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Association of *STAT3*, *CYP3A5,* and *ABCG2* Polymorphisms with Osimertinib-Induced Adverse Events in NSCLC Patients

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Running head: *STAT3*, *CYP3A5*, and *ABCG2* polymorphisms and adverse events of

osimertinib.

Abstract.

Background/Aim: Osimertinib is a key drug for treating *epidermal growth factor receptor* (*EGFR*) mutation-positive non-small cell lung cancer (NSCLC). Genetic differences may be associated with adverse events (AEs) induced by osimertinib. This retrospective observational multicenter study evaluated the association of genotypes, including *STAT3* -1697C>G, *CYP3A5* 6986A>G, and *ABCG2* 421C>A, with the incidence of osimertinib-induced AEs in patients with *EGFR* mutation-positive NSCLC. Materials and Methods: 85 patients treated with osimertinib (Institution A: 33, Institution B: 52) were enrolled in the study. Single nucleotide polymorphisms were determined by real-time PCR, and the incidence of AEs were compared for each genotype. Results: The paronychia incidences were 59% for the CC genotype, 19% for the CG genotype, and 19% for the GG genotype at *STAT3* -1697C>G. A genotype-related trend was observed (Cochran-Armitage test, p=0.009). Multivariate analysis showed that the CC genotype at *STAT3* -1697C>G and female were significant independent factors associated with paronychia (odds ratio (OR)=6.41, 95% confidence interval (CI)=1.94–21.20 and OR=3.40, 95%CI=1.03–11.22, respectively). The incidence of diarrhea was 53% for the CC genotype, 30% for the AC genotype, and 29% for the AA genotype at *ABCG2* 421C>A, and a genotype-related trend was observed (p=0.048). However, the CC genotype at *ABCG2* 421C>A was not a significant independent factor associated with diarrhea in the multivariate analysis. No significant associations were detected between other polymorphisms and the incidence of AEs. Conclusion: *STAT3* -

1697C>G may be a novel risk factor for osimertinib-induced paronychia in patients with NSCLC.

Osimertinib is a third-generation epidermal growth factor receptor-tyrosine kinase inhibitor (EGFR-TKI) that inhibits both active *EGFR* mutations and the resistant *EGFR* T790M mutation. Osimertinib is a first-line therapy for *EGFR* mutant-positive locally advanced or metastatic non-small cell lung cancer (NSCLC) (exon 19 deletion or L858R mutation); significant prolongation of progression-free survival (PFS) compared to first-generation EGFR-TKI has been demonstrated (1). For T790M-resistant mutant-positive NSCLC, osimertinib is used after disease progression following the use of first- or second-generation EGFR-TKIs; significant prolongation of PFS compared to platinum combination therapy has been demonstrated (2). Many adverse events (AEs), including diarrhea, acne-like skin rash, increased aspartate aminotransferase (AST)/ alanine transaminase (ALT) levels, and interstitial lung disease (ILD), were reported for osimertinib and first-generation EGFR-TKIs (1). The mechanisms of these AEs may be related to EGFR inhibition outside of tumor cells, but some aspects are not fully understood. Furthermore, the incidence of AEs is higher in the Japanese population (3,4), indicating that the frequency of TKI-induced AEs is race-related.

Osimertinib is metabolized by cytochrome P450 (CYP) 3A4/5 to the active metabolites AZ5104 and AZ7550, and total of them is equivalent to approximately 10% of osimertinib exposure (5, 6). Osimertinib and the two active metabolites are substrates of breast cancer resistance protein (BCRP; gene: *ABCG2*) (5). Inter-individual variability in the activities of these metabolizing enzymes and the transporter can affect osimertinib

pharmacokinetics. In addition, in a population pharmacokinetic study of osimertinib and AZ5104, several osimertinib-induced AEs, including rash, diarrhea, and QT prolongation, were found to be associated with the area under the plasma concentration curve (7). Thus, inter-individual variabilities in their activities may affect osimertinib pharmacokinetics and the incidence of osimertinib-induced AEs.

Signal transducer and activator of transcription 3 (STAT3) is involved in metastasis, migration, and various cellular responses to cytokines (8). Enhanced STAT3 activation is frequently observed in histological sections and cell lines of NSCLC. In addition, STAT3 inhibition in NSCLC cell lines significantly reduces survival. Thus, STAT3 is a therapeutic target in various solid tumors, including lung cancer (9). STAT3 may be a compensatory signaling pathway for EGFR inhibition and is associated with EGFR inhibitor sensitivity (10). On one hand, genetic polymorphisms in *STAT3* are associated with AEs, such as hand-foot skin reaction and stomatitis induced by TKI (11, 12), interstitial pneumonia induced by mTOR inhibitors (13), and overall toxicity induced by platinum-based chemotherapy (14). Thus, *STAT3* polymorphisms are potential predictors of the frequency of molecular targeted drug- and cytotoxic anticancer drug-related AEs.

Genetic polymorphisms in *STAT3*, *CYP3A5*, and *ABCG2* have been reported for a variety of drug pharmacokinetic and pharmacodynamic changes. However, the associations between osimertinib-induced AEs and these genetic polymorphisms have not been reported. The objective of this study was to evaluate the association of single nucleotide

polymorphisms (SNPs) in *STAT3*, *CYP3A5*, and *ABCG2* with the incidence of osimertinib-induced AEs in patients with *EGFR* mutation-positive advanced NSCLC.

Materials and Methods

Study design. This retrospective observational study was conducted at two Japanese hospitals (Kobe University Hospital and Nara Prefecture General Medical Center). The representative center, Kobe University Hospital, collected the anonymized case data and the frozen whole blood samples from each center.

Patients. Patients with *EGFR* mutation-positive advanced (Stage IV or recurrence) NSCLC who were treated with osimertinib between September 2018 and October 2021 and who have given consent to participate in the study were enrolled in the study. The eligibility criteria included available blood samples for DNA extraction and available follow-up data concerning treatment and prognosis. The exclusion criterion was intolerance to osimertinib with serious complications. Patient characteristics and laboratory data were collected at the initiation of osimertinib therapy. Patients baseline characteristics are provided in Table I.

Ethics. This study was approved by each institutional ethics committee (No. B200391 at the representative research institution) and complied with the Declaration of Helsinki and its later amendments or comparable ethical standards. All patients provided written informed consent.

Blood sampling and SNP genotyping assay. All frozen blood samples were sent to the analysis site. DNA was isolated from peripheral blood mononuclear cells using a NucleoSpin® Blood kit (MACHEREY-NAGEL, GmbH, Düren, Germany) according to the manufacturer's protocol. The *STAT3* -1697C>G (rs4796793), *CYP3A5* 6986A>G (rs776746), and *ABCG2* 421C>A (rs2231142) genotypes were determined using a TaqMan SNP Genotyping Assay (Thermo Fisher Scientific, Waltham, MA, USA) on a StepOnePlus Real-Time PCR System (Thermo Fisher Scientific). The manufacturer's recommended protocol was as follows: 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min.

Evaluation of clinical outcomes. Clinical data were collected from medical records by the research coordinator at each center. Osimertinib-induced AEs were defined as ILD, rash acneiform, paronychia, diarrhea, mucositis oral, increased AST/ ALT, increased creatinine, decreased neutrophil counts, and decreased platelet counts. AEs were graded according to the Common Terminology Criteria for Adverse Events version 5.0 (15). Severe AEs were defined as grade 3 or higher. AE incidences were determined from the initiation of osimertinib therapy to the end of therapy for each patient. If treatment was continued after March 2022, the follow-up was terminated on February 2022. If patients developed AEs at an ambulatory practice, the day of AE occurrence was defined as the date of the ambulatory visit. Responses were assessed by the treating physician based on the Response Evaluation Criteria in Solid Tumors version 1.1 (16). PFS, time to treatment failure (TTF), and osimertinib discontinuation attributable to disease progression or AE were evaluated as

efficacy outcomes for osimertinib therapy. PFS was defined as the time from the initiation of treatment to disease progression or death. TTF was defined as the period from the initiation of treatment to discontinuation of treatment for any reason. If osimertinib therapy was continued after March 2022, the follow up of PFS and TTF were also terminated in February 2022.

Statistical analysis. Categorical variables were compared using Fischer's exact tests. Continuous variables were compared using Mann–Whitney U tests. Categorical variables between ordinal groups were compared using Cochran–Armitage trend tests. The predictive significance of SNPs for AE incidence was evaluated by multivariate logistic regression analysis using patient characteristics with *P*-values less than 0.1 in the univariate analyses due to the lack of prior reports on factors associated with AEs. The cumulative incidence rate of AEs, PFS, and TTF was estimated using the Kaplan–Meier method, and significant differences were determined using the log-rank test. $p<0.05$ (two-tailed) was considered statistically significant. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan) (17), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). More precisely, it is a modified version of R commander designed to add statistical functions frequently used in biostatistics.

Results

Patient characteristics and incidence of AEs. 85 patients (Institution A: 33, Institution B: 52) were enrolled in the study. No patients met the exclusion criteria. Patient characteristics at the initiation of osimertinib therapy are shown in Table I. The *STAT3* -1697C>G*, CYP3A5* 6986A>G, and *ABCG2* 421C>A polymorphisms for all samples were successfully analyzed. The genotype frequencies did not deviate from the Japanese population according to the NCBI database (18). The median patient follow-up duration was 358 days (range=11–1,254 days); the follow-up in 34 patients was completed before the termination of osimertinib therapy.

Associations of AE incidence with each genetic polymorphism. The associations of AE incidences with each polymorphism genotype are shown in Table II. The incidence of paronychia was 59% (10/17) for the CC genotype, 19% (8/42) for the CG genotype, and 19% (5/26) for the GG genotype at *STAT3* -1697C>G, and there was a pharmacogenetic trend (Cochran-Armitage test, p=0.009). The incidence of osimertinib-induced paronychia was significantly higher in patients with the CC genotype compared with the incidence in patients with the G allele carrier at *STAT3* -1697C>G (Fisher's exact test, OR=5.88, 95% CI=1.67–22.19, p=0.002). The incidence of diarrhea was 53% (20/38) for the CC genotype, 30% (12/40) for the CA genotype, and 29% (2/7) for the AA genotype at *ABCG2* 421C>A, and there was a pharmacogenetic trend (Cochran-Armitage test, p=0.048). The incidence

of diarrhea was significantly higher in patients with the CC genotype compared with the incidence in patients with the A allele carrier at *ABCG2* 421C>A (Fisher's exact test, OR=2.59, 95% CI=0.98–7.04, *p*=0.045). Other AEs (including AST/ALT increase, creatinine increase, neutrophil count decrease, and platelet count decrease; not shown in Table II) were not significantly associated with any genotypes.

Patient characteristics at the initiation of osimertinib treatment according to AEs are shown in Tables III and IV. The incidence of paronychia was significantly higher in female patients compared with the incidence in male patients (*p*=0.047), and the incidence of diarrhea tended to be higher in patients with liver metastases compared with the incidence in patients without liver metastases (*p*=0.081). The results of the multiple logistic regression analyses are shown in Table V. The CC genotype at *STAT3* -1697C>G and female were significantly associated with paronychia (OR for *STAT3* genotype=6.41, 95% CI=1.94–21.20, *p*=0.002; OR for female=3.40, 95% CI=1.03–11.22, *p*=0.044). No genotypes or patient characteristics were significantly associated with diarrhea in the multiple logistic regression analysis.

Association of STAT3 genotypes with the cumulative incidence of paronychia, progression-free survival, and time-to-treatment failure. Time-to-event analyses according to the genotypes of *STAT3* -1697C>G are shown in Figure I. The cumulative incidence of paronychia was significantly earlier in patients with the CC genotype compared with the incidence in patients with the GG or CG genotypes at *STAT3* -1697C>G (*p*=0.003, Figure

IA). No significant differences in PFS and TTF were detected between patients with the CC genotype and patients with the GG or CG genotypes at *STAT3* -1697C>G (p=0.158 and p=0.783, respectively, Figures IB and IC). The proportion of dose reduction and interruption of osimertinib did not differ between the genotype groups (p=0.582, Figure IC). Other genotypes were not significantly associated with PFS and TTF.

Discussion

We evaluated the association of SNPs in *STAT3*, *CYP3A5*, and *ABCG2* genes with the incidence of osimertinib-induced AEs in patients with *EGFR* mutation-positive advanced NSCLC. Our results demonstrate that the CC genotype at *STAT3* -1697C>G is a pharmacogenetic risk factor for osimertinib-induced paronychia.

Significant differences in the incidence of AEs were not detected between our study patients and the Japanese subset of phase III trials comparing osimertinib with first-generation EGFR-TKIs (3) or the Japanese patients in the study to evaluate real-world osimertinib use (19). Although the exact mechanisms of EGFR-TKI-induced paronychia are unclear, periungual inflammation and abnormal differentiation of keratinocytes around the periungual tissue may trigger paronychia (20). In general, gender differences in nail brittleness have been reported (21). Nail brittleness is affected by nail dehydration, and dehydration is related to the lipid content of the nail matrix. With aging, especially in women,

cholesterol in the nail sheath tends to decrease, and women and the elderly are more likely to have weaker keratinocyte bridges between cells, so women are affected twice as frequently as men (21). Paronychia may also be affected by dehydration, consistent with the results of our multivariate analysis. Normally, the suprabasal layer shows increased expression of phosphorylated EGFR, the cyclin-dependent-kinase inhibitor p27 (p27KIP1), keratin 1 (KRT1), and STAT3, which are terminal differentiation markers (20, 22, 23). EGFR inhibition in basal keratinocytes leads to growth arrest and premature differentiation, as demonstrated by a shift in the localization of increased p27KIP1, KRT1, and STAT3 to the basal layer (20). EGFR inhibition also induces the release of inflammatory cytokines and chemokines. Inflammation induces apoptosis of keratinocytes, leading to a decrease in epidermal thickness or abnormal differentiation in the periungual lesion (20). STAT3 mediates cytokine stimulation of cellular responses leading to inflammation (24). Therefore, differences in STAT3 expression in keratinocytes can cause a variable response to EGFR inhibition-induced inflammation. Although ambiguity in the *STAT3* -1697C>G phenotype for expression level has been reported (13, 25), this SNP is located close to the promoter region and may affect STAT3 expression (26). Therefore, the higher incidence of paronychia in patients with the CC genotype in *STAT3* -1697C>G may be due to enhanced inflammatory responses to cytokines and chemokines resulting from changes in STAT3 expression. Furthermore, our previous study revealed a significant association between the GG genotype of *STAT3* -1697C>G (CC genotype in forward orientation of *STAT3* rs4796793)

and the incidence of hand-foot skin reaction (11). Interestingly, paronychia induced by osimertinib and hand-foot skin reaction induced by multiple TKIs showed inverse trends for the same SNP. Further experiments to elucidate the molecular phenotype of *STAT3* -1697C>G are necessary to prove these hypotheses.

Diarrhea is one of the most grueling AEs, leading to dehydration and electrolyte balance disruption, which reduce adherence to therapy. CC genotype at *ABCG2* 421C>A showed a trend of association with diarrhea in the multivariate analysis. The A allele at *ABCG2* 421C>A is associated with decreased BCRP function and a pharmacokinetic change in uric acid and rosuvastatin (27, 28). In a population pharmacokinetic study of osimertinib and its active metabolite (AZ5104), a linear relationship was demonstrated between their plasma exposure and safety endpoints, including rash, diarrhea, and QT prolongation (7). However, no associations of the *ABCG2* 421C>A genotype with plasma trough concentration or area under the curve of osimertinib were reported (29). Although osimertinib pharmacokinetics were not evaluated in our study, the association of diarrhea incidence with the genotype may not be based on differences in systemic exposure to osimertinib, because the AA genotype at *ABCG2* 421C>A is expected to reduce systemic exposure to the substrate. BCRP plays a role in the excretion of substrates to the intestinal and bile tract (30). The pharmacokinetic study of afatinib, a second-generation EGFR-TKI, showed that increased bile excretion may not be associated with diarrhea (31), therefore the same possibility might be applied to the osimertinib case. On the other hand, various

pharmacodynamic mechanisms have been proposed for EGFR-TKI-induced diarrhea, including mucosal atrophy associated with EGFR inhibition in intestinal epithelium, altered gut motility, colonic crypt damage, changes in the intestinal microflora, altered colonic transport, and the activation of apical membrane chloride channels in the intestinal epithelium (32, 33). Pharmacokinetic risk factors for osimertinib-induced diarrhea have not been identified, and our results may be confounded by multiple factors. Future studies are needed to determine the mechanism of EGFR-TKI-induced diarrhea.

ILD was not significantly associated with *STAT3* -1697C>G, *CYP3A5* 6986A>G, or *ABCG2* 421C>A. Although a previous report showed that the GG *STAT3* -1697C>G genotype is associated with the incidence of ILD for mTOR inhibitors (13), our results are not consistent with that report. Drug-related pneumonia has a variety of pathological patterns and causative drugs (34). Organizing pneumonia (OP), simple pulmonary eosinophilia (PEo), hypersensitivity pneumonia (HP), and diffuse alveolar damage (DAD) radiographic patterns have been reported for osimertinib-induced pneumonia at approximately the same frequency (35). The most frequently reported radiographic pattern of mTOR inhibitor-induced pneumonia is organizing pneumonia (36, 37). In addition, mTOR inhibitors are associated with asymptomatic pneumonitis requiring no specific therapies or drug interruptions (38, 39). The radiographic and pathological patterns of drug-related pneumonia induced by osimertinib may be different from pneumonia induced by mTOR inhibitors.

The therapeutic outcomes of osimertinib, including PFS and TTF, were not

significantly associated with any polymorphisms examined in this study. Several clinical trials to evaluate the safety and efficacy of compounds that target STAT3 have been conducted. Among the strategies directly targeting STAT3, the small molecule inhibitors OPB-31121 and OPB-111077 have dose-limiting toxicities and only modest antitumor activity in Phase I studies (40, 41). Another small molecule STAT3 inhibitor BBI608 showed no overall survival benefit in a Phase III study in patients with advanced colorectal cancer (42). AZD9150, an antisense oligonucleotide inhibiting STAT3, and synthetic oligonucleotides that selectively target the expression of mRNAs were well tolerated and efficacious in a phase Ib study in heavily pretreated lymphoma patients (43) and the currently ongoing Phase Ib/II trial in combination with checkpoint immunotherapies (NCT03421353). However, no clinical trials combining an EGFR-TKI with a direct inhibitor of STAT3 have been conducted in patients with lung cancer. The JAK1/2 and TBK1 inhibitor momelotinib, upstream of STAT3 signaling, in combination with erlotinib did not enhance clinical benefits over the historical data for erlotinib monotherapy in patients with EGFR-mutated NSCLC (44). In addition, in a population pharmacokinetic study of osimertinib, no significant relationship was found between exposure and efficacy (7). Therefore, our results and previous reports indicate that STAT3 was associated only with specific AEs, but not with the antitumor effects related to EGFR-TKI. Furthermore, it has been reported that overexpression of the anterior gradient-2 (AGR2), which is involved in oxidative protein folding in the endoplasmic reticulum, is associated with resistance to EGFR-TKI in NSCLC cell lines (45), and multiple genetic

polymorphisms may be potentially involved in treatment response in patients with NSCLC, using whole-exome sequencing (46). Thus, the efficacy of osimertinib may also be affected by multiple mediators or polymorphisms.

This study has several limitations. First, this was an observational pilot study including a limited number of patients, and the sample size was preferentially considered according to the number of recruitable patients. Therefore, the statistical power to detect the association of each genotype with other AEs in this study was inadequate. On the other hand, the statistical power of the post-hoc analysis for paronychia was 83.6%, which was considered adequate. In the future, sample size estimation focused on specific AEs and large exploratory clinical trials are required to confirm our hypothesis. Second, the association between STAT3 protein expression and the plasma concentration of osimertinib and its active metabolites were not examined. Third, we could not follow up the medical treatment and prognosis for all patients until the end of therapy, because we terminated the study before some patients had completed osimertinib therapy.

STAT3 -1697C>G polymorphism may be a significant risk factor for osimertinib-induced paronychia in Japanese patients with *EGFR* mutation-positive advanced NSCLC. In the future, we believe that information about genetic polymorphisms can help establish a mechanism-based prophylaxis for AEs.

Conflicts of Interest

Motoko Tachihara, M.D. received a research grant and lecture fees from AstraZeneca K.K. Other authors have no potential conflicts of interest to disclose.

Authors' Contributions

Conceptualization: M.T. and K.Y.; Data curation: M.T., T.H., M.Y, M.S., T.K., T.O., and N.Y; Conceptualization: M.T., and K.Y.; Formal Analysis: M.T., K.Y. and H.N.; Writing – original draft: M.T.; Funding acquisition: K.Y.; Project administration: K.Y.; Resources: H.N.; Supervision: M.T., T.I., S.I., T.O., and I.Y.; Writing – review & editing: M.T., K.Y., M.T., T.I., S.I., T.O., and I.Y.; All Authors have read and agreed to the published version of the manuscript.

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References

1. Soria JC, Ohe Y, Vansteenkiste J, Reungwetwattana T, Chewaskulyong B, Lee KH, Dechaphunkul A, Imamura F, Nogami N, Kurata T, Okamoto I, Zhou C, Cho BC, Cheng Y, Cho EK, Voon PJ, Planchard D, Su WC, Gray JE, Lee SM, Hodge R, Marotti M, Rukazenkov Y and Ramalingam SS; FLAURA Investigators: Osimertinib in untreated EGFR-mutated advanced non-small-cell lung cancer. N Engl J Med *378(2)*: 113-25, 2018. PMID: 29151359. DOI: 10.1056/NEJMoa1713137
2. Mok TS, Wu Y-L, Ahn M-J, Garassino MC, Kim HR, Ramalingam SS, Shepherd FA, He Y, Akamatsu H, Theelen WS, Lee CK, Sebastian M, Templeton A, Mann H, Marotti M, Ghiorghiu S and Papadimitrakopoulou VA; AURA3 Investigators: Osimertinib or platinum-pemetrexed in EGFR T790M-positive lung cancer. N Engl J Med *376(7)*: 629-40, 2017. PMID: 27959700. DOI: 10.1056/NEJMoa1612674
3. Ohe Y, Imamura F, Nogami N, Okamoto I, Kurata T, Kato T, Sugawara S, Ramalingam SS, Uchida H, Hodge R, Vowler SL, Walding A and Nakagawa K: Osimertinib versus standard-of-care EGFR-TKI as first-line treatment for EGFRm advanced NSCLC: FLAURA Japanese subset. Jpn J Clin Oncol *49(1)*: 29-36, 2019. PMID: 30508196. DOI: 10.1093/jjco/hyy179
4. Suh CH, Park HS, Kim KW, Pyo J, Hatabu H and Nishino M: Pneumonitis in advanced non-small-cell lung cancer patients treated with EGFR tyrosine kinase inhibitor: Meta-analysis of 153 cohorts with 15,713 patients: Meta-analysis of incidence and risk factors

of EGFR-TKI pneumonitis in NSCLC. Lung Cancer *123*: 60-69, 2018. PMID: 30089596. DOI: 10.1016/j.lungcan.2018.06.032

5. FDA: Tagrisso (osimertinib) prescribing information. Available at: http://www.accessdata.fda.gov/drugsatfda_docs/label/2017/208065s006lbl.pdf [Last accessed on July 14, 2022]
6. Dickinson PA, Cantarini MV, Collier J, Frewer P, Martin S, Pickup K and Ballard P: Metabolic disposition of osimertinib in rats, dogs, and humans: Insights into a drug designed to bind covalently to a cysteine residue of epidermal growth factor receptor. Drug Metab Dispos *44(8)*: 1201-12, 2016. PMID: 27226351. DOI: 10.1124/dmd.115.069203
7. Brown K, Comisar C, Witjes H, Maringwa J, de Greef R, Vishwanathan K, Cantarini M and Cox E: Population pharmacokinetics and exposure-response of osimertinib in patients with non-small cell lung cancer. Br J Clin Pharmacol *83(6)*: 1216-1226, 2017. PMID: 28009438. DOI: 10.1111/bcp.13223
8. Jin W: Role of JAK/STAT3 Signaling in the regulation of metastasis, the transition of cancer stem cells, and chemoresistance of cancer by epithelial–mesenchymal transition. Cells *9(1)*: 217, 2020. PMID: 31952344. DOI: 10.3390/cells9010217
9. Mohrherr J, Uras IZ, Moll HP and Casanova E: STAT3: Versatile functions in non-small cell lung cancer. Cancers (Basel) *12(5)*: 1107, 2020. PMID: 32365499. DOI: 10.3390/cancers12051107

10. Zulkifli AA, Tan FH, Putoczki TL, Stylli SS and Luwor RB: STAT3 signaling mediates tumor resistance to EGFR targeted therapeutics. Mol Cell Endocrinol *451*: 15-23, 2017. PMID: 28088467. DOI: 10.1016/j.mce.2017.01.010

11. Yamamoto K, Shinomiya K, Ioroi T, Hirata S, Harada K, Suno M, Nishioka T, Kume M, Makimoto H, Nakagawa T, Hirano T, Bito T, Nishigori C, Miyake H, Fujisawa M and Hirai M: Association of single nucleotide polymorphisms in *STAT3* with hand-foot skin reactions in patients with metastatic renal cell carcinoma treated with multiple tyrosine kinase inhibitors: A retrospective analysis in Japanese patients. Target Oncol *11(1)*: 93-99, 2016. PMID: 26300443. DOI: 10.1007/s11523-015-0382-9

12. Watanabe A, Yamamoto K, Ioroi T, Hirata S, Harada K, Miyake H, Fujisawa M, Nakagawa T, Yano I and Hirai M: Association of Single Nucleotide Polymorphisms in *STAT3*, *ABCB1*, and *ABCG2* with Stomatitis in Patients with Metastatic Renal Cell Carcinoma Treated with Sunitinib: A Retrospective Analysis in Japanese Patients. Biol Pharm Bull *40(4)*: 458-464, 2017. PMID: 28381801. DOI: 10.1248/bpb.b16-00875

13. Yamamoto K, Ioroi T, Shinomiya K, Yoshida A, Harada K, Fujisawa M, Omura T, Ikemi Y, Nakagawa S, Yonezawa A, Ogawa O, Matsubara K, Iwamoto T, Nishikawa K, Hayashi S, Tohara D, Murakami Y, Motoshima T, Jono H and Yano I: *STAT3* Polymorphism Associates With mTOR Inhibitor-Induced Interstitial Lung Disease in Patients With Renal Cell Carcinoma. Oncol Res *29(1)*: 11–23, 2022. PMID: 35016744. DOI: 10.3727/096504022X16418911579334

14. Gong WJ, Ma LY, Hu L, Lv YN, Huang H, Xu JQ, Huang DD, Liu RJ, Han Y, Zhang Y, Shi SJ and Wu SL: *STAT3* rs4796793 contributes to lung cancer risk and clinical outcomes of platinum-based chemotherapy. Int J Clin Oncol *24(5)*: 476-484, 2019. PMID: 30689078. DOI: 10.1007/s10147-018-01386-7

15. Common terminology criteria for adverse events, version 5.0; 2017. Available at: https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf [Last accessed on December 17, 2022]

16. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, Rubinstein L, Shankar L, Dodd L, Kaplan R, Lacombe D and Verweij J: New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer *45(2)*: 228-47, 2009. PMID: 19097774. DOI: 10.1016/j.ejca.2008.10.026

17. Kanda Y: Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant *48(3)*: 452–458, 2013. PMID: 23208313. DOI: 10.1038/bmt.2012.244

18. NCBI dbSNP. Available at: http://www.ncbi.nlm.nih.gov/snp/ [Last accessed on September 5, 2022]

19. Sakata Y, Sakata S, Oya Y, Tamiya M, Suzuki H, Shibaki R, Okada A, Kobe H, Matsumoto H, Yokoi T, Sato Y, Uenami T, Saito G, Tsukita Y, Inaba M, Ikeda H, Arai D, Maruyama H, Hara S, Tsumura S, Morinaga J and Sakagami T: Osimertinib as first-line

treatment for advanced epidermal growth factor receptor mutation-positive non-small-cell lung cancer in a real-world setting (OSI-FACT). Eur J Cancer *159*: 144-153, 2021. PMID: 34749119. DOI: 10.1016/j.ejca.2021.09.041

20. Lacouture ME: Mechanisms of cutaneous toxicities to EGFR inhibitors. Nat Rev Cancer *6(10)*: 803-812, 2006. PMID: 16990857. DOI: 10.1038/nrc1970
21. Shemer A and Daniel CR 3rd: Common nail disorders. Clin Dermatol *31(5)*: 578-86, 2013. PMID: 24079587. DOI: 10.1016/j.clindermatol.2013.06.015
22. Kozuki T: Skin problems and EGFR-tyrosine kinase inhibitor. Jpn J Clin Oncol *46(4)*: 291–298, 2016. PMID: 26826719. DOI: 10.1093/jjco/hyv207
23. Yorulmaz A and Yalcin B: Panitumumab-induced paronychia: A Case Report and a Brief Review of the Literature. Skin Appendage Disord *7(2)*: 123-126, 2021. PMID: 33796558. DOI: 10.1159/000512036
24. Atsumi T, Singh R, Sabharwal L, Bando H, Meng J, Arima Y, Yamada M, Harada M, Jiang JJ, Kamimura D, Ogura H, Hirano T and Murakami M: Inflammation amplifier, a new paradigm in cancer biology. Cancer Res *74(1)*: 8-14, 2014. PMID: 24362915. DOI: 10.1158/0008-5472.CAN-13-2322
25. Schrama D, Ugurel S, Sucker A, Ritter C, Zapatka M, Schadendorf D and Becker JC: *STAT3* single nucleotide polymorphism rs4796793 SNP does not correlate with response to adjuvant IFNα therapy in stage III melanoma patients. Front Med (Lausanne) *1*: 47, 2014. PMID: 25593920. DOI: 10.3389/fmed.2014.00047

26. Ito N, Eto M, Nakamura E, Takahashi A, Tsukamoto T, Toma H, Nakazawa H, Hirao Y, Uemura H, Kagawa S, Kanayama H, Nose Y, Kinukawa N, Nakamura T, Jinnai N, Seki T, Takamatsu M, Masui Y, Naito S and Ogawa O: *STAT3* polymorphism predicts interferon-alfa response in patients with metastatic renal cell carcinoma. J Clin Oncol *25(19)*: 2785-2791, 2007. PMID: 17602083. DOI: 10.1200/JCO.2006.09.8897

27. Matsuo H, Takada T, Ichida K, Nakamura T, Nakayama A, Ikebuchi Y, Ito K, Kusanagi Y, Chiba T, Tadokoro S, Takada Y, Oikawa Y, Inoue H, Suzuki K, Okada R, Nishiyama J, Domoto H, Watanabe S, Fujita M, Morimoto Y, Naito M, Nishio K, Hishida A, Wakai K, Asai Y, Niwa K, Kamakura K, Nonoyama S, Sakurai Y, Hosoya T, Kanai Y, Suzuki H, Hamajima N and Shinomiya N: Common defects of ABCG2, a high-capacity urate exporter, cause gout: a function-based genetic analysis in a Japanese population. Sci Transl Med *1(5)*: 5ra11, 2009. PMID: 20368174. DOI: 10.1126/scitranslmed.3000237

28. Zhang W, Yu BN, He YJ, Fan L, Li Q, Liu ZQ, Wang A, Liu YL, Tan ZR, Fen-Jiang, Huang YF and Zhou HH: Role of BCRP 421C>A polymorphism on rosuvastatin pharmacokinetics in healthy Chinese males. Clin Chim Acta *373(1-2)*: 99-103, 2006. PMID: 16784736. DOI: 10.1016/j.cca.2006.05.010

29. Yokota H, Sato K, Sakamoto S, Okuda Y, Fukuda N, Asano M, Takeda M, Nakayama K and Miura M: Effects of CYP3A4/5 and ABC transporter polymorphisms on osimertinib plasma concentrations in Japanese patients with non-small cell lung cancer. Invest New Drugs *40(6)*: 1254-1262, 2022. PMID: 36149549. DOI: 10.1007/s10637-022-01304-9

30. Kawahara I, Nishikawa S, Yamamoto A, Kono Y and Fujita T: Assessment of contribution of BCRP to intestinal absorption of various drugs using portal-systemic blood concentration difference model in mice. Pharmacol Res Perspect *8(1)*: e00544, 2020. PMID: 31988753. DOI: 10.1002/prp2.544

31. Yokota H, Sato K, Sakamoto S, Okuda Y, Asano M, Takeda M, Nakayama K and Miura M: Relationship between Plasma Concentrations of Afatinib and the Onset of Diarrhea in Patients with Non-Small Cell Lung Cancer. Biology (Basel) *10(10)*: 1054, 2021. PMID: 34681153. DOI: 10.3390/biology10101054

32. Hirsh V, Blais N, Burkes R, Verma S and Croitoru K: Management of diarrhea induced by epidermal growth factor receptor tyrosine kinase inhibitors. Curr Oncol *21(6)*: 329-36, 2014. PMID: 25489260. DOI: 10.3747/co.21.2241

33. Khurana S, Nath SK, Levine SA, Bowser JM, Tse CM, Cohen ME and Donowitz M: Brush border phosphatidylinositol 3-kinase mediates epidermal growth factor stimulation of intestinal NaCl absorption and Na+/H+ exchange. J Biol Chem *271(17)*: 9919-27, 1996. PMID: 8626628. DOI: 10.1074/jbc.271.17.9919

34. Conte P, Ascierto PA, Patelli G, Danesi R, Vanzulli A, Sandomenico F, Tarsia P, Cattelan A, Comes A, De Laurentiis M, Falcone A, Regge D, Richeldi L and Siena S: Drug-induced interstitial lung disease during cancer therapies: expert opinion on diagnosis and treatment. ESMO Open *7(2)*: 100404, 2022. PMID: 35219244. DOI: 10.1016/j.esmoop.2022.100404

35. Sato Y, Sumikawa H, Shibaki R, Morimoto T, Sakata Y, Oya Y, Tamiya M, Suzuki H, Matsumoto H, Yokoi T, Hashimoto K, Kobe H, Hino A, Inaba M, Tsukita Y, Ikeda H, Arai D, Maruyama H, Hara S, Tsumura S, Sakata S and Fujimoto D: Drug-related pneumonitis induced by osimertinib as first-line treatment for epidermal growth factor receptor mutation-positive non-small cell lung cancer: A real-World setting. Chest *162(5)*: 1188-1198, 2022. PMID: 35661746. DOI: 10.1016/j.chest.2022.05.035

36. Fehrenbach U, Rodríguez-Laval V, Jann H, Fernández CMP, Pavel M and Denecke T: Everolimus-induced pneumonitis in neuroendocrine neoplasms: correlation of CT findings and clinical signs. Acta Radiol *62(8)*: 1006-1015, 2021. PMID: 32819165. DOI: 10.1177/0284185120950100

37. Johkoh T, Lee KS, Nishino M, Travis WD, Ryu JH, Lee HY, Ryerson CJ, Franquet T, Bankier AA, Brown KK, Goo JM, Kauczor HU, Lynch DA, Nicholson AG, Richeldi L, Schaefer-Prokop CM, Verschakelen J, Raoof S, Rubin GD, Powell C, Inoue Y and Hatabu H: Chest CT diagnosis and clinical management of drug-related pneumonitis in patients receiving molecular targeting agents and immune checkpoint inhibitors: a position paper from the Fleischner Society. Radiology *298(3)*: 550-566, 2021. PMID: 33434111. DOI: 10.1148/radiol.2021203427

38. Duran I, Siu LL, Oza AM, Chung TB, Sturgeon J, Townsley CA, Pond GR, Seymour L and Niroumand M: Characterisation of the lung toxicity of the cell cycle inhibitor temsirolimus. Eur J Cancer *42(12)*: 1875-1880, 2006. PMID: 16806903. DOI:

10.1016/j.ejca.2006.03.015

39. White DA, Camus P, Endo M, Escudier B, Calvo E, Akaza H, Uemura H, Kpamegan E, Kay A, Robson M, Ravaud A and Motzer RJ: Noninfectious pneumonitis after everolimus therapy for advanced renal cell carcinoma. Am J Respir Crit Care Med *182(3)*: 396-403, 2010. PMID: 20194812. DOI: 10.1164/rccm.200911-1720OC

40. Bendell JC, Hong DS, Burris HA 3rd, Naing A, Jones SF, Falchook G, Bricmont P, Elekes A, Rock EP and Kurzrock R: Phase 1, open-label, dose-escalation, and pharmacokinetic study of STAT3 inhibitor OPB-31121 in subjects with advanced solid tumors. Cancer Chemother Pharmacol *74(1)*: 125-30, 2014. PMID: 24819685. DOI: 10.1007/s00280-014-2480-2

41. Tolcher A, Flaherty K, Shapiro GI, Berlin J, Witzig T, Habermann T, Bullock A, Rock E, Elekes A, Lin C, Kostic D, Ohi N, Rasco D, Papadopoulos KP, Patnaik A, Smith L and Cote GM: A First-in-human phase I study of OPB-111077, a small-molecule STAT3 and oxidative phosphorylation inhibitor, in patients with advanced cancers. Oncologist *23(6)*: 658-e72, 2018. PMID: 29511132. DOI: 10.1634/theoncologist.2017-0325

42. Jonker DJ, Nott L, Yoshino T, Gill S, Shapiro J, Ohtsu A, Zalcberg J, Vickers MM, Wei AC, Gao Y, Tebbutt NC, Markman B, Price T, Esaki T, Koski S, Hitron M, Li W, Li Y, Magoski NM, Li CJ, Simes J, Tu D and O'Callaghan CJ: Napabucasin versus placebo in refractory advanced colorectal cancer: a randomized phase 3 trial. Lancet Gastroenterol Hepatol *3(4)*: 263-270, 2018. PMID: 29397354. DOI: 10.1016/S2468-

1253(18)30009-8

43. Reilley MJ, McCoon P, Cook C, Lyne P, Kurzrock R, Kim Y, Woessner R, Younes A, Nemunaitis J, Fowler N, Curran M, Liu Q, Zhou T, Schmidt J, Jo M, Lee SJ, Yamashita M, Hughes SG, Fayad L, Piha-Paul S, Nadella MVP, Xiao X, Hsu J, Revenko A, Monia BP, MacLeod AR and Hong DS: STAT3 antisense oligonucleotide AZD9150 in a subset of patients with heavily pretreated lymphoma: results of a phase 1b trial. J Immunother Cancer *6(1)*: 119, 2018. PMID: 30446007. DOI: 10.1186/s40425-018-0436-5
44. Padda SK, Reckamp KL, Koczywas M, Neal JW, Kawashima J, Kong S, Huang DB, Kowalski M and Wakelee HA: A phase 1b study of erlotinib and momelotinib for the treatment of EGFR-mutated, tyrosine kinase inhibitor-naive metastatic non-small cell lung cancer. Cancer Chemother Pharmacol *89(1)*: 105-115, 2022. PMID: 34773474. DOI: 10.1007/s00280-021-04369-0
45. Luu TT, Bach DH, Kim D, Hu R, Park HJ and Lee SK: Overexpression of AGR2 Is associated with drug resistance in mutant non-small cell lung cancers. Anticancer Res *40(4)*: 1855-1866, 2020. PMID: 32234873. DOI: 10.21873/anticanres.14139
46. Nastase A, Lupo A, Laszlo V, Damotte D, Dima S, Canny E, Alifano M, Popescu I, Klepetko W and Grigoroiu M: Platinum drug sensitivity polymorphisms in stage III non-small cell lung cancer with invasion of mediastinal lymph nodes. Cancer Genomics Proteomics *17(5)*: 587-595, 2020. PMID: 32859637. DOI: 10.21873/cgp.20215

Figure legends

Figure I. Time-to-event analysis according to the genotypes of *signal transducer and activator of transcription 3* (*STAT3*) -1697C>G. Time to event analyses were performed using Kaplan–Meier curves. The solid line represents patients carrying the GG and CG genotypes of *STAT3* -1697C>G, and the dotted line represents patients carrying the CC genotype. (A) The cumulative incidence of paronychia for each genotype group. (B) Progression-free survival for each genotype group. (C) Time-to-treatment failure for each genotype group. The inset table in (C) presents the rate of dose reduction or interruption of osimertinib attributable to any reasons according to each genotype. *p*-Values in the figures and table were calculated using the log-rank test and Fisher's exact test, respectively.

Table I. Patient characteristics at the initiation of osimertinib treatment.

		n=85	
Institution			
Institution A, n (%)		33	39%
Institution B, n (%)		52	61%
Characteristics			
Age (years), median (range)		71	46-94
Sex (Female), n (%)		51	60%
Body weight (kg), median (range)		54.0	38.7-87.8
PS, n (%)	0-1	82	96%
	2-3	3	4%
Histological type, n (%)	Adenocarcinoma	85	100%
Stage, n (%)	IV	64	75%
	Recurrence	21	25%
Liver metastasis (yes), n (%)		10	12%
Brain metastasis (yes), n (%)		22	26%
History of surgery, n (%)		20	24%
History of irradiation of lung fields, n (%)		13	15%
History of ICI treatments, n (%)		2	2%
History of TKI treatments, n (%)		12	14%
History of chemotherapy, n (%)		14	16%

PS: Performance status; ICI: immune checkpoint inhibitor; TKI: tyrosine kinase inhibitor.

Table II. Association of incidence of adverse events by osimertinib with the genotype of related gene polymorphisms.

Genotype		Total AEs			ILD			Rash acneiform			Paronychia			Diarrhea			Mucositis oral		
		None	≥G1	*p*-Value	None	≥G1	*p*-Value	None	≥G1	*p*-Value	None	≥G1	*p*-Value	None	≥G1	*p*-Value	None	≥G1	*p*-Value
STAT3 -1697C>G	CC	0	17		15	2		4	13		7	10		9	8		13	4	
	CG	1	41	0.061	31	11	0.521	17	25	0.556	34	8	0.009	27	15	0.850	31	11	0.936
	GG	3	23		24	2		9	17		21	5		15	11		20	6	
CYP3A5 6986A>G	AA	0	5		5	0		2	3		2	3		2	3		3	2	
	AG	0	30	0.111	26	4	0.151	7	23	0.243	21	9	0.092	18	12	0.465	21	9	0.195
	GG	4	46		39	11		21	29		39	11		31	19		40	10	
ABCG2 421C>A	CC	3	35		31	7		13	25		29	9		18	20		30	8	
	CA	0	40	0.660	34	6	0.832	14	26	0.734	28	12	0.590	28	12	0.048	28	12	0.792
	AA	1	6		5	2		3	4		5	2		5	2		6	1	

AEs: Adverse events; ILD: interstitial lung disease; G1: grade 1.

Table III. Patient characteristics at the initiation of osimertinib therapy according to the paronychia.

	Non-paronychia (n=62)	Paronychia (n=23)	*p*-Value
Institution			
Institution A, n (%)	22 (35%)	11 (48%)	0.326
Institution B, n (%)	40 (65%)	12 (52%)	
Characteristics			
Age (years), median (range)	72.5 (46-94)	69 (49-83)	0.292
Female, n (%)	33 (53%)	18 (78%)	0.047
Body weight (kg), median (range)	54.0 (39.0-82.4)	58.3 (38.7-87.8)	0.921
PS: 0–1, n (%)	59 (95%)	23 (100%)	0.560
Histological type: Adeno, n (%)	62 (100%)	23 (100%)	-
Stage: IV, n (%)	46 (74%)	18 (78%)	0.784
Liver metastasis, n (%)	9 (15%)	1 (4%)	0.274
Brain metastasis, n (%)	17 (27%)	5 (22%)	0.782
History of surgery, n (%)	16 (26%)	4 (17%)	0.568
History of irradiation of lung fields, n (%)	8 (13%)	5 (22%)	0.325
History of ICI therapies, n (%)	1 (2%)	1 (4%)	0.470
History of TKI therapies, n (%)	8 (13%)	4 (17%)	0.727
History of chemotherapy, n (%)	8 (13%)	6 (26%)	0.189

PS: Performance status; ICI: immune checkpoint inhibitor; TKI: tyrosine kinase inhibitor.

Table IV. Patient characteristics at the initiation of osimertinib therapy according to diarrhea.

	Non-diarrhea (n=51)	Diarrhea (n=34)	*p*-Value
Institution			
Institution A	16 (31%)	17 (50%)	0.113
Institution B	35 (69%)	17 (50%)	
Characteristics			
Age (years), median (range)	73 (52-94)	70.5 (46-86)	0.489
Female, n (%)	33 (65%)	18 (53%)	0.652
Body weight (kg), median (range)	56.1 (39.0-87.8)	52.4 (38.7-82.4)	0.407
PS: 0–1, n (%)	50 (98%)	32 (94%)	0.561
Histological type: Adeno, n (%)	51 (100%)	34 (100%)	-
Stage: IV, n (%)	41 (80%)	23 (68%)	0.207
Liver metastasis, n (%)	3 (6%)	7 (21%)	0.081
Brain metastasis, n (%)	15 (29%)	7 (21%)	0.452
History of surgery, n (%)	10 (20%)	10 (29%)	0.310
History of irradiation of lung fields, n (%)	9 (18%)	4 (12%)	0.549
History of ICI therapies, n (%)	0 (0%)	2 (6%)	0.157
History of TKI therapies, n (%)	8 (16%)	4 (12%)	0.755
History of chemotherapy, n (%)	8 (16%)	6 (18%)	1.000

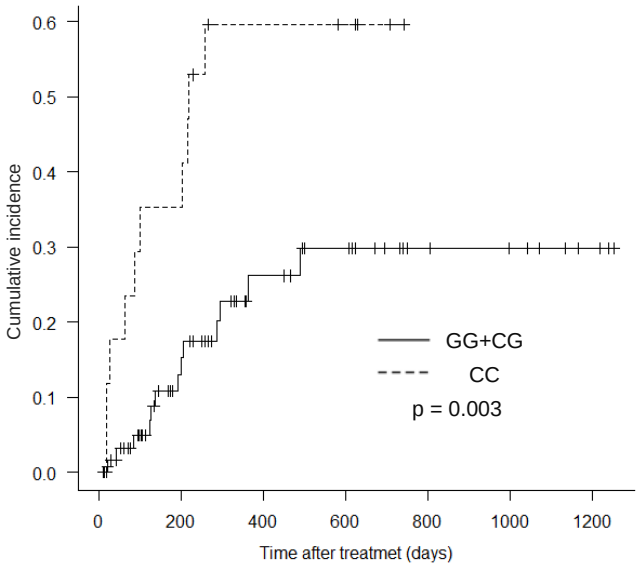
PS: Performance status; ICI: immune checkpoint inhibitor; TKI: tyrosine kinase inhibitor.

Table V. Multivariate logistic regression analysis for paronychia and diarrhea incidence.

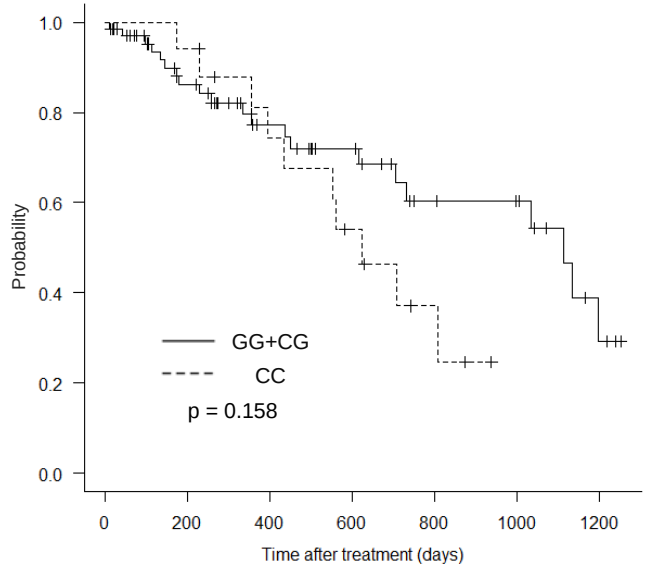
Analysis for paronychia incidence	Odds ratio	95% CI	*p*-Value
CC genotype of *STAT3* -1697C>G	6.41	1.94-21.20	0.002
Female	3.40	1.03-11.22	0.044
Analysis for diarrhea incidence	Odds ratio	95% CI	*p*-Value
CC genotype of *ABCG2* 421C>A	2.47	0.99-6.16	0.052
Liver metastasis	3.78	0.88-16.40	0.075

95% CI: 95% Confidence interval.

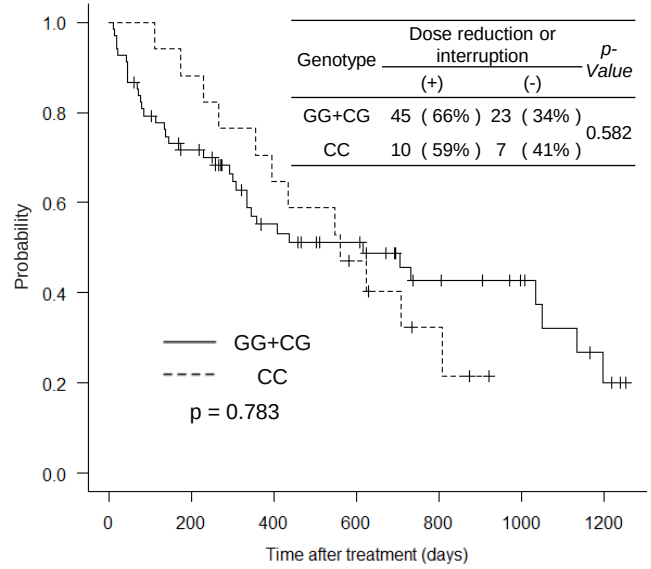
(A) Cumulative incidence of paronychia



(B) Progression-free survival



(C) Time-to-treatment failure



Genotype	Dose reduction or interruption (+)	Dose reduction or interruption (-)	*p-Value*
GG+CG	45 (66%)	23 (34%)	0.582
CC	10 (59%)	7 (41%)	