

## Target sequencing of cancer-related genes in early esophageal squamous neoplasia resected by endoscopic resection in Japanese patients

### SUPPLEMENTARY MATERIALS

**Supplementary Table 1: Mutations detected in adjacent normal tissues**

| Cases   | Depth | Gene          | Mutation     | Tumor tissue |        | Adjacent normal tissue |        |
|---------|-------|---------------|--------------|--------------|--------|------------------------|--------|
|         |       |               |              | Coverage     | VF (%) | Coverage               | VF (%) |
| ESCC_10 | EP    | <i>TP53</i>   | p.Phe270Leu  | 1638         | 2.1    | 2000                   | 3.1    |
| ESCC_16 | EP    | <i>TP53</i>   | p.Arg273Cys  | 1997         | 45.9   | 141                    | 0.7    |
| ESCC_17 | EP    | <i>APC</i>    | p.Gln1294Ter | 316          | 5.4    | 79                     | 1.3    |
| ESCC_26 | EP    | <i>TP53</i>   | p.Pro72Ala   | 1999         | 2.8    | 2000                   | 1.4    |
| ESCC_3  | EP    | <i>NOTCH1</i> | p.Val1578del | 1947         | 3.0    | 1941                   | 1.2    |
| ESCC_3  | EP    | <i>PTEN</i>   | p.Pro246Leu  | 1994         | 7.1    | 1990                   | 1.9    |
| ESCC_36 | EP    | <i>TP53</i>   | p.Pro72Ala   | 711          | 3.7    | 278                    | 2.9    |
| ESCC_4  | EP    | <i>TP53</i>   | p.Arg342Ter  | 1999         | 2.7    | 1999                   | 1.5    |
| ESCC_4  | EP    | <i>EGFR</i>   | p.Gly721Ser  | 1999         | 4.2    | 2000                   | 1.0    |
| ESCC_40 | EP    | <i>TP53</i>   | p.Arg110Leu  | 1994         | 21.3   | 1993                   | 1.3    |
| ESCC_1  | LPM   | <i>NOTCH1</i> | p.Val1578del | 1931         | 1.6    | 1137                   | 0.1    |
| ESCC_18 | LPM   | <i>TP53</i>   | p.Pro72Ala   | 1999         | 2.9    | 228                    | 3.1    |
| ESCC_21 | LPM   | <i>NOTCH1</i> | p.Val1578del | 1958         | 2.7    | 1040                   | 1.2    |
| ESCC_21 | LPM   | <i>TP53</i>   | p.Glu198Ter  | 1995         | 53.7   | 1410                   | 0.1    |
| ESCC_24 | LPM   | <i>TP53</i>   | p.Arg110Cys  | 740          | 2.2    | 1999                   | 1.3    |
| ESCC_32 | LPM   | <i>TP53</i>   | p.Pro278Ser  | 2000         | 72.8   | 2000                   | 1.6    |
| ESCC_34 | LPM   | <i>TP53</i>   | p.Pro72Ala   | 1999         | 3.0    | 1999                   | 3.7    |
| ESCC_38 | LPM   | <i>TP53</i>   | p.Pro72Ala   | 1997         | 4.3    | 1841                   | 3.9    |
| ESCC_5  | LPM   | <i>RB1</i>    | p.Arg556Gln  | 1999         | 3.7    | 94                     | 1.1    |
| ESCC_20 | MM    | <i>SMAD4</i>  | p.Glu337Gly  | 502          | 3.2    | 354                    | 1.1    |
| ESCC_41 | MM    | <i>TP53</i>   | p.Arg306Ter  | 2000         | 38.9   | 1356                   | 1.2    |
| ESCC_42 | MM    | <i>TP53</i>   | p.Pro72Ala   | 1999         | 4.5    | 1991                   | 5.9    |
| ESCC_52 | MM    | <i>TP53</i>   | p.Pro72Ala   | 2000         | 4.6    | 2000                   | 4.3    |
| ESCC_19 | SM1   | <i>TP53</i>   | p.Arg213Ter  | 1963         | 2.9    | 1978                   | 1.0    |
| ESCC_19 | SM1   | <i>CDKN2A</i> | p.Arg80Ter   | 1997         | 2.4    | 1995                   | 1.3    |

Abbreviations: VF: variant frequency; EP: invasion limited to the epithelium; LPM: invasion limited to lamina propria mucosae; MM: tumor invading into the muscularis mucosa; SM1: invasion limited to the upper third of the submucosa.

**Supplementary Table 2: Gene alterations between IEN and superficial ESCC**

|                       | IEN             | EP/LPM/MM        | <i>P</i> -value |   |
|-----------------------|-----------------|------------------|-----------------|---|
|                       | ( <i>n</i> = 7) | ( <i>n</i> = 47) |                 |   |
| Mutation              |                 |                  |                 |   |
| <i>TP53</i>           | 6               | 39               | 0.60            | a |
| <i>CDKN2A</i>         | 2               | 9                | 0.98            | a |
| <i>TP53/CDKN2A</i>    | 6               | 41               | 0.81            | a |
| CNV                   |                 |                  |                 |   |
| Average number of CNV | 2.571           | 3.822            | 0.47            | b |

Abbreviations: a, Yates's correction for continuity; b, *t*-test.

**Supplementary Table 3: Association between *CYP2A6* polymorphisms and *TP53* mutation**

|                                       | Mutation detected | Mutation undetected | <i>P</i> -value |
|---------------------------------------|-------------------|---------------------|-----------------|
|                                       | ( <i>n</i> = 47)  | ( <i>n</i> = 7)     |                 |
| Brinkman Index (BI), median ± Q       | 38 (81%)          | 3 (43%)             | 0.049*          |
| <i>CYP2A6</i> , GG/GA+AA/undetermined | 31/9/7            | 5/2/0               | 0.726           |

\*Fisher's exact test

In *CYP2A6* SNP analysis, TaqMan SNP assay (SNP ID: rs1137115, ThermoFisher) was performed.

|                | Early lesions<br>This study (n=54)<br>Mutation (%) | Advanced ESCCs<br>Database (n=227)<br>Mutation (%) |    |
|----------------|--|--|----|
| <i>TP53</i>    | 87.0   | 68.7   | ** |
| <i>CDKN2A</i>  | 20.4   | 3.5  | ** |
| <i>APC</i>     | 5.6  | 1.8  |    |
| <i>PTEN</i>    | 3.7  | 3.5  |    |
| <i>KDR</i>     | 3.7  | 2.2  |    |
| <i>STK11</i>   | 3.7  | 0.9  |    |
| <i>KIT</i>     | 3.7  | 0.9  |    |
| <i>CSF1R</i>   | 3.7  | 0.9  |    |
| <i>VHL</i>     | 3.7  | 0  | *  |
| <i>RB1</i>     | 1.9  | 7.9  |    |
| <i>PIK3CA</i>  | 1.9  | 6.6  |    |
| <i>SMARCB1</i> | 1.9  | 0.9  |    |
| <i>BRAF</i>    | 1.9  | 0.9  |    |
| <i>FLT3</i>    | 1.9  | 0.4  |    |
| <i>EGFR</i>    | 1.9  | 0.4  |    |
| <i>CTNNB1</i>  | 1.9  | 0.4  |    |
| <i>PDGFRA</i>  | 1.9  | 0  |    |
| <i>HRAS</i>    | 1.9  | 0  |    |
| <i>FGFR3</i>   | 1.9  | 0  |    |
| <i>NOTCH1</i>  | 0  | 8.4  |    |
| <i>FBXW7</i>   | 0  | 3.5  |    |
| <i>ERBB4</i>   | 0  | 2.6  |    |
| <i>SMO</i>     | 0  | 1.8  |    |
| <i>PTPN11</i>  | 0  | 1.3  |    |
| <i>ATM</i>     | 0  | 1.3  |    |
| <i>ALK</i>     | 0  | 1.3  |    |
| <i>MLH1</i>    | 0  | 0.9  |    |
| <i>JAK2</i>    | 0  | 0.9  |    |
| <i>FGFR2</i>   | 0  | 0.9  |    |
| <i>CDH1</i>    | 0  | 0.9  |    |
| <i>NPM1</i>    | 0  | 0.4  |    |
| <i>MET</i>     | 0  | 0.4  |    |
| <i>HNF1A</i>   | 0  | 0.4  |    |
| <i>FGFR1</i>   | 0  | 0.4  |    |
| <i>EZH2</i>    | 0  | 0.4  |    |
| <i>ABL1</i>    | 0  | 0.4  |    |
| <i>SRC</i>     | 0  | 0  |    |
| <i>SMAD4</i>   | 0  | 0  |    |
| <i>RET</i>     | 0  | 0  |    |
| <i>NRAS</i>    | 0  | 0  |    |
| <i>MPL</i>     | 0  | 0  |    |
| <i>KRAS</i>    | 0  | 0  |    |
| <i>JAK3</i>    | 0  | 0  |    |
| <i>IDH2</i>    | 0  | 0  |    |
| <i>IDH1</i>    | 0  | 0  |    |
| <i>GNAS</i>    | 0  | 0  |    |
| <i>GNAQ</i>    | 0  | 0  |    |
| <i>GNA11</i>   | 0  | 0  |    |
| <i>ERBB2</i>   | 0  | 0  |    |
| <i>AKT1</i>    | 0  | 0  |    |

\* Fisher's exact test

**Supplementary Figure 1: The somatic aberration status between our early esophageal neoplastic lesions and advanced ESCCs (internet-based data) was compared.** The somatic aberration data of 227 advanced ESCCs in 50 cancer-related genes analyzed in our study was retrieved from the online database (cBioPortal for Cancer Genomics [<http://cbioportal.org>]; mutation data of the Cancer Genome Atlas [TCGA] and International Cancer Genome Consortium [ICGC] were available) [13]. The only evident difference between these two groups was in the expression of three genes: *CDKN2A*, *TP53*, and *VHL*.