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SHORT COMMUNICATION

Effectiveness of a genetic test panel designed for gynecological cancer: an exploratory study

Koichi Ida[1] · Tsutomu Miyamoto[1] · Shotaro Higuchi[1] · Hodaka Takeuchi[1] · Satoshi Yamada[1] · Motoki Ono[1] · Hiroshi Nishihara[2] · Tanri Shiozawa[1]

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Abstract

To increase diagnostic efficiency and cost-effectiveness, we performed an exploratory genetic test using a newly designed panel containing 28 actionable and druggable genes, alterations in which are frequently reported in gynecological cancers (TANRE-G, Targeted variants ANalysis RElated to Gynecological cancers). Samples consisted of the formalin-fixed, paraffin-embedded tissue of endometrial (4 cases), cervical (3 cases), and ovarian (4 cases) carcinomas. The sequencing procedure was performed using Ion PGM in our institute with related sequencing kits, and data were analyzed using ClinVar. The present system achieved more than 2500 reads in all tumor samples, and enabled a copy number variation analysis. Results showed that actionable and druggable mutations were detected in 82% (9/11) and 64% (7/11) of cases, respectively, which was similar to other commercially available genetic tests. The amplification of MYC and KRAS was also detected. The analysis cost for each sample was JPY 94,000 (USD 850). These results demonstrate the potential of the TANRE-G panel as an effective tool for examining genetic alterations in gynecological cancers.

Keywords Genetic test · Endometrial carcinoma · Cervical carcinoma · Ovarian carcinoma · Gene panel

Introduction

Recent advances in next-generation sequencing (NGS) have enabled comprehensive genetic variants to be analyzed in a clinical setting as precision medicine, particularly for cancer [1]. A precision approach leads cancer patients to targeted therapies directed against specific genomic driver alterations in tumors identified with NGS-based testing [2]. Testing contents has recently involved more comprehensive gene panels instead of disease-focused gene panels [3]. In Japan, several types of NGS-based genetic tests are currently available; most use panels containing 100–200 comprehensive genes, and cost between 60,000 and 100,000 JPY (5000–8000 USD), which is not covered by medical insurance.

Due to prevailing precision approaches [4], we have encountered a number of "actionable" alterations, defined as alterations potentially targetable with established or investigational therapeutics directly or indirectly [5]. However, there are currently only a few FDA-approved drugs, i.e., those targeting angiogenesis, homologous recombination deficiencies, and microsatellite instabilities [4].

While precision approaches offer useful diagnostic strategies for cancer patients, the following issues remain unsolved. Since there are many targeting genes on popular NGS cancer panels, including unfamiliar variants, such as variants of uncertain significances (VUSs), difficulties are often associated with interpreting the data obtained [6]. The handling of these "incidental" findings creates ethical dilemmas. Therefore, multiple and comprehensive cancer panels may currently result in many useless variants for patients, which inevitably causes confusion. Furthermore, although the cost of sequencing has gradually been decreasing [7], the cancer panels used in clinical sequencing are still too

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✉ Tsutomu Miyamoto
tmiya@shinshu-u.ac.jp

[1] Department of Obstetrics and Gynecology, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto 390-8621, Japan

[2] Genomics Unit, Keio Cancer Center, Keio University School of Medicine, 3-S5, Institute of Integrated Medical Research, 35 Shinanomachi, Shinjuku-Ku, Tokyo 160-8582, Japan

expensive for many terminal cancer patients and their families to request. However, the number of studies on the costs of clinical sequencing for advanced cancer patients is currently limited, and we often encounter difficulties understanding real transactions in clinical settings.

In addition, although multiple clinical studies evaluating precision medicine revealed that between 30 and 50% of patients had actionable mutations, only between 3 and 13% received treatments that had been adapted to individual druggable mutations [8]. However, even if druggable mutations are detected in the cancer panels of patients, it may be impossible to take targeted drugs covered by health insurance in many cases, or only a very small number of these patients may discover and join clinical trials offering suitable therapies. In some cases, these patients may eventually select a drug recommended by an expert meeting as off-label use at their expense, which often has been priced at astronomical levels, i.e., USD 70,000 to 130,000 for a course of treatment [9].

To address these issues, we designed a genetic testing approach focused exclusively on 28 actionable and druggable genes in gynecological cancers named Targeted variants ANalysis RElated to Gynecological cancers (TANRE-G), and retrospectively performed clinical sequencing as an experimental trial. This exploratory research aimed to evaluate the cost-effectiveness and feasibility of clinical sequencing by the TANRE-G panel and show the prospects of this gynecologically specific panel.

Materials and methods

Design

TANRE-G is based on the Ion AmpliSeq DNA assay workflow (Thermo Fisher Scientific, Wilmington, DE, USA). Amplicons ($n = 1471$) were designed with Ion AmpliSeq DNA Designer. The 28 target genes of TANRE-G comprised actionable and potentially actionable genes, harboring frequent variants of oncogenic driver and tumor suppressor genes reported in gynecological cancers, and were selected based on the following databases: CanDL; https://candl.osu.edu/, CANCER GENOME INTERPRETER; https://www.cancergenomeinterpreter.org/home, CIViC; https://civicdb.org/home, OncoKB; http://oncokb.org/#/, and ClinicalTrials.gov; https://clinicaltrials.gov/ (Table 1). The TP53 gene was omitted from this panel because p53 variants did not have any impact on further treatment.

Patient and samples

This study was a single-center case series with retrospective data collection conducted with the approval of the Ethics Committee of Shinshu University (approval No.591). Subjects were ensured of their right to opt out prior to the start. Eleven refractory or relapsed gynecological cancer cases (four endometrial, four ovarian, and three cervical cancers) were selected from patients who underwent surgery at Shinshu University Hospital between 2016 and 2017. Median age was 57 years (44–78), 6 out of 11 cases were stage III–IV, and only 2 cases received neoadjuvant chemotherapy. Formalin-fixed paraffin-embedded (FFPE) tissues removed surgically and fixed within 48 h were used in the following working flow.

Library preparation, emulsion PCR, and enrichment

Tumor tissues (as test samples) and normal myometrial tissues (as normal references) were collected by needle microdissection from several 10-µm-thick FFPE tissue sections. The ratio of tumor cells was confirmed to be between 60 and 80% across all samples. The genomic DNA of each sample was extracted using the MagMAX FFPE DNA/RNA Ultra Kit (Thermo Fisher Scientific, Wilmington, DE) according to the manufacturer's instructions. The concentration and quality of extracted DNAs were checked using the Qubit dsDNA HS Assay Kit with the Qubit 3.0 Fluorometer (Thermo Fisher Scientific) and quantitative PCR (qPCR) with the TaqMan FFPE DNA QC Assay (Thermo Fisher Scientific), respectively. Libraries were generated from 10 ng of DNA per sample using the Ion AmpliSeq Library Kit 2.0 (Thermo Fisher Scientific), quantified by applying a qPCR analysis with the Ion Library TaqMan Quantitation Kit (Thermo Fisher Scientific), and pooled to an equimolar concentration of 20 pM for template preparation. Template preparation, which comprised emulsion PCR, the enrichment of beads containing the template, and chip loading, was performed using the Ion Chef system and Ion PGM Hi-Q View Chef Kit following the manual (Thermo Fisher Scientific).

Sequencing and data analysis

The sequencing of multiplexed templates was performed using the Ion PGM system on Ion 318 v2 chips with the Ion PGM Hi-Q View Chef Kit (Thermo Fisher Scientific) according to the manufacturer's instructions. A primary data analysis was performed by Torrent Suite Software v5.8 (Thermo Fisher Scientific) with default settings using hg19 as reference genome data. The data of normal DNA were only used individually for the subtraction of germline alterations, such as SNPs. Variant calling was executed using the Torrent Variant Caller plugin (5.8.0.19) in the Torrent Server and visualized using Integrative Genomics Viewer Version 5.01 (0) (Broad Institute). After the sequence was aligned and filtered on a tumor-normal pipeline, a variant analysis

Table 1 Selected actionable or potentially actionable genes in a 28-gene panel and therapeutic implications

Gene	Mutation type	Potential therapeutic implications
AKT1	Activating mutations	Treatment with AKT or mTOR inhibitors
APC	Inactivating mutations	Treatment with Tankylase inhibitors
ARID1A	Inactivating mutations	Treatment with EZH2 or PI3 K inhibitors
ASXL1	Inactivating mutations	Treatment with HDAC inhibitors
ATM	Inactivating mutations	Treatment with PARP inhibitors
ATR	Inactivating mutations	Treatment with PARP inhibitors
BAP1	Inactivating mutations	Treatment with HDAC inhibitors
BLM	Inactivating mutations	Treatment with PARP inhibitors
BRCA1	Inactivating mutations	Treatment with PARP inhibitors
BRCA2	Inactivating mutations	Treatment with PARP inhibitors
CHEK2	Inactivating mutations	Treatment with Chk2 inhibitors
ERBB2	Amplification/activating mutations	Treatment with HER2 inhibitors
FANCA	Inactivating mutations	Treatment with PARP inhibitors
FBXW7	Inactivating mutations	Treatment with mTOR inhibitors
KRAS	Activating mutations	Treatment with MEK inhibitors
MAPK1	Activating mutations	Treatment with ERK inhibitors
MLH1	Inactivating mutations	Treatment with immune checkpoint inhibitors
MRE11	Inactivating mutations	Treatment with PARP inhibitors
MSH2	Inactivating mutations	Treatment with immune checkpoint inhibitors
MSH6	Inactivating mutations	Treatment with immune checkpoint inhibitors
MYC	Amplification/activating mutations	Treatment with BET inhibitors
PALB2	Inactivating mutations	Treatment with PARP inhibitors
PIK3CA	Activating mutations	Treatment with PI3 K, AKT, or mTOR inhibitors
PIK3R1	Inactivating mutations	Treatment with PI3 K, AKT, or mTOR inhibitors
PMS2	Inactivating mutations	Treatment with immune checkpoint inhibitors
PTEN	Inactivating mutations	Treatment with PI3 K, AKT, or mTOR, inhibitors
RAD51B	Inactivating mutations	Treatment with PARP inhibitors
TSC1	Inactivating mutations	Treatment with mTOR inhibitors

mTOR mammalian target of rapamycin, *EZH2* enhancer of zeste homolog 2, *HDAC* histone deacetylases, *PARP* poly (ADP-ribose) polymerase, *BET* bromodomain and extra-terminal

and annotations were performed by IonReporter software 5.6 with default settings. Candidate variants selected by these processes were filtered based on the predicted impact on protein function by SIFT (Sorting Intolerant From Tolerant, http://provean.jcvi.org/index.php) and Polyphen-2 (Polymorphism Phenotyping version 2, http://genetics.bwh.harvard.edu/pph2/). These functionally affected variants were finally defined as 'Pathogenic', 'Likely Pathogenic', or others by ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/). Furthermore, if the candidate variant was a frameshift or nonsense mutation affecting known functional domains of a tumor suppressor gene, it was also regarded as 'Pathogenic' regardless of the registration in ClinVar.

Prediction of copy number alterations

Copy number alterations (CNAs) were detected using an Ion Reporter Software Copy Number Variation Analysis (Thermo Fisher Scientific) based on a hidden Markov model [10]. CNAs with precision scores ≥ 10 were included in the analysis according to the manufacturer's instructions. The copy number of the tumor cells was calculated by correcting with the ratio of tumor cells in the tissue section. According to previous studies, copy numbers of ≥ 6 were defined as high-level amplification, while copy numbers of 3–5 were regarded as moderate amplification [11]. In the present study, high-level and moderate amplifications of oncogenic driver genes were defined as "Likely pathogenic" variations.

Confirmation of genetic alterations

Each variant sequence detected by the above workflow was confirmed by Sanger sequencing. Immunohistochemistry (IHC) was performed to confirm CNAs. Primers for Sanger sequencing were designed using Primer Designer Tool online (Thermo Fisher Scientific) or Primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). IHC was performed using 3-μm-thick FFPE tissue sections as

described previously [12]. Antibodies for MYC (9E10, 1:200 dilution, Novus Biologicals, Littleton) and KRAS (12063-1-AP, 1:300 dilution, ProteinTech) were used as the primary antibody.

Statistical analysis

The significance of differences between the TANRE-G panel and NCC Oncopanel was evaluated with a two-tailed Fisher's exact test. A p value of < 0.05 was considered to be significant. All statistical analyses were performed using R software (https://www.r-project.org/).

Results

We evaluated the performance of the TANRE-G panel in the retrospective exploratory analysis of 11 gynecological FFPE tumor-normal samples. All samples successfully underwent the targeted sequencing of genes on the TANRE-G panel. A TANRE-G assay generated approximately 5.5 million reads per specimen with the following characteristics for tumor and normal tissues: mean coverage depths in tumor and normal amplicons were approximately 3400 × and 350 ×, respectively; mean coverage depths in 86% of all tumor amplicons and 82% of all normal tissue amplicons were > 500 × and > 100 ×, respectively; and < 1% of tumor amplicons and < 3% of normal tissue amplicons were < 20 × in their mean coverage depths.

Somatic variants detected

Table 2 shows 32 candidate somatic variants in 8/11 (72%) cases from sequencing with the TANRE-G panel. Each number of variation type was as follows: missense 22 (69%); nonsense 6 (19%); amplification 3 (9%); frameshift-deletion 1 (3%). Regarding 'Pathogenic' or 'Likely pathogenic' somatic alterations as actionable somatic alterations, 24/32 (75%) actionable variants were detected. The gene having the most frequent variations was PIK3CA with seven variants, followed by PTEN with five variants. Most of these gene alterations, especially those defined as 'Pathogenic' were consistently with previous reports in each primary cancer, and were shown in pBioPortal (http://www.cbioportal.org/) and/or COSMIC (https://cancer.sanger.ac.uk/cosmic) (Supplementary Table 1). Twenty-four candidate somatic variants were extracted in 4/4 (100%) cases of endometrial cancer. Eighteen (18/24; 75%) of these were actionable variants, most of which were related to an aberrant PI3 K pathway by PIK3CA or PTEN mutations (4/4 cases; 100%) or an aberrant Receptor Tyrosine Kinase (RTK)/RAS signaling pathway by ERBB2 or KRAS variants (3/4 cases; 75%). In 3/4 (75%) of ovarian cancer cases, six candidate somatic variants were identified, and 3/6 (50%) were actionable variants; PIK3CA and ARID1A variants in one (25%) clear cell ovarian carcinoma case, and BRCA2 gene in one (25%) high-grade serous ovarian carcinoma case. In Case 7 with the BRCA2 variation, the tumor relapsed four times, however, she has been successfully treated with platinum-based chemotherapy followed by PARP inhibitors as maintenance therapy. Only one (33%) out of the three GAS cases had actionable variants in the KRAS gene. All actionable somatic mutations were validated by Sanger sequencing.

Although the target region of TANRE-G panel is not large enough (0.14 Mb) to precisely calculate the tumor mutation burden (TMB, mutation frequency/Mb), that of case 1 and 4 were 62 and 82/Mb, respectively. Therefore, TMB data obtained by TANRE-G panel may be applied to select the candidates for MSI testing.

CNAs were assessed in 5 cases (45%) under the detection algorithm. We detected three moderate amplifications as actionable variants in two driver oncogenes: One was MYC (copy number = 3 in case 6, and copy number = 5 in case 7), the other was KRAS (copy number = 7 in case 9). This KRAS amplification may explain the high variant allele frequency (VAF) of it (Table 2). These results were confirmed by IHC (Supplementary Fig. 1). The specimens from cases 8 and 11 were immunostained as a control for MYC and KRAS due to their normal copy number, respectively. The direct material cost for an analysis of one sample by TANRE-G, including chemicals, was approximately USD 850 (JPY 94,000), which is less expensive than other gene panels (e.g., FoundationOne CDx: USD 5800, MI Tomor Seek: USD 3500).

Comparison with another panel assay

In a comparison of the TANRE-G panel with the NCC Oncopanel for the first 131 cases [13], at least one pathogenic somatic variant was observed in 8/11 (73%) cases in the TANRE-G panel, whereas 104/131 (79%) were noted in the NCC Oncopanel (p value, 0.700). Furthermore, at least one actionable somatic variant was observed in 8/11 (73%) cases in the TANRE-G panel, whereas 59/131 (45%) were noted in the NCC Oncopanel (p value, 0.115). Neither panel showed a significant difference in detecting pathogenic or actionable somatic variants, which indicates that the TANRE-G panel is a similar or alternative test to the NCC Oncopanel, at least in gynecological clinical settings.

Discussion

NGS has enabled us to perform personalized oncologic strategies for targeted therapy. We herein conducted exploratory research on a customized, cost-effective NGS panel

Table 2 Candidate variants detected in 11 cases using the TANRE-G panel

Case	Histology	Gene	VAF	Nucleotide change	Protein change	Variation type	Clinical significance
Endometrial cancer (n = 4)							
Case 1	EC, Grade 1	*PIK3CA*	0.50	c.1636C > G	p.Q546E	Missense	Pathogenic
		PIK3CA	0.39	c.3140A > G	p.H1047R	Missense	Pathogenic
		PTEN	0.55	c.758T > A	p.I253 N	Missense	Uncertain significance
		PTEN	0.32	c.419T > G	p.L140*	Nonsense	Pathogenic
Case 2	EC, Grade 2	*ATM*	0.83	c.9023G > A	p.R3008H	Missense	Likely pathogenic
		KRAS	0.42	c.35G > A	p.G12D	Missense	Pathogenic
		PTEN	0.29	c.389G > A	p.R130Q	Missense	Pathogenic
		PIK3CA	0.43	c.3140A > G	p.H1047R	Missense	Pathogenic
Case 3	EC, Grade 2	*ERBB2*	0.44	c.2305G > T	p.A769Y	Missense	Pathogenic
		FBXW7	0.17	c.1514G > T	p.R505L	Missense	N/A
		PIK3CA	0.25	c.331A > G	p.K111E	Missense	Likely pathogenic
Case 4	EC, Grade 3	*APC*	0.34	c.3340C > T	p.R1114*	Nonsense	Pathogenic
		APC	0.39	c.4495G > T	p.G1499*	Nonsense	Pathogenic
		APC	0.40	c.4760C > A	p.S1587*	Nonsense	Pathogenic
		ARID1A	0.41	c.5965C > T	p.R1989*	Nonsense	Pathogenic
		ATR	0.37	c.4094A > C	p.D1365A	Missense	N/A
		ATR	0.37	c.4051C > T	p.R1351 W	Missense	N/A
		FBXW7	0.39	c.328G > T	p.E110*	Nonsense	Pathogenic
		PIK3CA	0.31	c.333G > T	p.K111 N	Missense	Likely pathogenic
		PIK3CA	0.35	c.3062A > G	p.Y1021C	Missense	Likely pathogenic
		PIK3R1	0.40	c.1067T > C	p.L356S	Missense	N/A
		PTEN	0.39	c.389G > A	p.R130Q	Missense	Pathogenic
		PTEN	0.43	c.407G > A	p.C136Y	Missense	Pathogenic
		RAD51B	0.42	c.317T > G	p.I106S	Missense	N/A
Ovarian cancer (n = 4)							
Case 5	CCC	*ARID1A*	0.46	c.6265delC	p.L2089 fs	Frameshift-deletion	Pathogenic
		PIK3CA	0.47	c.331A > G	p.K111E	Missense	Likely pathogenic
Case 6	LGSC	*MRE11A*	0.35	c.751A > G	p.I251 V	Missense	N/A
		MYC				Amplification	Likely pathogenic
Case 7	HGSC	*BRCA2*	0.84	c.8023A > G	p.I2675 V	Missense	Pathogenic
		MYC				Amplification	Likely pathogenic
Case 8	HGSC	No variant					–
Cervical cancer (n = 3)							
Case 9	GAS	*KRAS*	0.58	c.35G > T	p.G12 V	Missense	Pathogenic
		KRAS				Amplification	Likely pathogenic
Case 10	GAS	No variant					–
Case 11	GAS	No variant					–

VAF variant allele frequency, *EC* endometrioid carcinoma, *CCC* clear cell carcinoma, *LGSC* low-grade serous carcinoma, *HGSC* high-grade serous carcinoma, *GAS* mucinous carcinoma, gastric type, *N/A* not applicable

*Termination codon

"TANRE-G" for the identification of relevant somatic alterations specifically in gynecological cancers. We performed a retrospective analysis of archival tumor-normal FFPE samples in recurrence or refractory cases. We effectively identified mutations through deep targeted sequencing using the Ion Torrent platform and its useful annotation software Ion Reporter [14]. The prevalence of detecting actionable or potentially actionable mutations in TANRE-G was similar to that in existing panels. For example, actionable mutations were detected in 45% in the NCC Oncopanel [13], 47% in Foundation One, and 36% in MSK-IMPACT [15].

Endometrial cancer is expected to have the most actionable mutations in TANRE-G. Likewise, a recent study revealed that the prevalence of actionable mutations or

CNAs in all cancers was the highest (98%) in endometrial cancer, resulting in the cost of finding at least one actionable mutation for this cancer to be the second lowest among all cancers (USD 5,897) [16].

Drugs for potentially actionable mutations may be developed in rapid succession. The use of a comprehensive panel may be advantageous for unintentionally identifying new actionable drugs already approved for other diseases with the same mutations. However, basket trials on one drug for a single mutation in a number of tumors have only recently started [4]; therefore, further time is needed to obtain information, which results in delays in the treatment of patients using these drugs. Therefore, we consider TANRE-G to be a beneficial and cost-effective choice, particularly for gynecological patients.

One issue needs to be addressed; when a higher number of genes is adopted in one panel, more VUSs are also found, which results in difficulties judging pathogenicity in clinical settings. We selected candidate genes that did not cause increases in germline or somatic VUSs because there are many functionally unknown variants that may be pathogenetic [17]. In general, the fewer genes mounted in gene panels, the fewer VUSs detected. Based on our results in TANRE-G, we only found one VUS in case 1 (Table 2), thereby confirming the pathogenicity of VUSs.

Following the recommendations of the American College of Medical Genetics and Genomics (ACMG), we do not necessarily inform patients of all results of VUSs. However, we need to consider that changes in VUSs to actionable ones in the near future may be detected by upcoming new methods, such as whole genome clinical sequencing, and this information may be provided to patients.

Thus, TANRE-G is a simple gene panel test. By using this panel, we may examine the minimum necessary genes, judge whether they are actionable, and consider the next treatment policy. Furthermore, CNV and TMB may be used as references. The discovery rate of actionable genes is expected to be high from the present results, suggesting lower costs. Therefore, this may become an excellent option, particularly for gynecological cancer patients in the local area.

In conclusion, TANRE-G proved to be a clinical model that is a reliable, fast, and cost-effective targeted NGS panel. Many selected genes are useful for finding new candidate variants to target or new functional therapies, but this may potentially be very rare. In contrast, many VUSs are identified that provide patients with helpful or sometimes harmful information. We currently cannot offer patients sufficient consultations on the genes listed in ACMG secondary findings [18] because of the lack of genetic counselors, particularly in Japan (approximately 200 counselors). However, as discussed above, our exploratory TANRE-G panel may become a shortcut to resolving these issues by merely defining gynecological target genes. We consider the effectiveness of our panel to support its clinical application to advanced gynecological cancer patients in near future.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the Ethics Committee of Shinshu University (approval No.591) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Blanket consents had been obtained from all individual participants included in this study for using their resected tissue samples to any studies with anonymization. It was accepted in the Ethics Committee that the additional consent was unnecessary from any participants because of analyzing only somatic alterations of the genes on the TANRE-G panel. All individual participants were ensured of their right to opt out prior to the start.

References

1. Gagan J, Van Allen EM. Next-generation sequencing to guide cancer therapy. Genome Med. 2015;29:80.
2. Garraway LA, Verweij J, Ballman KV. Precision oncology: an overview. J Clin Oncol. 2013;31:1803–5.
3. Dong L, Wang W, Li A, Kansal R, Chen Y, Chen H, Li X. Clinical next generation sequencing for precision medicine in cancer. Curr Genom. 2015;16:253–63.
4. Barroilhet L, Matulonis U. The NCI-MATCH trial and precision medicine in gynecologic cancers. Gynecol Oncol. 2018;148:585–90.
5. Meric-Bernstam F, Brusco L, Shaw K, Horombe C, Kopetz S, Davies MA, Routbort M, Piha-Paul SA, Janku F, Ueno N, Hong D, De Groot J, Ravi V, Li Y, Luthra R, Patel K, Broaddus R, Mendelsohn J, Mills GB. Feasibility of large-scale genomic testing to facilitate enrollment Onto genomically matched clinical trials. J Clin Oncol. 2015;33:2753–62.
6. Van Allen EM, Wagle N, Levy MA. Clinical analysis and interpretation of cancer genome data. J Clin Oncol. 2013;31:1825–33.
7. Dong L, Wang W, Li A, Kansal R, Chen Y, Chen H, Li X. Clinical next generation sequencing for precision medicine in cancer. Curr Genom. 2015;16:253–63.
8. Tannock IF, Hickman JA. Limits to personalized cancer medicine. N Engl J Med. 2016;375:1289–94.
9. Experts in Chronic Myeloid Leukemia. The price of drugs for chronic myeloid leukemia (CML) is a reflection of the unsustainable prices of cancer drugs: from the perspective of a large group of CML experts. Blood. 2013;121:4439–42.
10. Kader T, Goode DL, Wong SQ, Connaughton J, Rowley SM, Devereux L, Byrne D, Fox SB, Mir Arnau G, Tothill RW, Campbell IG, Gorringe KL. Copy number analysis by low coverage whole genome sequencing using ultra low-input DNA from

formalin-fixed paraffin embedded tumor tissue. Genome Med. 2016;8:121.
11. Ohshima K, Hatakeyama K, Nagashima T, Watanabe Y, Kanto K, Doi Y, Ide T, Shimoda Y, Tanabe T, Ohnami S, Ohnami S, Serizawa M, Maruyama K, Akiyama Y, Urakami K, Kusuhara M, Mochizuki T, Yamaguchi K. Integrated analysis of gene expression and copy number identified potential cancer driver genes with amplification-dependent overexpression in 1454 solid tumors. Sci Rep. 2017;7:641.
12. Ohya A, Yamanoi K, Shimojo H, Fujii C, Nakayama J. Gastric gland mucin-specific O-glycan expression decreases with tumor progression from precursor lesions to pancreatic cancer. Cancer Sci. 2017;108:1897–902.
13. Tanabe Y, Ichikawa H, Kohno T, Yoshida H, Kubo T, Kato M, Iwasa S, Ochiai A, Yamamoto N, Fujiwara Y, Tamura K. Comprehensive screening of target molecules by next-generation sequencing in patients with malignant solid tumors: guiding entry into phase I clinical trials. Mol Cancer. 2016;15:73.
14. Deshpande A, Lang W, McDowell T, Sivakumar S, Zhang J, Wang J, San Lucas FA, Fowler J, Kadara H, Scheet P. Strategies for identification of somatic variants using the Ion Torrent deep targeted sequencing platform. BMC Bioinform. 2018;19:5.
15. 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 LGT, Omuro AM, Raj N, Razavi P, Shoushtari AN, Shukla N, Soumerai TE, Varghese AM, Yaeger R, Coleman J, Bochner B, Riely GJ, Saltz LB, Scher HI, Sabbatini PJ, Robson ME, Klimstra DS, Taylor BS, Baselga J, Schultz N, Hyman DM, Arcila ME, Solit DB, Ladanyi M, Berger MF. Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. Nat Med. 2017;23:703–13.
16. Shen C, Meric-Bernstam F, Su X, Mendelsohn J, Giordano S. Prevalence of actionable mutations and copy number alterations and the price of a genomic testing panel. Oncotarget. 2016;7:71686–95.
17. Foley SB, Rios JJ, Mgbemena VE, Robinson LS, Hampel HL, Toland AE, Durham L, Ross TS. Use of whole genome sequencing for diagnosis and discovery in the cancer genetics clinic. EBioMedicine. 2015;2:74–81.
18. 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.