and 1 in *MSH6*) (11%), all of which were disclosed to each patient by the physicians and certified genetic counselors. Two patients wished confirmatory single-site test and were found to be pathogenic.

3.11 Tumor Mutational Burden

The TMB representing the number of somatic variants per Mb is a biomarker to estimate the efficacy of immune checkpoint inhibitors. In 2019, Pembrolizumab was approved for adult and pediatric patients with TMB ≥10 [21]. In this study, we defined a threshold of 10 mutations/Mb as TMB-High and found that 7 cases (1 cervical cancer, 3 endometrial cancer, and 3 ovarian cancer) were TMB-high (Fig. 3.3). One cervical cancer patient exhibited TMB of 14.3/Mb. This patient received concurrent chemoradiation as primary treatment and the sample was obtained from the recurrent site in the heart. Careful caution is required to address TMB-high, as treatment by either chemotherapy or irradiation may increase the

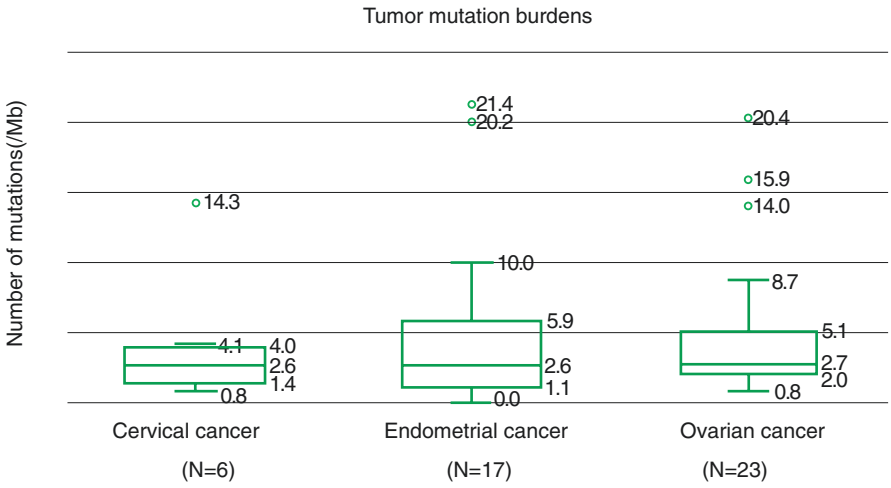

Fig. 3.3 Tumor mutational burdens in gynecological cancers. (**a**) Distribution of the somatic tumor mutational burden (TMB), defined as a number of coding mutations per megabase. The threshold for hypermutation is 10 mutations/Mb. A plot of 206.2/Mb in endometrial cancer is an ultra-mutated case with a variant of *POLE*

number of mutations. In endometrial cancer cases, three patients exhibited TMB-high (206.2/Mb, 21.4/Mb, and 20.2/Mb). One of the three patients harbored pathogenic germline variant of *MSH2*, one with a variant of uncertain significance (VUS) of *MSH2*, and the remaining one with somatic variants of *POLE* (206.2/Mb). In ovarian cancer, three cases were TMB-high. Two of them harbored somatic variants of *POLE*, which might induce hypermutation genotype, although one of the *POLE* variants was uncertain for pathogenicity. The remaining one patient with TMB-high (20.4/Mb) was initially diagnosed as double primary cancer (endometrial cancer and ovarian cancer) and harbored a germline VUS of *MSH2*. We confirmed that her sample from endometrial cancer also exhibited TMB-high with overlapping genotype. Therefore, we amended the diagnosis as endometrial cancer with metastasis to the ovary.

3.12 Discussion

Our study confirmed that NGS-based CGP, using the TOP panel, is useful to identify "actionable" mutations in gynecological malignancies. It has been uncertain which types of gynecological cancers are suitable for CGP, especially for the RNA panel. Here, we revealed by our cohort that the TOP panel, a twin-panel system of DNA and RNA, can efficiently detect molecular profiling of gynecological

malignancies, including identification of gene fusions. More than 90% of all patients harbored at least one clinically annotated gene alteration and 46% harbored actionable alterations (Tier1 or Tier2). The ratio of actionability is higher than that from the pan-cancer analysis (actionability rate at 32.2%) [6].

Allele-specific analysis of CNV is useful to identify homozygous deletion (and uniparental disomy) of tumor suppressor genes [6, 22]. Another merit of TOP is the RNA panel. The junction-capture method enabled us to accurately and cost-effectively detect hundreds of fusion genes, as well as aberrantly spliced transcripts [6]. The RNA panel can detect novel gene fusions if one of the constituent genes is targeted by the capture panel. Although the clinical actionability of the fusion gene may be limited in gynecological carcinomas [23, 24], detection of *ROS1* gene fusion in one cervical cancer suggested that exploring gene fusions may lead to the best-personalized therapy. In addition, the identification of fusion genes using junction-capture RNA sequencing may be useful for molecular diagnosis of sarcomas, which are characterized by various fusion genes [16, 25, 26]. We identified two novel gene fusions in uterine sarcomas in this study. Although these two alterations were not clinically actionable, the existence of a gene fusion was useful to differentially diagnose high-grade endometrial stromal sarcoma from undifferentiated uterine sarcoma.

By using the TOP panel, we constructed the infrastructure of a clinical sequencing laboratory (with ISO15189 certification) within The University of Tokyo Hospital and established an expert annotation team to properly assess the results of sequencing data and provide final reports to each patient. Building this in-house clinical sequencing system should be advantageous to further propel personalized medicine by the cutting-edge technology.

The low rate of clinical trial enrollment after CGP is still a major problem [1, 4, 6, 27]. In this study, only one patient with an oncogenic variant of *PIK3CA* was enrolled in the clinical of AKT inhibitor, although we included cancer patients regardless of the patients' status (both nonrecurrent patients and those who receive standardized treatment can be enrolled). Larger numbers of basket-type clinical trials are anticipated to provide more options for personalized therapy. We conducted the prospective TOP analysis as Advanced Medical Care Category B (Japanese medical system: Senshin-iryou B) in 200 patients toward approval from the Ministry of Health, Labor and Welfare from August 2018 (Patient accrual was completed in December 2019) (UMIN000033647). The data of the 200 patients will disclose the ratio of the actionability in patients who (almost) finished all the standardized treatments. We believe that the TOP panel system will accelerate personalized medicine and broaden cancer treatment options for cancer patients in the near future.

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References

1. Zehir A, Benayed R, Shah RH, Syed A, Middha S, Kim HR, Srinivasan P, Gao J, Chakravarty D, Devlin SM, Hellmann MD, Barron DA, Schram AM, Hameed M, Dogan S, Ross DS, Hechtman JF, DeLair DF, Yao J, Mandelker DL, Cheng DT, Chandramohan R, Mohanty AS, Ptashkin RN, Jayakumaran G, Prasad M, Syed MH, Rema AB, Liu ZY, Nafa K, Borsu L, Sadowska J, Casanova J, Bacares R, Kiecka IJ, Razumova A, Son JB, Stewart L, Baldi T, Mullaney KA, Al-Ahmadie H, Vakiani E, Abeshouse AA, Penson AV, Jonsson P, Camacho N, Chang MT, Won HH, Gross BE, Kundra R, Heins ZJ, Chen HW, Phillips S, Zhang H, Wang J, Ochoa A, Wills J, Eubank M, Thomas SB, Gardos SM, Reales DN, Galle J, Durany R, Cambria R, Abida W, Cercek A, Feldman DR, Gounder MM, Hakimi AA, Harding JJ, Iyer G, Janjigian YY, Jordan EJ, Kelly CM, Lowery MA. Morris: mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. Nat Med. 2017;23:703–13.
2. Allegretti M, Fabi A, Buglioni S, Martayan A, Conti L, Pescarmona E, Ciliberto G, Giacomini P. Tearing down the walls: FDA approves next generation sequencing (NGS) assays for actionable cancer genomic aberrations. J Exp Clin Cancer Res. 2018;37:47.
3. Colomer R, Mondejar R, Romero-Laorden N, Alfranca A, Sanchez-Madrid F, Quintela-Fandino M. When should we order a next generation sequencing test in a patient with cancer? E Clin Med. 2020;25:100487.
4. Sunami K, Ichikawa H, Kubo T, Kato M, Fujiwara Y, Shimomura A, Koyama T, Kakishima H, Kitami M, Matsushita H, Furukawa E, Narushima D, Nagai M, Taniguchi H, Motoi N, Sekine S, Maeshima A, Mori T, Watanabe R, Yoshida M, Yoshida A, Yoshida H, Satomi K, Sukeda A, Hashimoto T, Shimizu T, Iwasa S, Yonemori K, Kato K, Morizane C, Ogawa C, Tanabe N, Sugano K, Hiraoka N, Tamura K, Yoshida T, Fujiwara Y, Ochiai A, Yamamoto N, Kohno T. Feasibility and utility of a panel testing for 114 cancer-associated genes in a clinical setting: a hospital-based study. Cancer Sci. 2019;110:1480–90.
5. Ebi H, Bando H. Precision oncology and the universal health coverage system in Japan. JCO Precis Oncol. 2019;3:1–10.
6. Kohsaka S, Tatsuno K, Ueno T, Nagano M, Shinozaki-Ushiku A, Ushiku T, Takai D, Ikegami M, Kobayashi H, Kage H, Ando M, Hata K, Ueda H, Yamamoto S, Kojima S, Oseto K, Akaike K, Suehara Y, Hayashi T, Saito T, Takahashi F, Takahashi K, Takamochi K, Suzuki K, Nagayama S, Oda Y, Mimori K, Ishihara S, Yatomi Y, Nagase T, Nakajima J, Tanaka S, Fukayama M, Oda K, Nangaku M, Miyazono K, Miyagawa K, Aburatani H, Mano H. Comprehensive assay for the molecular profiling of cancer by target enrichment from formalin-fixed paraffin-embedded specimens. Cancer Sci. 2019;110:1464–79.
7. Kohno T, Nakaoku T, Tsuta K, Tsuchihara K, Matsumoto S, Yoh K, Goto K. Beyond ALK-RET, ROS1 and other oncogene fusions in lung cancer. Transl Lung Cancer Res. 2015;4:156–64.
8. Vellichirammal NN, Albahrani A, Banwait JK, Mishra NK, Li Y, Roychoudhury S, Kling MJ, Mirza S, Bhakat KK, Band V, Joshi SS, Guda C. Pan-cancer analysis reveals the diverse landscape of novel sense and antisense fusion transcripts. Mol Ther Nucl Acids. 2020;19:1379–98.

9. Davies KD, Doebele RC. Molecular pathways: ROS1 fusion proteins in cancer. Clin Cancer Res. 2013;19:4040–5.
10. Schram AM, Chang MT, Jonsson P, Drilon A. Fusions in solid tumours: diagnostic strategies, targeted therapy, and acquired resistance. Nat Rev Clin Oncol. 2017;14:735–48.
11. Drilon A, Jenkins C, Iyer S, Schoenfeld A, Keddy C, Davare MA. ROS1-dependent cancers - biology, diagnostics and therapeutics. Nat Rev Clin Oncol; 2020.
12. Al-Salama ZT, Keam SJ. Entrectinib: first global approval. Drugs. 2019;79:1477–83.
13. Vuong HG, Nguyen TQ, Nguyen HC, Nguyen PT, Ho ATN, Hassell L. Efficacy and safety of crizotinib in the treatment of advanced non-small-cell lung cancer with ROS1 rearrangement or MET alteration: a systematic review and meta-analysis. Target Oncol. 2020;15:589–98.
14. Marcus L, Lemery SJ, Keegan P, Pazdur R. FDA approval summary: pembrolizumab for the treatment of microsatellite instability-high solid tumors. Clin Cancer Res. 2019;25:3753–8.
15. Marabelle A, Le DT, Ascierto PA, Di Giacomo AM, De Jesus Acosta A, Delord JP, Geva R, Gottfried M, Penel N, Hansen AR, Piha-Paul SA, Doi T, Gao B, Chung HC, Lopez-Martin J, Bang YJ, Frommer RS, Shah M, Ghori R, Joe AK, Pruitt SK, Diaz LA Jr. Efficacy of pembrolizumab in patients with noncolorectal high microsatellite instability/mismatch repair-deficient cancer: results from the phase II KEYNOTE-158 study. J Clin Oncol. 2020;38:1–10.
16. Chiang S, Cotzia P, Hyman DM, Drilon A, Tap WD, Zhang L, Hechtman JF, Frosina D, Jungbluth AA, Murali R, Park KJ, Soslow RA, Oliva E, Iafrate AJ, Benayed R, Ladanyi M, Antonescu CR. NTRK fusions define a novel uterine sarcoma subtype with features of fibrosarcoma. Am J Surg Pathol. 2018;42:791–8.
17. Oda K, Tanikawa M, Sone K, Mori-Uchino M, Osuga Y, Fujii T. Recent advances in targeting DNA repair pathways for the treatment of ovarian cancer and their clinical relevance. Int J Clin Oncol. 2017;22:611–8.
18. Bercow AS, Eisenhauer EL. Screening and surgical prophylaxis for hereditary cancer syndromes with high risk of endometrial and ovarian cancer. J Surg Oncol. 2019;120:864–72.
19. Kalia SS, Adelman K, Bale SJ, Chung WK, Eng C, Evans JP, Herman GE, Hufnagel SB, Klein TE, Korf BR, McKelvey KD, Ormond KE, Richards CS, Vlangos CN, Watson M, Martin CL, Miller DT. Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. Genet Med. 2017;19:249–55.
20. Pujol P, Vande Perre P, Faivre L, Sanlaville D, Corsini C, Baertschi B, Anahory M, Vaur D, Olschwang S, Soufir N, Bastide N, Amar S, Vintraud M, Ingster O, Richard S, Le Coz P, Spano JP, Caron O, Hammel P, Luporsi E, Toledano A, Rebillard X, Cambon-Thomsen A, Putois O, Rey JM, Herv C, Zorn C, Baudry K, Galibert V, Gligorov J, Azria D, Bressac-de Paillerets B, Burnichon N, Spielmann M, Zarca D, Coupier I, Cussenot O, Gimenez-Roqueplo AP, Giraud S, Lapointe AS, Niccoli P, Raingeard I, Le Bidan M, Frebourg T, Rafii A, Genevive D. Guidelines for reporting secondary findings of genome sequencing in cancer genes: the SFMPP recommendation. Eur J Hum Genet. 2018;26:1732–42.
21. Subbiah V, Solit DB, Chan TA, Kurzrock R. The FDA approval of pembrolizumab for adult and pediatric patients with tumor mutational burden (TMB): a decision centered on empowering patients and their physicians. Ann Oncol. 2020;31:1115–8.
22. Sato-Otsubo A, Sanada M, Ogawa S. Single-nucleotide polymorphism array karyotyping in clinical practice: where, when, and how? Semin Oncol. 2012;39:13–25.
23. Hu X, Wang Q, Tang M, Barthel F, Amin S, Yoshihara K, Lang FM, Martinez-Ledesma E, Lee SH, Zheng S, Verhaak RGW. Tumor Fusions: an integrative resource for cancer-associated transcript fusions. Nucleic Acids Res. 2018;46:D1144–9.
24. Wang J, Dean DC, Hornicek FJ, Shi H, Duan Z. RNA sequencing (RNA-Seq) and its application in ovarian cancer. Gynecol Oncol. 2019;152:194–201.
25. Hrzenjak A, Moinfar F, Tavassoli FA, Strohmeier B, Kremser ML, Zatloukal K, Denk H. JAZF1/JJAZ1 gene fusion in endometrial stromal sarcomas: molecular analysis by reverse transcriptase-polymerase chain reaction optimized for paraffin-embedded tissue. J Mol Diagn. 2005;7:388–95.

26. Hoang L, Chiang S, Lee CH. Endometrial stromal sarcomas and related neoplasms: new developments and diagnostic considerations. Pathology. 2018;50:162–77.
27. Flaherty KT, Gray RJ, Chen AP, Li S, McShane LM, Patton D, Hamilton SR, Williams PM, Iafrate AJ, Sklar J, Mitchell EP, Harris LN, Takebe N, Sims DJ, Coffey B, Fu T, Routbort M, Zwiebel JA, Rubinstein LV, Little RF, Arteaga CL, Comis R, Abrams JS, O'Dwyer PJ, Conley BA. Molecular landscape and actionable alterations in a genomically guided cancer clinical trial: national cancer institute molecular analysis for therapy choice (NCI-MATCH). J Clin Oncol; 2020.