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Exploration of germline variants responsible for adverse events of crizotinib in *anaplastic lymphoma kinase*-positive non-small cell lung cancer by target-gene panel sequencing

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ABSTRACT

Objectives: Crizotinib is a standard treatment for advanced *anaplastic lymphoma kinase* (*ALK*)- or *ROS1*-fusion-gene-positive non-small cell lung cancer; however, serious adverse events (AEs), including elevated alanine aminotransferase (ALT)/aspartate aminotransferase (AST) and interstitial lung disease (ILD), develop occasionally. Here, we evaluated relationships between clinically significant crizotinib-associated AEs and germline variations.

Materials and Methods: DNA obtained from 75 patients allowed selection of 147 genes according to function, exon identification and sequencing, and determination of germline single nucleotide variants (SNVs). Correlations between clinically significant AEs and presence of germline variants were estimated by Fisher's exact test.

Abbreviations: AE, adverse event; ALK, anaplastic lymphoma kinase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BWA, Burrows–Wheeler Aligner; CI, confidence interval; CNR, copy number ratio; CNV, copy number variation; EPHX1, epoxide hydrolase 1; FCGR2A, Fc fragment of IgG receptor IIa; ILD, interstitial lung disease; indel, insertion/deletion; NGS, next-generation sequencing; NSCLC, non-small cell lung cancer; PCR, polymerase chain reaction; OR, odds ratio; PGx, pharmacogenomics; PK, pharmacokinetics; SAM, Sequence Alignment/Map format; SNP, single nucleotide polymorphism; SNV, single nucleotide variant; TCF7L2, transcription factor 7-like 2

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Results: We defined clinically significant AEs as grade 4 hematological toxicity, grade ≥3 non-hematological toxicity, and any grade of ILD. These AEs were observed in 26 patients (35%), with elevated AST/ALT (15%) the most common, followed by neutropenia (5%), ILD (4%), and thromboembolic events (4%). Nonsynonymous SNVs in *epoxide hydrolase 1* (*EPHX1*) [odds ratio (OR): 3.86; p = 0.0009) and transcription factor 7-like 2 (*TCF7L2*) (OR: 2.51; p = 0.025) were associated with the presence of clinically significant AEs.
Conclusion: Nonsynonymous *EPHX1* and *TCF7L2* SNVs might be associated with clinically significant crizotinib-associated AEs. These data indicated that target-gene sequencing could be feasible for predicting anticancer-agent toxicity, and that germline multi-gene information might be useful for predicting patient-specific AEs to promote precision medicine.

1. Introduction

Activating rearrangements of the *anaplastic lymphoma kinase* (*ALK*) gene have been found in several cancers, including non-small cell lung cancer (NSCLC). *ALK*-fusion-gene-positive (*ALK*-positive) cases are observed in ~5% of NSCLC patients [1], with *ROS1* proto-oncogene receptor tyrosine kinase also activated by chromosomal rearrangement in NSCLC [2]. Crizotinib has shown favorable clinical activity in the treatment of *ALK*- and *ROS1*-positive NSCLC patients [3–6]. Frequently occurring treatment-related adverse events (AEs) following crizotinib treatment include vision abnormalities, diarrhea, nausea, vomiting, constipation, elevated transaminase levels, edema, upper respiratory infection, dysgeusia, and dizziness. Grade ≥3 AEs, including neutropenia, elevated transaminase levels, fatigue, interstitial lung disease (ILD), pneumonia, and electrocardiogram QTc prolongation, are also reported to occasionally occur [4,4,5,6].

To date, there have been no studies evaluating risk factors, including patient background, ethnic differences, and genetic profiling, for AEs associated with crizotinib treatment. To investigate genetic risks for crizotinib-associated AEs, we conducted an open-label, multicenter, observational study to evaluate relationships between clinically significant crizotinib-associated AEs and germline variations, as well trough concentrations of crizotinib at steady state.

2. Materials and methods

2.1. Study population

Key inclusion criteria were as follows: advanced NSCLC patients harboring the *ALK*-fusion gene, those planned to receive 250 mg of crizotinib twice daily or who were currently under crizotinib treatment, aged ≥20 years, and adequate organ function (serum total bilirubin ≤ 2.0 mg/dL, aspartate aminotransferase (AST) ≤ 150 IU/L, alanine aminotransferase (ALT) ≤ 150 IU/L, serum creatinine ≤ 2.0 mg/dL, and SpO_2 ≥ 90%). Exclusion criteria were as follows: concomitant treatment with other anticancer agents, radiotherapy, or surgery; ILD; inability to swallow tablets; gastrointestinal disorders, such as watery diarrhea, intestinal paresis, ileus, or a history of gastrectomy or intestinal resection, that could affect crizotinib ingestion or absorption; intake of drugs or food that could act as potent cytochrome P450 3A4 or P-glycoprotein inhibitors or inducers; and women of child-bearing age, unless using effective contraception.

2.2. Study design and outcome

The study was performed under an open-label, multicenter, observational design to evaluate the pharmacokinetics (PK)/pharmacodynamics/pharmacogenomics (PGx) of crizotinib in Japanese patients with *ALK*-positive NSCLC. The primary objective was to evaluate relationships between clinically significant crizotinib-associated AEs within 8 weeks of starting crizotinib and germline single nucleotide variants (SNVs) in these patients using next-generation sequencing (NGS). Secondary objectives were to investigate the relationship between PK parameters and pharmacodynamics, including toxicities. This study (UMIN000010768) was designed and carried out at the National Cancer Center (NCC) Hospital (Tokyo, Japan) and participating institutions. The protocol was approved by the institutional review board of the NCC Hospital, and the study was performed in accordance with the ethical principles stated in the Declaration of Helsinki. All patients provided written informed consent.

2.3. Treatment and assessments

Patients received the standard crizotinib dose of 250 mg twice daily and continued to receive therapy until disease progression, clinical deterioration, or intolerable AEs that did not improve with dose adjustment. The *ALK*-fusion gene was confirmed by immunohistochemistry staining, fluorescence *in situ* hybridization, or reverse transcription polymerase chain reaction (PCR) in each institution. Blood samples for PK analysis were collected after administration of crizotinib on days 15 (trough concentration) and at the point of clinically significant AEs. AEs were evaluated using the Common Terminology Criteria for Adverse Events version 4.0.

2.4. PGx analysis

2.4.1. Clinical samples and DNA extraction

High-molecular-weight DNA was extracted from blood samples of *ALK*-positive NSCLC patients using a QIAamp DNA blood mini kit (Qiagen, Venlo, Netherlands).

2.4.2. Target-capture probe design for PGx analysis

Genes (n = 147) were selected for this study among those related to drug metabolism, drug transport, immunotherapy, and other pharmacologic functions of interest and used to create a capture library (NCC_PGx Panel v1) targeting the genes or regions (Supplementary Table 1). The capture probes were custom designed using SureDesign software (Agilent Technologies, Santa Clara, CA, USA). The NCC_PGx Panel v1 contained a total of 30,513 probes covering 96.4% (averaged) of the regions for the 108 SNVs, 186 promoter regions, and 147 coding sequences + untranslated regions, with the total region targeted comprising 994.164 kbp.

2.4.3. Target-capture library preparation and sequencing

One microgram of high-molecular-weight DNA was fragmented using a Covaris S2 ultrasonicator (Covaris, Inc., Woburn, MA, USA) to produce an average fragment size ranging from 150 bp to 200 bp. The sequencing libraries were prepared by DNA end-repair, A-tailing, and ligation of SureSelect adapter oligos using SureSelect XT reagents (Agilent Technologies) according to manufacturer instruction. Pre-capture PCR amplification of the adapter-ligated library was performed for five cycles on 1 μg DNA or 10 cycles for 200 ng DNA. Amplified DNA (~750 ng) was hybridized with the above capture library (NCC_PGx Panel v1) for 24 h for target enrichment. Post-capture amplification was performed for 13 or 14 cycles using Herculase II polymerase (Agilent Technologies) with SureSelect indexing post-capture PCR primers (Agilent Technologies). Purification was performed with Agencourt AMPure XP beads (Beckman Coulter, Brea, CA, USA). Quantification

and size distribution of the amplified libraries were determined using TapeStation (Agilent Technologies) and the KAPA library quantification kit (Kapa Biosystems, Wilmington, MA, USA).

Approximately 20 libraries per lane were mixed to 20 pM each and sequenced using the Illumina HiSeq 2000 platform in 100-bp paired-end mode (Illumina, San Diego, CA, USA). A sequencing run generated ~16 million to ~29 million successful reads on each lane of a flow cell, achieving an average of 91% coverage of the targeted regions to a depth of ≥500 × . We used the UCSC Genome Browser (human GRCh37/hg19; Feb. 2009 assembly; https://genome.ucsc.edu/) for sequence mapping.

2.4.4. Variant call procedures

Detailed methods used in this study to call SNVs/insertion or deletions (indels) and to correlate the SNV/indel/copy number variation (CNV) with clinically significant AEs are shown in Supplementary Figure 1.

2.4.5. SNV calling

Paired-end reads were aligned to the human reference genome (GRCh37) by the Burrows–Wheeler Aligner (BWA) [7]. The following reads were removed using an in-house program: 1) low mapping-quality reads, 2) PCR-duplication reads, 3) those with a low alignment score [the "AS" tag in Sequence Alignment/Map (SAM) format] and a mismatch rate (a percentage of the number of mismatches to read length) ≥ 5% of the reads, and 4) those with a high suboptimal-alignment score (the "XS" tag in SAM format) and a mismatch rate ≥ 5% of the reads.

The germline SNVs/indel call analysis pipeline was based on Genome Analysis Tool Kit (GATK; v3.5; https://software.broadinstitute.org/gatk/) best practices (Broad Institute, Cambridge, MA, USA). After running indel realignment and base quality score recalibration using IndelRealigner and BaseRecalibrator in GATK, samples were subjected to HaplotypeCaller runs in GVCF mode. The gVCF files for each sample were merged by GenotypeGVCF. The following calls were filtered by Variant Filtration: 1) SNV quality by depth (QD < 2.0), Phred-scaled p-value using Fisher's exact test to detect strand bias (FS > 60.0), mapping quality (MQ < 40.0), MappingQualityRankSumTest (MQRankSum < −12.5), ReadPosRankSumTest (ReadPosRankSum < −8.0), or StrandOddsRatio (SOR > 4.0); and 2) INDEL: QD < 2.0, FS > 200.0, ReadPosRankSum < −20.0, or SOR > 10.0. The following calls were filtered using an in-house program: calls in repeat regions and indel calls in homopolymers. These calls were annotated by ANNOVAR [8] and extracted as nonsynonymous /nonsense/splicing/frameshift calls.

2.4.6. CNV calling

We analyzed germline CNVs based on the targeted sequencing data using our in-house algorithm. The methods used in this study to call CNVs and determine their correlation with clinically significant AEs are shown in Supplementary Figure 2. First, we eliminated 500 of 2264 probes, resulting in sequences having > 90% identity at different positions according to NCBI BLASTN analysis (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE = BlastSearch) in order to avoid misalignment. The reads were then aligned to the human reference genome (GRCh37) using the BWA. The copy number ratio (CNR) for each gene was estimated by dividing the average sequence coverage by the average sequence coverage of the entire region. The CNR was then normalized by dividing the average CNRs of all samples. A normalized CNR < 0.5 was considered a deletion, whereas a normalized CNR > 1.5 was considered an amplification.

2.4.7. Verification of SNV and CNV candidates

We validated SNV and CNV calls using TaqMan single nucleotide polymorphism (SNP) genotyping assays and TaqMan copy number assays (Applied Biosystems, Waltham, MA, USA).

Table 1
Patient characteristics (n = 78).

		No. Patients
Sex	Male/Female	26 / 52
Age (y)	Median (range)	54 (26–83)
ECOG PS	0 / 1 / 2 / 3	35 / 33 / 5 / 5
Histological type	Adeno / Non-small / Squamous	73 / 4 / 1
Clinical stage	IIIA / IIIB / IV / recurrence	1 / 3 / 57 / 17
Height (cm)	Median (range)	159.4 (141.0–179.6)
Weight (kg)	Median (range)	54.1 (37.5–85.1)
BSA (m^2)	Median (range)	1.53 (1.25–2.01)
Smoking status	Never / Ex-smoker	50 / 28
Prior treatment	Surgery	18
	Definitive radiotherapy	8
	Palliative radiotherapy	26
	Cytotoxic chemotherapy	48
	Molecular targeted agent	7
Enrolment	Before / After starting crizotinib	47 / 31

BSA, body surface area; ECOG PS, Eastern Cooperative Oncology Group performance status.

2.5. Analysis of genetic variants correlating with clinically significant AEs in crizotinib-treated ALK-positive NSCLC patients

We used Fisher's exact test to construct a 2 × 2 contingency table displaying the number of reference alleles and variant alleles associated with toxicity and non-toxicity at each SNV/indel call. We tested deletion and amplification separately as follows: 1) the number of samples with deletions and others associated with toxicity and non-toxicity, and 2) the number of samples with amplifications and others associated with toxicity and non-toxicity. Calls were selected according to the p-value and odds ratio determined by Fisher's exact test.

3. Results

3.1. Patient characteristics

A total of 78 patients were enrolled between March 2013 and October 2014. Patient characteristics are listed in Table 1. Twenty six patients were male and 52 were female, with a median age of 54 years (range: 26–83 years). A majority of patients were diagnosed with adenocarcinoma (94.6%). The median body weight was 54.1 kg (range: 37.5–85.1 kg), and the median body surface area was 1.53 m^2 (range: 1.25–2.01 m^2). Forty-seven patients enrolled before starting crizotinib therapy, and 31 patients enrolled during crizotinib therapy under the following conditions: they continued crizotinib therapy at a dose of 250 mg twice daily and with no onset of clinically significant AEs. Three patients could not be evaluated due to patient withdrawal (n = 1) and early AEs (n = 2).

3.2. Clinically significant AEs and treatment delivery

We defined clinically significant AEs as follows: grade 4 hematological toxicity, grade ≥3 non-hematological toxicity, and any grade of ILD. Clinically significant AEs are listed in Table 2. Common events included elevated AST/ALT levels (37.5%), neutropenia (16.7%), ILD (12.5%), and thromboembolic events (12.5%), including pulmonary embolism (n = 2) and deep vein thrombosis (n = 1). There was one treatment-related death as a result of ILD. Thirty six (46%) patients needed dose reductions of crizotinib due to AEs. There was no significant relationship between patient characteristics and clinically significant AEs (data not shown).

3.3. Relationships between clinically significant AEs and crizotinib concentrations

Blood samples for PK analysis were collected after administration of

Table 2
Clinically significant adverse events (n = 75).

Events	Gr 1–2	Gr 3	Gr 4	Gr 5	Total (%)
Elevated AST/ALT	NA	9	2	0	11 (15)
Neutropenia	NA	NA	4	0	4 (5)
ILD	1	1*	0	1	3 (4)
Thromboembolic event	NA	3*	0	0	3 (4)
Esophagitis	NA	2	0	0	2 (3)
QTc prolongation	NA	2	0	0	2 (3)
Elevated CPK	NA	1	0	0	1 (1)
Interstitial nephritis	NA	1	0	0	1 (1)
Total	1	19	6	1	27 (36)

One patient had two AEs of grade 3 ILD and grade 3 deep vein thrombosis.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; CPK, creatine phosphokinase; Gr, grade; ILD, interstitial lung disease; NA, not available.

crizotinib on day 15, and we evaluated relationships between trough concentrations of crizotinib at steady state and clinically significant AEs. We found that steady state crizotinib trough concentrations did not differ significantly between patients with clinically significant AEs [429 ng/mL (95% confidence interval [CI]: 361–509 ng/mL)] and those without [378 ng/mL (95% CI: 313–456 ng/mL)] (Mann–Whitney *U* test; p = 0.365).

3.4. PGx analysis using NGS

We then evaluated 147 drug-related genes using the NCC_PGx Panel v1 by NGS. High-molecular-weight DNA extracted from blood samples of 75 *ALK*-positive NSCLC patients enabled detection of 3,585 SNVs/indels, of which 3,177 SNV/indels were registered in the dbSNP database (https://www.ncbi.nlm.nih.gov/SNP/). Functional SNVs included the following: 450 nonsynonymous SNVs in 134 genes, 364 synonymous SNVs in 126 genes, seven nonsense SNVs in seven genes, 23 splicing variants in 16 genes, 16 indels with a frameshift in five genes, and six indels without a frameshift in four genes (Table 3).

3.5. Significant genetic alteration profiles relating to clinically significant AEs

Fisher's exact test was performed to measure correlations between crizotinib-associated clinically significant AEs (Table 2) and genes harboring damaging SNPs and CNVs in the 75 patients. The presence of nonsynonymous SNVs in *epoxide hydrolase 1* (*EPHX1*) and *transcription factor 7-like 2* (*TCF7L2*) significantly correlated with the occurrence of any crizotinib-associated clinically significant AEs (Table 4).

Table 3
Germline SNVs/indels in the 75 *ALK*-positive NSCLC patients.

	Total (n)	SNV types	dbSNP144 (novel)	Gene with SNVs/indels
SNVs/indels	83,357	3585	3177 (408)	
Nonsynonymous	10,414	450	413 (37)	134
Synonymous	8,953	364	345 (19)	126
Nonsense	30	7	6 (1)	7
Splicing	295	23	20 (3)	16
Indel with frameshift	825	16	14 (2)	5
Indel without frameshift	115	6	6 (0)	4
5′-UTR	3,841	193	160 (33)	85
3′-UTR	13,381	622	548 (74)	119
Upstream	11,819	431	366 (65)	108
Downstream	1,040	44	33 (11)	24
Intergenic	2,360	83	70 (13)	27
Intronic	26,697	1192	1,070 (122)	147

ALK, anaplastic lymphoma kinase; dbSNP, database of short genetic variations; Indel, insertion or deletion; NSCLC, non-small cell lung cancer; SNV, single nucleotide variant; UTR, untranslated region.

Table 4
Significant genetic alteration profiles relating to all clinically significant AEs.

Gene	Residue change	p	OR	95% CI
EPHX1	H139R	0.0087	3.86	1.43–12.07
TCF7L2	H401Q	0.025	2.51	1.15–5.65

AE; adverse event; CI, confidence interval; EPHX1, epoxide hydrolase 1; OR, odds ratio; TCF7L2, transcription factor 7-like 2.

Table 5
Significant genetic alteration profiles associated with elevated AST/ALT levels.

Gene	Residue change	P	OR	95% CI
DPYD	I543V	0.0050	3.95	1.45–10.97
SLC19A1	H27R	0.017	3.20	1.20–8.31
FCGR2A	H167R	0.020	3.30	1.18–8.69
EPHX1	H139R	0.037	3.28	1.06–10.81
EDN1	K197N	0.039	2.70	1.02–6.95

AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; DPYD, dihydropyrimidine dehydrogenase; EDN1, endothelin 1; EPHX1, epoxide hydrolase 1; FCGR2A, Fc fragment of IgG receptor IIa; OR, odds ratio; SLC19A1, solute carrier family 19 member 1.

Additionally, nonsynonymous SNVs in *dihydropyrimidine dehydrogenase*, *solute carrier family 19 member 1*, *Fc fragment of IgG receptor IIa (FCGR2A)*, *EPHX1*, and *endothelin 1* were significantly detected in patients presenting with elevated AST/ALT levels (Table 5). We also detected CNVs; however, there was no significant relationship between the presence of CNVs in any target gene and the occurrence of clinically significant AEs (data not shown).

4. Discussion

Crizotinib is a standard treatment for advanced *ALK*- and *ROS1*-positive NSCLC [6,9]. Serious AEs, such as elevated AST/ALT levels and ILD, develop occasionally. Identification of a biomarker enabling prediction of crizotinib-associated AEs would be clinically efficacious for both patients and physicians. In recent years, several studies reported identification of oncogenic drivers through genetic profiling of lung cancer using a tumor-genotype panel [10,11]. However, there have been no reports evaluating relationships between AEs associated with the use of anticancer agents and genetic profiling using target-gene panel sequencing, especially for germline variants. Therefore, we evaluated relationships between crizotinib-associated clinically significant AEs and germline variations by NGS.

We selected 147 genes according to their physiological and pharmacological functions, finding that nonsynonymous SNVs in *EPHX1* and *TCF7L2* were associated with the presence of clinically significant AEs. This represents the first report of these nonsynonymous SNVs related to AEs associated with anticancer drugs. Previously, we suggested the possibility that ATP-binding cassette subfamily B member 1 SNVs might affect the PKs of erlotinib and crizotinib and thereby influence toxicity development [12,13]. However, these SNVs were not associated with the presence of all clinically significant AEs in the present study. *EPHX1* is a critical biotransformation enzyme that converts epoxides from degraded aromatic compounds to trans-dihydrodiols, which can be conjugated and excreted from the body [14]. Significant associations were reported between *EPHX1* SNVs and the risk of lung and other cancer types in various populations [15–17]. *TCF7L2* is a protein-coding gene, the product of which plays a key role in the Wnt-signaling pathway and has been implicated in blood-glucose homeostasis. Diseases associated with *TCF7L2* mutations include type 2 diabetes mellitus, colorectal cancer, and many other cancer types [18,19]. We were unable to identify direct correlations between crizotinib treatment and nonsynonymous *EPHX1* and *TCF7L2* SNVs in the literature.

Liver dysfunction is a common AE in ~15% of patients using crizotinib according to phase III clinical trials [4]. A previous report described crizotinib-induced fatal fulminant acute liver failure due to liver-cell necrosis in a Caucasian patient [20]. *EPHX1* expression in mammals is generally highest in the liver, followed by the adrenal gland, lung, kidney, and intestine [21]. Therefore, this suggests a possible connection between nonsynonymous EPHX1 SNVs and liver dysfunction. Previous studies reported dose-independent liver dysfunction associated with immune-related mechanisms and possibly similar to lapatinib-induced hepatotoxicity associated with specific human leukocyte antigen polymorphisms within major histocompatibility complex proteins [22,23]. In the present study, we observed statistically significant associations between the presence of nonsynonymous SNVs in *FCGR2A* and elevated AST/ALT levels in patients. Antibody dependent cellular cytotoxicity mediated by the Fc fragment of the IgG receptor on immune cells, such as macrophages and natural killer cells, plays an important role in the antitumor effect of IgG1 antibodies [24]. Additionally, esophagitis was reported as a crizotinib-associated AE in a patient with *ALK*-positive NSCLC [25–28]. Pathologic findings from esophageal biopsies demonstrated active esophagitis with surface ulcerations, fibrin purulent exudate, and granulated-tissue formation [27]. It is possible that these findings resulted from immunological responses against crizotinib related to the nonsynonymous SNVs identified in the present study.

We recruited 10 patients exhibiting poor performance status (PS; PS2–3; 12.8%) for this study, among which four displayed clinically significant AEs (40%): two esophagitis, one elevated AST/ALT, and one QTc prolongation. We noted that the frequency of occurrence of these clinically significant AEs was equivalent to the total frequency (36%); therefore, we do not recognize these patients as a source of bias in this study.

Our study has several limitations. The AEs might be associated with many patient factors, including their general condition, organ function, and concomitant medications, and we could not explain all clinically significant AEs associated with the nonsynonymous SNVs. This analysis was performed using data from AEs identified in single crizotinib-treated cohort from the Japanese population. We believe that our data offers an overview of germline multi-gene information related to AEs associated with anticancer agents; however, these nonsynonymous SNVs should be validated in other datasets. To validate these nonsynonymous SNVs, we conducted a multicenter study on the PKs and PGxs of first-line afatinib in patients aged ≥75 years and presenting with advanced NSCLC involving epidermal growth factor receptor mutation (UMIN000017050). It would also be interesting and valuable to compare the results of similar studies of Japanese and Western populations on the basis of these genotypes potentially associated with influencing immunological responsiveness. Furthermore, functional evaluation of these genotypes using mouse models should be performed in future work.

This study showed that target-gene sequencing represents a feasible method for predicting the toxicity of anticancer agents and a cost-effective technique with significant statistical power for analyzing small-to-medium-sized cohorts as compared with whole-exome or genome sequencing. We were unable to predict AEs associated with anticancer drugs using only pharmacological approaches; therefore, exhaustive genetic analyses of germline alterations in multiple genes, including those associated with the immune response, using NGS represented a complementary and valuable technique. Germline multi-gene information might be useful for predicting AEs in each patient in order to develop safe treatment strategies as precision medicine in the clinic.

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Conflicts of interest

The authors declare no conflict of interest.

Availability of data and material

NCBI Gene ID: 2052. (https://wwwncbinlmnihgov/gene?Db=gene&Cmd=ShowDetailView&TermToSearch=2052).

UCSC Genome Browser on Human Feb. 2009 (human GRCh37/hg19; Feb. 2009 assembly; https://genome.ucsc.edu/)

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Appendix A. Supplementary data

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