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Precision Medicine Clinic: Molecular Tumor Board

Therapeutic Potential of Afatinib for Cancers with *ERBB2* (*HER2*) Transmembrane Domain Mutations G660D and V659E

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Abstract

We previously reported on a family with hereditary lung cancer, in which a germline mutation in the transmembrane domain (G660D) of avian erythroblastic leukemia viral oncogene homolog 2 (erb-b2 receptor tyrosine kinase 2) (*ERBB2*; human epidermal growth factor receptor 2 [HER2]) seemed to be responsible for the cancer predisposition. Although few data are available on treatment, anti-ERBB2 therapeutic agents may be effective for *ERBB2*-mutant cancers. The familial lung cancer patient in one of the authors' institutes developed bone metastasis with enlarging lung tumors and was treated with the ERBB2 inhibitor afatinib. We also encountered a patient with ampullary adenocarcinoma with *ERBB2* G660D and S310F comutations in another institute of the authors', revealed by comprehensive genomic profiling. This patient was then treated with afatinib and also achieved transitory response. We also searched for *ERBB2* transmembrane mutations in various types of cancers in PubMed, The Cancer Genome Atlas (TCGA), and the Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) database. Besides our two cases, two patients with V659E mutations were found via PubMed. Three potential patients were found in TCGA. In addition, MSK-IMPACT allowed identification of three additional urothelial carcinomas with G660D mutations and two lung adenocarcinomas with V659E mutations. Our experience suggests that establishing a database of integrated information regarding the clinical genome and therapeutic outcome of patients with recurrent but less common mutations is essential to implement precision oncology. ***The Oncologist*** 2018;23:150–154

Key Points

- Rare but targetable mutations such as avian erythroblastic leukemia viral oncogene homolog 2 (erb-b2 receptor tyrosine kinase 2) (*ERBB2*; human epidermal growth factor receptor 2 [*HER2*]) transmembrane domain (TMD) mutations can be detected by comprehensive genomic profiling.
- Afatinib may be effective for patients with cancer with *ERBB2* (*HER2*) TMD mutations.
- In order to implement precision oncology, it is important to establish a database of integrated information regarding the clinical genomes and therapeutic outcomes of patients with recurrent but less common mutations.

Patient Stories

Patient 1

Patient 1, the familial lung cancer patient in Okayama University Hospital, was a 56-year-old woman with the avian erythroblastic leukemia viral oncogene homolog 2 (erb-b2 receptor tyrosine kinase 2) (*ERBB2*; human epidermal growth factor receptor 2 [*HER2*]) G660D mutation described in our previous report [1]. She developed bone metastasis with enlarging multiple lung tumors. She was a light smoker with a 1.2-pack-year smoking history. She received carboplatin and pemetrexed but developed progressive disease. We examined the effect of afatinib on *ERBB2* G660D-transfected cells, leading to the inhibition of phosphorylation of ERBB2 G660D protein [2]. Based on our *in vitro* findings, we administered afatinib (40 mg per day) to Patient 1 using Health Insurance Claims Review and Reimbursement Service Board. Informed consent was obtained from the patient. Although a grade 3 adverse effect of an acne-like rash appeared, the lesions in lung have shown a partial response (Fig. 1), and those in the bone have remained stable for the past 16 months with a reduced afatinib dose (20 mg per day). Clinical responses

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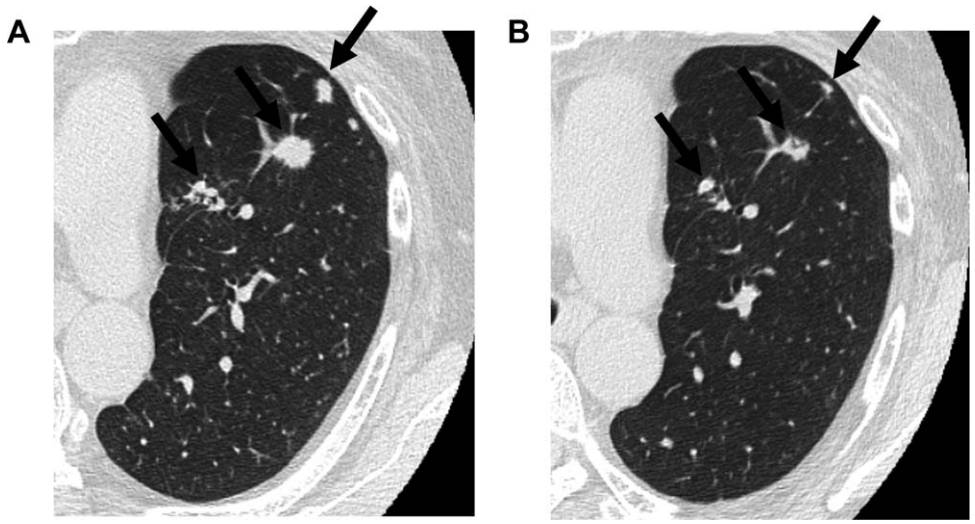

Figure 1. Effect of afatinib in Patient 1 with an *ERBB2* G660D mutation. Computed tomography scan of the chest before **(A)** and after **(B)** treatment with afatinib. Shrinkage of multiple lung nodules (arrows) was observed.

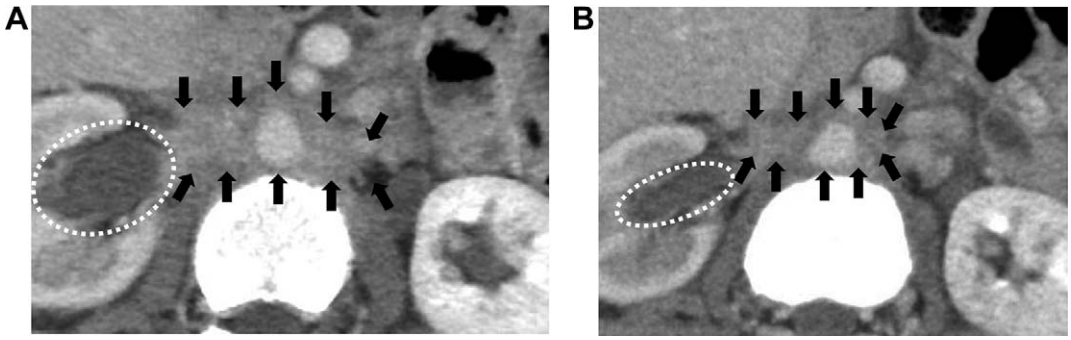

Figure 2. Effect of afatinib in Patient 2 with *ERBB2* G660D and S310F comutations. Computed tomography scan of the abdomen before **(A)** and after **(B)** treatment with afatinib. Shrinkage of the metastatic region of the para-aorta (region circled with arrows) and improvement of hydronephrosis (region circled with dots) were observed.

were based on Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 criteria.

Patient 2

In Kyoto University Hospital, a 52-year-old woman, Patient 2, developed a postoperative metastatic lung nodule originating from adenocarcinoma of the ampulla of Vater. She had no smoking history. She had undergone curative resection followed by adjuvant chemotherapy. She developed recurrent disease in the lung 15 months after surgery and received gemcitabine and cisplatin because of progressive disease. Finally, she became resistant to the standard regimen for biliary tract cancer, and comprehensive genomic profiling, as described below, was performed on the resected primary tumor. Because we identified the same mutation, *ERBB2* G660D, in Patient 2, as described below, we treated her with afatinib after the approval of the Ethics Committee. The treatment regimens were approved by the Institutional Review Board, and informed consent was obtained from the patient. The results of afatinib therapy are described in the Patient Update section, and the imaging findings are shown in Figure 2.

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Comprehensive genomic profiling using the OncoPrime panel test, which sequences the entire coding region of 215 genes and examines the rearrangement of 17 frequently rearranged genes with clinical and preclinical relevance in human cancers [3], is performed for cancer patients in both Okayama University Hospital and Kyoto University Hospital, and the results are discussed at each institute. Patients are presented by the physician, and the board members, including doctors and bioinformaticians, discuss the results of the OncoPrime panel test and suggest any applicable drugs.

Genotyping Results and Interpretation of the Molecular Results

The OncoPrime [3] panel test was performed on the resected primary tumor in Patient 2. Detailed method was shown in the previous report [3]. OncoPrime identified *ERBB2* G660D and S310F mutations in Patient 2. As the allele frequency result from OncoPrime for *ERBB2*-S310F was 0.568, there was a possibility of germline mutation for S310F. We performed Sanger sequencing for both codons G660 and S310 using tumor and peripheral blood specimens. The ratio of the heights of mutant and wild type waves in the sequencing electropherogram for S310F in the tumor specimen was almost 1:1, whereas the variant allele was not detected in the specimen of peripheral blood for either G660 or S310, confirming that both G660D and S310F were somatic mutations, which suggested the presence of focal amplification in the locus of S310F. OncoPrime also

Table 1. Characteristics of patients with tumors harboring mutations in *ERBB2* residues G660 and V659

No.	Sex	Age	Primary site	Stage	*ERBB2* mutation	Location of mutation	Histology	Histological features	Source
1	F	53	Lung	IB	G660D	TMD	Adenocarcinoma	Mixed invasive mucinous and nonmucinous	Yamamoto et al. [1]
2	F	77	Colon	III	G660D	TMD	Adenocarcinoma	NOS, well to moderately differentiated	cBioPortal
3	M	67	Bladder	IV	G660D	TMD	Urothelial carcinoma	High grade papillary, NOS	MSK-IMPACT
4	F	52	Ampulla of Vater	IIB	G660D + S310F	TMD + ECD	Adenocarcinoma	Pancreatobiliary type, well differentiated	Kyoto University
5	M	65	Bladder	0a	G660D + S310F	TMD + ECD	Urothelial carcinoma	High grade papillary, NOS	MSK-IMPACT
6	M	74	Bladder	I	G660D + S310F	TMD + ECD	Urothelial carcinoma	High grade papillary with micropapillary features	MSK-IMPACT
7	F	64	Cervix	IB	G660R	TMD	Adenocarcinoma	Invasive, endocervical (usual) type	cBioPortal
8	F	38	Lung	IV	V659E	TMD	Adenocarcinoma	N.A.	Serra et al. [9]
9	M	56	Lung	IA	V659E	TMD	Adenocarcinoma	Lepidic predominant	Yamamoto et al. [1]
10	M	46	Lung	IV	V659E	TMD	Adenocarcinoma	Micropapillary predominant	MSK-IMPACT
11	F	70	Lung	IV	V659E	TMD	Adenocarcinoma	Acinar, with mucinous and clear cell features	MSK-IMPACT
12	F	62	Lung	IA	V659E	TMD	Adenocarcinoma	N.A.	Wang et al. [10]
13	F	72	Stomach	IIIB	V659D + T862A	TMD + KD	Adenocarcinoma	Tubular (intestinal) type	cBioPortal

Abbreviations: ECD, extracellular domain; F, female; KD, kinase domain; M, male; MSK-IMPACT, Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets; No, number; NOS, not otherwise specified; N.A., not available; TMD, transmembrane domain.

reported a *CTNNB1* T41A variant in this patient. At a joint conference of the members from both institutions, we shared the information that afatinib had been effective for Patient 1. In addition, a previous report strongly suggested that afatinib might be effective for disease with *ERBB2* S310F mutations [4]. As described in the Patient Stories section, we treated Patient 2 with afatinib after the approval of the Ethics Committee.

Functional and Clinical Significance of *ERBB2* Transmembrane Domain Mutations

Genomic and functional analyses suggested that the germline mutation at the transmembrane domain (TMD) of *ERBB2* (G660D) in Patient 1 was responsible for familial lung cancer [1, 2]. We also found one patient with sporadic lung adenocarcinoma with an *ERBB2* V659E mutation [1]. Furthermore, afatinib inhibited the phosphorylation of ERBB2 G660D or V659E protein in *ERBB2* G660D- or V659E-transfected cells [2]. TMD mutations V659E and G660D are located within the glycine zipper motif The^{652}-X_3-Ser^{656}-X_3-Gly^{660}, a tandem variant of the GG4-like motif, at the N-terminal portion of the transmembrane domain, and it is reported that this motif is critically related to the dimerization of ERBB2 [5, 6].

We searched for *ERBB2* TMD mutations in various type of cancers in PubMed and The Cancer Genome Atlas (TCGA) via cBioPortal [7]. We also performed a search for the Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) clinical sequencing cohort [8]. Rare but recurrent mutations of the *ERBB2* TMD occurred at residues G660 and V659, as summarized in Table 1. Besides our patient in the previous report [1], only two patients with V659E mutation were found via the PubMed database [9, 10]. Out of 147 studies and datasets, three potential patients were found in TCGA. In addition, five patients with *ERBB2* TMD mutations were identified in the MSK-IMPACT database. We have no detailed data on afatinib treatment for the *ERBB2* TMD-mutant cases shown in Table 1.

A schematic figure of *ERBB2* with the locations of other missense or in-frame mutations in lung cancer via cBioPortal is shown in Figure 3A. *ERBB2* mutations exist in the extracellular domain (ECD), TMD, and tail domain, in addition to the kinase domain (KD). Although little is known of the significance of the *ERBB2* mutations in the ECD and tail domain, there is a possibility of conformational changes via these mutations like those in the KD and TMD, resulting in the increased activity of ERBB2. The MEK/ERK signaling pathway is stimulated by dimerization of ERBB2 [11]. *ERBB2* amplification may also increase kinase activity. However, conformational changes do not occur via amplification itself, and increased kinase activity is dependent on the extent of the amplification. In non-small cell lung cancer, *ERBB2* mutation and amplification are mutually exclusive [12].

Potential Strategies to Target the Pathway and Implications for Clinical Practice

Recurrent but less common mutations, such as *ERBB2* TMD mutations, have potential for targeting therapy. However, it may be difficult to develop a treatment strategy, as the functional data on rare mutations are limited. In the current study, it was useful to share information interinstitutionally regarding the clinical genomes and therapeutic outcomes of patients

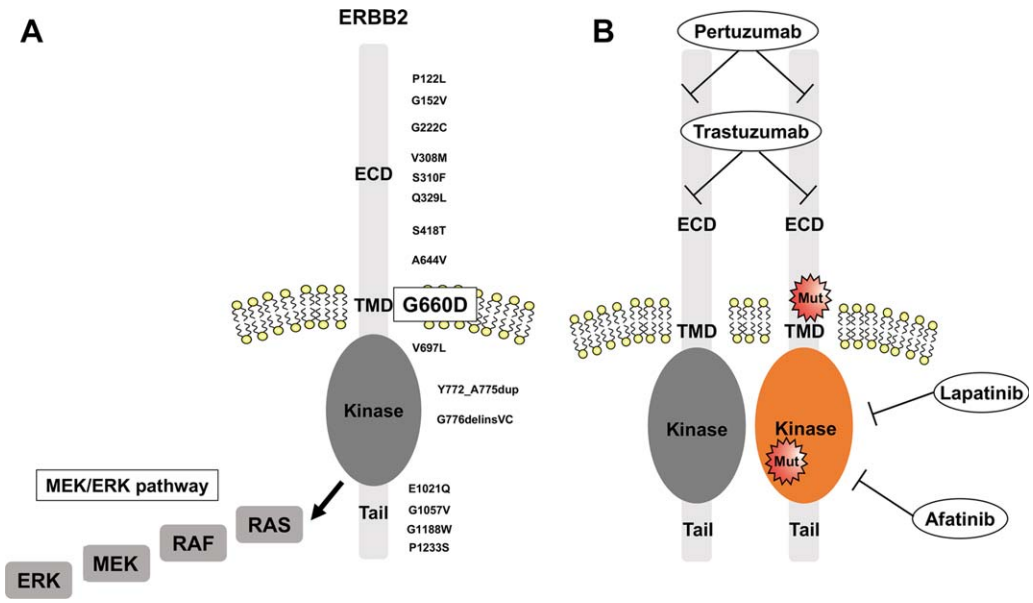

Figure 3. Schema of ERBB2. **(A)**: The G660D mutation is located in the *ERBB2* transmembrane domain. Other missense or in-frame mutations are also shown. The MEK/ERK signaling pathway is stimulated by dimerization of ERBB2. **(B)**: Mechanisms of monoclonal antibodies, such as trastuzumab, and tyrosine kinase inhibitors (TKIs), such as afatinib, for ERBB2. Trastuzumab and other monoclonal antibodies bind to the ECD and prevent dimerization of ERBB2, whereas afatinib and other TKIs bind to the kinase domain (KD) and inhibit ERBB2. The KD is activated in *ERBB2* KD- or TMD-mutant tumors regardless of ECD status.

Abbreviations: ECD, extracellular domain; ERBB2, avian erythroblastic leukemia viral oncogene homolog 2 (erb-b2 receptor tyrosine kinase 2); ERK, extracellular signal-regulated kinases; MEK, MAPK-ERK kinase; Mut, mutation; RAF, rapidly accelerated fibrosarcoma proto-oncogene, serine/threonine kinase; RAS, rat sarcoma viral oncogene homolog; TMD, transmembrane domain.

with these rare mutations in order to perform the appropriate targeting therapy.

The development of comprehensive genomic profiling has opened the door for precision medicine. Our findings demonstrate that *ERBB2* G660D is an actionable mutation for afatinib therapy. Although clinical response to lapatinib has been reported in a patient with *ERBB2* V659E-mutant lung cancer [9], our data suggest that responses to ERBB2-targeted agents are not uniform across all patients with these *ERBB2* mutations. Regarding Patient 1, the candidate alterations were restricted to 29 variants by comparing the results of whole-exome sequencing, and the *ERBB2* TMD mutation was picked up using five types of standardized deleteriousness scores (SIFT [13], PolyPhen-2 [14], LRT [15], MutationTaster [16], and phyloP [17]). According to these scores, *SRP54*, *MIPOL1*, *SLC26A1*, *IDUA*, and *SYTL5* were considered possible causative genes other than *ERBB2*, although the impact of these variants seems to be limited. As for Patient 2, the *CTNNB1* T41A variant, which was mainly reported in soft tissue tumors, was identified in addition to *ERBB2*. *CTNNB1* is responsible for Wnt signaling, suggesting that another pathway was also activated in this case. Identified mutations other than *ERBB2* may affect the variable responsiveness to afatinib. Additionally, skin toxicity was observed in Patient 1 and not in Patient 2. As there was an association between the therapeutic effect of afatinib and skin toxicity in *EGFR*-mutant lung cancer [18], we consider that there may also be a relationship between them in *ERBB2* TMD-mutant tumors.

As for ERBB2-targeting therapy, possible options are small-molecule inhibitors and monoclonal antibodies (Fig. 3B). There are several ERBB2-targeting tyrosine kinase inhibitors (TKIs), such as lapatinib, neratinib, pyrotinib, and poziotinib, in addition to afatinib. Those TKIs have a potential to inhibit *ERBB2* KD-mutant tumors, as they directly bind to the KD of ERBB2. *ERBB2* TMD-mutant tumors also respond to those TKIs, as TMD mutations favor a kinase-active conformation [5, 6, 19]. On the other hand, trastuzumab and other monoclonal antibodies target the ECD, preventing the dimerization of ERBB2. Because the KD is constitutively activated in *ERBB2* KD-mutant tumors [20] even if dimerization of ERBB2 is inhibited by trastuzumab or other monoclonal antibodies, their antiproliferative effect may be limited. In *ERBB2* TMD-mutant tumors, the effect of trastuzumab and other antibodies may also be limited, as dimerization of ERBB2 is predicted to be stable even if trastuzumab or other antibodies bind to the ECD [19]. Therefore, ERBB2-targeting TKIs such as afatinib have a therapeutic advantage over trastuzumab and other monoclonal antibodies.

Patient Update

For Patient 2, there was a tendency of slight size reduction of the metastatic para-aortic lymph node at 2.4 months after afatinib introduction that resulted in obvious clinical improvement of stenotic hydronephrosis (Fig. 2). Although the effect of afatinib was observed, the evaluation of the lesions was a stable disease, according to RECIST version 1.1 criteria. Patient 2 finally experienced progression of disease 4 months later. Skin rash was not observed.

Conclusion

We experienced two cases of patients with carcinoma with *ERBB2* TMD mutation who showed clinical improvement with afatinib. The treatment strategy for Patient 2 was based on our experience of afatinib treatment for Patient 1, which was accomplished by sharing information of such rare mutations interinstitutionally. Our experience of sharing treatment outcomes highlights the importance of establishing an integrated

database of genomic and clinical information, including therapeutic outcomes, to implement precision oncology medicine.

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Disclosures

Shinichi Toyooka: Boehringer-Ingelheim (RF); **Katsuyuki Kiura**: Chugai Pharmaceutical, Pfizer, Novaltis Pharma, Taiho Pharmaceutical, Eli Lilly, Ono Pharmaceutical (RF); AstraZeneca, Boehringer-Ingelheim, Nippon Kayaku, Daiichi Sankyo, Shionogi, Taiho Pharmaceutical, Ono Pharmaceutical (H); **Marc Ladanyi**: Boehringer-Ingelheim, AstraZeneca (C/A). The other authors indicated no financial relationships.

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