This study revealed a potential role of HER2 VUS identified by clinical next-generation sequencing in a cancer patient.

In this study, we performed the functional analyses of HER2 variants of uncertain significance (VUS) identified by the clinical next-generation sequencing (NGS) from a cancer patient. We found that HER2 G776S mutation affects oncogenic malignant potential as well as drug sensitivity in colon cancer cells. These analytical methods may be exploited to develop selective personalized cancer medicines for patients.

**Introduction and Aim**

Clinical NGS identifies some VUS as well as various actionable mutations in tumors. The aim of this study was to clarify the potential roles of unknown gene mutations identified using clinical NGS.

**Method**

(A) Construction of plasmid pCDNA3.1 vectors including cDNA encoding HER2 wild type or mutant G776S produced by site-directed mutagenesis. (B) Transfection of these plasmids into colon cancer cells (COLO 320: neither EGFR nor HER2 are highly expressed or mutated). Antibiotic selection and fluorescence activated cell sorting (FACS) were used to select cells stably expressing HER2.

**Results**

Figure 3. HER2 mutant G776S colon cancer cells showed increased phosphorylation of HER2, as well as anchorage-independent growth. (A) HER2 protein expression and phosphorylation in HER2-expressing cells (B) Soft agar colony formation assay.

Figure 4. The effect of EGFR-TKI (Gefitinib) and HER2-TKI (Afatinib, Lapatinib) in HER2-expressing cells. (A) Afatinib highly suppressed phosphorylation of HER2 in HER2 mutant G776S-expressing cells, while Gefitinib did not. (B) Both Lapatinib and Afatinib strongly inhibited the anchorage-independent cell growth in HER2 mutant G776S-expressing cells, while Gefitinib did not.

**Discussion**

HER2 G776S mutation increased malignant potential in colorectal cancer cells. HER2 TKIs are potent and selective against HER2 mutant G776S-expressing cells.