Clinical utility of Next-Generation Sequencing-Based Panel Testing under the Universal Health Care System in Japan: A Retrospective Analysis at a Single University Hospital

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Figure S1. CONSORT diagram of patients enrolled in the study. MTB; molecular tumor board.
Figure S2. Top 20 frequent genomic alterations with the treatment recommendation.

Figure S3. The operation workflow for evaluating and nominating presumed germline findings from tumor-only sequencing panel.

The workflow is adapted from the proposal of the Japan Agency for Medical Research and Development (AMED) study group concerning the information transmission process in genomic medicine. APC; adenomatous polyposis coli, BRCA1; breast cancer susceptibility gene 1, BRCA2; breast cancer susceptibility gene 2, RB1; retinoblastoma 1, TP53; tumor protein P53
Table S1. Actionable alterations according to cancer type.

| Cancer Type          | No. of patients with actionable mutation, n | No. of actionable mutation, n | Level of Evidence, n (%) |
|----------------------|--------------------------------------------|------------------------------|--------------------------|
|                      | Total                                       | 168                         | 70 (64.8)                |
|                      | Colorectal                                  | 45                          | 19 (42.4)                |
|                      | Sarcomas                                    | 22                          | 8 (36.4)                 |
|                      | Pancreatic                                   | 18                          | 4 (22.2)                 |
|                      | Gastric                                     | 13                          | 2 (15.4)                 |
|                      | Ovarian                                     | 11                          | 5 (35.5)                 |
|                      | Bile Duct                                   | 9                           | 5 (55.6)                 |
|                      | Esophageal                                  | 8                           | 4 (50.0)                 |
|                      | Breast                                      | 7                           | 6 (85.7)                 |
|                      | Cervical                                    | 6                           | 2 (33.3)                 |
|                      | Small intestinal                            | 5                           | 4 (80.0)                 |
|                      | Endometrial                                 | 3                           | 3 (100.0)                |
|                      | Non-Small Cell Lung                        | 3                           | 2 (66.6)                 |
|                      | Brain                                       | 3                           | 2 (66.6)                 |
|                      | Melanoma                                    | 3                           | 2 (66.6)                 |
|                      | Unknown Primary                             | 3                           | 1 (33.3)                 |
|                      | Hepatocellular Carcinoma                    | 3                           | 1 (33.3)                 |
|                      | Neuroblastoma                               | 3                           | 0 (0)                    |
|                      | Kidney                                      | 1                           | 0 (0)                    |
|                      | Prostate                                    | 1                           | 0 (0)                    |
|                      | Urinary Tract                               | 1                           | 0 (0)                    |

Note: The table shows the number of patients with actionable mutations and the distribution of actionable mutations by cancer type, along with the level of evidence for each mutation.
**Table S2.** List of cases that underwent genomically matched treatment beyond standard of care based on MTB recommendation.

| Cancer Type | Age | Sex | Targeted gene | LE | Treatment | Institution      | Clinical Benefit |
|-------------|-----|-----|----------------|----|-----------|------------------|------------------|
| Endometrial | 62  | F   | ATM            | 3B | Clinical trial; ATR inhibitor Clinical trial; JAK inhibitor | Outside hospital | NA              |
|             |     |     | JAK1           |     |           | Our hospital       | NA              |
| Colorectal  | 43  | F   | TMB-H          | 1  | Clinical trial; PD-1 inhibitor | Our hospital | NA              |
| Gastric     | 68  | M   | FGFR2 amp      | 4  | Clinical trial; FGFR2 inhibitor | Our hospital | NA              |
| Bile duct   | 69  | M   | FGFR2 amp      | 4  | Clinical trial; FGFR2 inhibitor | Our hospital | NA              |
| Bile duct   | 75  | F   | ERBB2 amp      | 3B | Off label; Trastuzumab/Pertuzumab Off label; Trastuzumab deruxtecan | Our hospital | Yes             |
| Colorectal  | 59  | F   | ERBB2 amp      | 2  | Off label; Trastuzumab/Pertuzumab | Our hospital | No              |

amp: amplification, ATM: ataxia telangiectasia mutated, ERBB2: erbB-2 receptor tyrosine-protein kinase, FGFR2: fibroblast growth factor receptor 2, JAK1: janus kinase 1, MTB: molecular tumor board, TMB-H: tumor mutation burden high, LE: level of evidence.

**Table S3.** Patients’ preference for receiving presumed germline finding.

|                           | Yes (n) | No (n) |
|---------------------------|---------|--------|
| Wanting to receive         | 166     | 2      |
| presumed germline finding* |         |        |
| Sharing information         | 156     | 8      |
| with family members**      |         |        |

*For minor patients, answers are obtained from their parents/guardians with patients’ assent. **exclude minor patients.

**Table S4.** Presumed germline finding gene list used for assessing F1CDx.

| Gene  | Major Phenotype                                      |
|-------|------------------------------------------------------|
| APC   | FAP                                                  |
| ATM   | Breast cancer                                        |
| BAP1  | Malignant Mesothelioma etc.                         |
| BRCA1 | HBOC                                                 |
| BRCA2 | HBOC                                                 |
| BRIP1 | Ovarian cancer                                       |
| CDH1  | Diffuse gastric cancer                               |
| CDK4  | Melanoma                                             |
| CDKN2A| Melanoma/Pancreatic cancer                           |
| CHEK2 | Breast cancer                                        |
| FH    | Hereditary Leiomyomatosis and Renal Cell cancer      |
| FLCN  | Birt-Hogg-Dube syndrome                              |
| MEN1  | MEN1                                                 |
| MET   | GIST                                                 |
| MLH1  | Lynch syndrome                                       |
| MSH1  | Lynch syndrome                                       |
| MSH6  | Lynch syndrome                                       |
| MUTYH | MAP                                                  |
NBN  Breast cancer
NF1  NF1
NF2  NF2
PALB2  Breast cancer
PMS2  Lynch syndrome
POLD1  Colon cancer
POLE  Colon cancer
PTEN  PTEN hamartoma
RAD51C  Ovarian cancer
RAD51D  Ovarian cancer
RB1  Retinoblastoma
RET  MEN2
SDHA  HPPS
SDHAF2  HPPS
SDHB  HPPS
SDHC  HPPS
SDHD  HPPS
SMAD4  Juvenile Polyposis
STK11  Peutz-Jeghers syndrome
TGFBR2  Loeys-Dietz syndrome
TP53  Li-Fraumeni syndrome
TSC1  Tuberous Sclerosis
TSC2  Tuberous Sclerosis
VHL  VHL
WT1  WT1-related Wilms tumor

APC; adenomatous polyposis coli, ATM; ataxia telangiectasia mutated, BAPI; BRCA1 associated protein 1, BRCA1; breast cancer susceptibility gene 1, BRCA2; breast cancer susceptibility gene 2, BRIP1; BRCA1 interacting protein C-terminal helicase 1, CDH1; cadherin-1, CDK4; cyclin dependent kinase 1, CDKN2A; cyclin Dependent Kinase Inhibitor 2A, CHEK2; checkpoint kinase 2, FAP; familial adenomatous polyposis, FH; fumarate hydratase, FLCN; folliculin, GIST; gastrointestinal stromal tumor, HBOC; hereditary breast and ovarian cancer, HPPS; hereditary pheochromocytoma/paraganglioma syndrome, MAP; MUTYH-Associated polyposis, MENI; multiple endocrine neoplasia type1, MEN2; multiple endocrine neoplasia type2, MET; hepatocyte growth factor receptor, MLH1; MutL homolog 1, MSH2; MutS Homolog 2, MSH6; mutS homolog 6, MUTYH; mutY homolog, NBN; nibrin, NF1; neurofibromatosis type1, NF2; neurofibromatosis type2, PALB2; partner and localizer of BRCA2, PMS2; PMS1 homolog 2, POLD1; DNA polymerase delta 1, POLE; DNA polymerase epsilon 1, PTEN; phosphatase and tensin homolog, RAD51C; RAD51 homolog C, RAD51D; RAD51 homolog D, RB1; retinoblastoma 1, RET; rearranged during transfection, SDHA; succinate dehydrogenase complex flavoprotein subunit A, SDHAF2; succinate dehydrogenase complex assembly factor 2, SDHb; succinate dehydrogenase complex subunit B, SDHC; succinate dehydrogenase complex subunit C, SDHD; succinate dehydrogenase complex subunit D, SMAD4; mothers against decapentaplegic homolog 4, STK11; serine/threonine kinase 11, TGFBR2; transforming growth factor beta receptor 2, TP53; tumor protein P53, TSC1; tuberous sclerosis complex 1, TSC2; tuberous sclerosis complex 2, VHL; von Hippel-Lindau, WT1; Wilms’ tumor 1,