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Polymorphism AGT2 (rs4762) is involved in development of dermatologic adverse events: Proof-of-concept in hepatocellular carcinoma patients treated with sorafenib.

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The present work is a collaborative and multicenter study that has been developed in collaboration with the BCLC group, Liver Unit, Hospital Clinic where the candidate spent a fellowship period between April 1st, 2021 and October 31st, 2021.

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SUMMARY

Introduction:

Dermatologic adverse events (DAEs) are associated to better outcome of patients with hepatocellular carcinoma (HCC) in a variety of tyrosine kinase inhibitors (TKIs) treatments. The exact mechanisms associated with the development of DAEs are unknown although several studies pointed to a possible direct skin-toxicity of TKIs or an immune-mediated reaction triggered by the oncologic treatment. As is the case in other conditions, individual genetic variants may partially explain a higher risk of DAEs.

Objective:

To evaluate the contribution of several gene variants to the risk of developing DAEs in HCC patients treated with TKIs.

Methods:

We first analyzed 27 Single-nucleotide polymorphisms (SNPs) from 12 genes selected as potential predictors of adverse event (AE) development in HCC patients treated with sorafenib (BCLC-1 cohort). Three additional cohorts were analyzed for AGT1 (rs699) and AGT2 (rs4762) polymorphisms - initially identified as predictors of DAEs: BCLC-2

(n=79), Northern Italy (n=221) and Naples (n=69) cohorts. The relation between SNPs and dermatological AEs (DAEs) and death were assessed by means of univariate and multivariate Cox regression models, and presented with hazard ratios and their 95% confidence intervals (95% CI).

Results:

The BCLC-1 cohort showed that patients with arterial hypertension (AHT) [HR: 1.61; p-value=0.007] and/or AGT SNPs had an increased risk of DAEs. Thereafter, AGT2 (rs4762) AA genotype was found to be linked to a statistically significant increased probability of DAEs [HR= 5.97; p-value=0.0201, AA vs GG] in the Northern Italy cohort by the multivariate analysis adjusted for BCLC stage, ECOG-PS, diabetes and AHT. The value of this genetic marker was externally validated in the cohort combining the BCLC1, BCLC2 and Naples cohorts [HR=3.12 (95%CI: 1.2 -8.14), p-value=0.0199, AGT2 (rs4762) AA vs AG genotype and HR=2.73 (95%CI: 1.18- 6.32) p-value=0.0188, AGT2 (rs4762) AA vs GG genotype]. None of the other gene variants tested were found to be associated with risk of DAE development.

Conclusion:

DAE development in HCC patients receiving TKIs could be explained by AGT2 (rs4762) gene variant. If validated in other antioncogenic treatments, it might be envisioned as a good prognosis or predictive marker.

List of Abbreviations: HCC: Hepatocellular carcinoma; AHT: Arterial Hypertension; AGT: Angiotensinogen gene; ACE: Angiotensin-Converting Enzyme gene; RAS: Renin-Angiotensin System; tRAS: tissue Renin-Angiotensin System; HCV: Hepatitis C Virus; HBV: Hepatitis B Virus; HIV: human immunodeficiency virus; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: Gamma glutamyl transpeptidase; IQR: Interquartile range; ECOG-PS: Eastern Cooperative Oncology Group Performance Status; BCLC: Barcelona Clinic Liver Cancer; INR: International Normalized Ratio; AE: Adverse Events; DAE: dermatologic adverse events; eDAE: early DAE (within the first 60 days); SNPs: Single-nucleotide polymorphisms; TKI: tyrosine kinase inhibitors; DTP: Data Transfer Protocol; AASLD: American Association for the Study of Liver Diseases; CTCAE: Common Terminology Criteria for Adverse Events; PCR: Polymerase Chain Reaction; DNA: deoxyribonucleic acid; PBMCs: peripheral blood mononucleated cells; 95%CI: 95% confidence intervals; HR: Hazard Ratio; OS: Overall survival.

INTRODUCTION

Treatment related dermatologic adverse events (DAEs) are reported in a variety of oncological therapies. The profile and timing of on-target skin adverse events (AEs) differs across treatments and cancer types. In this regard, the hand-foot skin reaction (HFSR) reported in patients receiving tyrosine kinase inhibitors (TKI) resembles the already described hand-foot syndrome (HFS) related to some cytotoxic chemotherapies.[1,2] Moreover, several studies have described the association between DAE development and better patient outcome in different therapies[TKI, monoclonal antibody directed against EGFR[3] or immunotherapy[4,5]] and different cancer types such as colorectal, renal, prostate, non-small cell lung and breast cancer as well as melanoma and hepatocellular carcinoma (HCC).[6]Therefore, it appears that the association between DAE development and better clinical outcome is observed regardless of the cancer type and oncological treatment.

Although there are several hypotheses explaining the potential mechanisms of DAE development, the exact mechanisms remain unknown. Previous studies postulated that direct skin-toxicity of TKI to could depend on drug secretion into eccrine glands[7] somehow mimicking the already described detection of doxorubicin in treated

patients' sweat.[8] Apart from other speculative explanations, inhibition of proangiogenic pathways could potentially prevent vascular repair mechanisms from functioning correctly and causing HFSR in high pressure areas that may be repeatedly exposed to subclinical trauma.[9] This hypothesis could apply mainly to anti-angiogenic treatments but would exclude other therapies. Considering other drug treatments, a study on immune checkpoint inhibitors (ICIs) therapy in non–small cell lung cancer patients suggested that T cells would recognize antigens shared by both lung tumors and skin.[10] Consequently, treatment would target both organs thus leading to tumor regression associated with autoimmune skin toxic effects. However, the low frequency of tumors harboring potent neoantigens clearly compromises the rationale of this hypothesis. More recently, a study published by Ruiz-Pinto and colleagues[11] described the association between CDH4 genetic variants with the risk of developing capecitabine-induced HFS. In that study, CDH4 gene downregulation negatively impacted skin barrier function.

In 2018, a study from the BCLC group demonstrated that 91.6% of HCC patients who received sorafenib and achieved complete radiological response also developed DAEs within the first 2 months of treatment.[12,13] Other recent data from the BCLC group allowed to identify a potential role of TKI in peripheral immune cell population

profile modification towards a more pro-inflammatory behavior and phenotype.[14] Thus, we envision skin toxicity as a consequence of an immune-mediated reaction triggered by the oncologic treatment in patients prone to develop this side effect.

In order to uncover potential mechanisms underlying individual genetic susceptibility to AEs with clinical implications for risk prediction, we first analyzed 27 Single-Nucleotide Polymorphisms (SNPs) in 12 different genes as potential predictors of AE development in a BCLC1 cohort of 82 HCC patients treated with sorafenib. Upon the identification of the potential relevance of the angiotensin genes, which include the AGT1 (rs699) and AGT2 (rs4762), as predictors of DAEs, we further explored the association in three additional cohorts: a second BCLC cohort (n=79), a Northern Italy cohort (n=221) and a Naples cohort (n=69).

PATIENTS AND METHODS

Four cohorts of patients were analyzed in this study. Two prospective cohorts from Barcelona Clinic Liver Cancer (BCLC-1 and BCLC-2) and two additional cohorts from Northern Italy (Milan, Bologna, Meldola (FC) and Cagliari Hospitals) and Naples (Figure 1).

The study was approved by the institutional review board of each center (HCB/2009/4755, HCB/2015/0352, Ethical Board 2 480_2018 and CE/2014/193) and complied with the provisions of the Good Clinical Practice guidelines and the Declaration of Helsinki. A Data Transfer Protocol (DTP) was written according to the European regulation (General Data Protection Regulation (GDPR) 2016/679) and approved by each cohort responsible.

Patients' eligibility

BCLC-1 cohort

This cohort included patients referred to BCLC between February 2009 and March 2015 for sorafenib treatment.

Inclusion criteria were: 1) HCC diagnosed according to EASL guidelines[15]; 2) advanced HCC following the BCLC staging system or patients with earlier stages who could not benefit from treatments of

higher priority; 3) normal liver or compensated cirrhosis with preserved liver function (Child-Pugh score <7 points without clinical ascites and/or encephalopathy; 4) Performance status 0-1; 5) controlled arterial hypertension (AHT) and/or stable peripheral vascular disease; 6) adequate hematologic profile (platelet count > 60x109/L; hemoglobin> 8.5g/dL; and prothrombin time > 50%); 7) adequate hepatic function (albumin > 2.8g/dL; total bilirubin <=3 mg/dL; and alanine and aspartate aminotransferases <=5 times the upper limit of the normal range) 8) adequate renal function(serum creatinine <=1.5 times the upper limit of the normal range).

Exclusion criteria were: 1) myocardial infarction in the last year or active ischemic heart disease; 2) acute variceal bleeding in the last month; 3) severe peripheral arterial disease; 4) arrhythmia under treatment with drugs different from beta-blockers or digoxin; 5) uncontrolled ascites; 6) encephalopathy. All patients provided written informed consent before enrolment.

Follow-up

Clinical and laboratory assessments were done monthly and radiologic tumor evaluation at week 4 and every 8 weeks thereafter. Unscheduled visits due to adverse events occurred according to patients' needs.

DAEs were graded according to version 3.0 of the CTCAE of the National Cancer Institute, during treatment and 30 days after the last dose. We focused on the DAEs within the first 60 days (eDAE) +/-7days of treatment, which determined dose modification.

BCLC-2 cohort

This cohort included patients referred to BCLC between June 2015 and August 2018 for sorafenib treatment.

The inclusion and exclusion criteria as well as the follow-up of this cohort was the same as for the BCLC1 cohort.

Northern Italy cohort

The Northern Italy cohort included patients with HCC treated with sorafenib prospectively enrolled between July 2008 and June 2018 in four tertiary centers in Italy whose data have already been published in several multicenter studies on sorafenib treatment.[16,17] Briefly, all patients with advanced HCC or intermediate-stage HCC refractory to or unsuitable for locoregional therapies, either histologically proven or diagnosed according to the AASLD guidelines (American Association for the Study

of Liver Diseases 2005) and receiving sorafenib were eligible for our analysis. Exclusion criteria were those established by the Italian Medicines Agency (AIFA), i.e., a performance status score >2 and clinical decompensation. All patients received sorafenib with the standard schedule (400 mg bid continuously) with dose reduction applied as clinically indicated.

Follow-up

Follow-up consisted of a physical examination and complete blood count every 3 weeks and CT/MRI scanning every 8 weeks or as clinically indicated. Each visit included recording of AEs, clinical laboratory tests, physical examination, and assessment of vital signs. At any time during treatment, the patient could have direct access to physicians for AE management. Safety was assessed in all patients who received at least one dose of sorafenib; AEs were graded according to the National Cancer Institute's Common Terminology Criteria (version 3.0CTCAE). Hepatic function deterioration was defined as a Child-Pugh score increase ≥2 points, being evaluated at each visit and at predefined time points of week 12 and 24 of therapy. For the aim of the study, independently of clinical practice, we focused on the AEs which determined dose modification

within the first 30 and 60 days of treatment, respectively. Treatment with sorafenib was continued until disease progression, unacceptable toxicity, or death. In each patient, the medical history, physical examination, blood cell count, serum chemistries, coagulation and alpha-fetoprotein levels were obtained at baseline and every 4 weeks thereafter.

Naples cohort

This cohort included patients referred to the Gastroenterology Unit of the University Hospital Federico II of Naples between January 2014 and December 2019 for sorafenib treatment.

Inclusion criteria were: 1) HCC diagnosed according to EASL guidelines[15]; 2) advanced HCC following the BCLC staging system or patients with earlier stages who could not benefit from treatments of higher priority; 3) normal liver or compensated cirrhosis with preserved liver function (Child-Pugh score ≤7 points without clinical ascites and/or encephalopathy; 4) Performance status 0-1; 5) controlled arterial hypertension (AHT) and/or stable peripheral vascular disease; 6) adequate hematologic profile (platelet count > 30x103/L; hemoglobin> 8.5g/dL; and INR < 1.7; 7) adequate hepatic function (albumin > 2.8g/dL; total bilirubin <3 mg/dL; and alanine and aspartate aminotransferases <5 times

the upper limit of the normal range) 8) adequate renal function (serum creatinine <1.5 times the upper limit of the normal range).

Exclusion criteria were: 1) myocardial infarction in the last year or active ischemic heart disease; 2) acute variceal bleeding in the last month; 3) severe peripheral arterial disease; 4) arrhythmia under treatment with drugs different from beta-blockers or digoxin; 5) uncontrolled ascites; 6) encephalopathy. All patients provided written informed consent before enrolment.

Follow-up

Clinical and laboratory assessments were done monthly and radiologic tumor evaluation at week 8 and every 8 weeks thereafter. Unscheduled visits due to adverse events occurred according to patients' needs.

DAEs were graded according to version 3.0 of the CTCAE of the National Cancer Institute, during treatment and 30 days after the last dose. We focused on the DAEs within the first 60 days (eDAE) +/- 7 days of treatment, which determined dose modification.

Genomic DNA (gDNA) purification

gDNA was purified from isolated peripheral blood mononucleated cells (PBMCs) in BCLC cohorts of patients and from 500 μL of whole frozen blood in the Naples cohort. gDNA purification was performed using PureLink gDNA mini kit (Invitrogen, Thermo Fisher Scientifics) following manufacturer's instructions.

Patient genotyping

BCLC-1 cohort

Patients were genotyped for a series of SNPs in IL23R, IL17, FOXP3, VEGF, AGT, PLA2G12A, IL-8, AT1R, ANGPT2, TNF-a, GNB3, IL-6 genes. SNPs were selected according to reported associations with susceptibility to cardiovascular disease, hypertension, stroke, inflammatory pathways or even cancer development.

Twenty ngr of gDNA were used for each SNP reaction. All SNPs were evaluated by means of TaqMan predesigned genotyping Assays (Applied Biosystems, Thermo Fisher Scientifics) and the procedure was performed following manufacturer's instructions.

Briefly, TaqMan® MGB probes from the Genotyping Assay provide a fluorescent signal for the amplification of each allele. SNP genotyping uses a 60 sec extension time at 60ºC for 40 cycles. Real-time PCR

software plots the results of the allelic discrimination data as a scatter plot of Allele 1 (VIC® dye) versus Allele 2 (FAM™ dye). Each well of the 96-well reaction plate is represented as an individual point on the allelic discrimination plot. Positive controls were used for each homozygote and heterozygote genotypes.

Patients from the BCLC-2, Northern Italy and Naples cohorts were genotyped for 2 single-nucleotide polymorphisms (SNPs) of the AGT-gene [AGT1 (rs699) and AGT2 (rs4762)] by using the TaqMan endpoint-genotyping assay, following the same techniques as previously described.

Statistical analysis

The statistical methods and analysis of this study were performed by Víctor Sapena and reviewed by Ferran Torres from Hospital Clínic de Barcelona.

Quantitative variables were expressed as median and interquartile range [IQR 25th-75th percentiles]. Categorical variables were described as absolute frequencies and percentages (%).

Time to event variables were expressed as median and 95% confidence intervals (95%CI) using the Kaplan-Meier method. Log-rank test was

used to compare Kaplan-Meier curves. Univariate and multivariate Cox regression models were used to estimate Hazard Ratios (HR) and 95% CI to evaluate the increased probability of developing grade II or early dermatologic events (eDAE), DAE or death according to each Single Nucleotide Polymorphism (SNP). The multivariate adjusting factors were previously selected according to their clinical relevance, these were BCLC stage (A or B vs C), ECOG-PS (0 vs >=1), history of AHT (No vs Yes) and history of diabetes (No vs Yes). An analysis using 67 days as the landmark time point was used to calculate the OS according to eDAE.

The level of significance was set at the two-tailed 5% level and all analyses and data base integration structure were performed with SAS 9.4 software (SAS Institute, Cary, NC, USA).

RESULTS

This study includes 82 patients from the BCLC-1 cohort, 79 from the second BCLC-2 cohort, 221 from the Northern Italy cohort, and 69 from the Naples cohort.

Baseline characteristics

Tables 1, 2 and 3 describe the characteristics, overall survival (OS) and follow-up at the time of locking the database (December 2019) and the AE rates of all patients included in the study.

BCLC-1 cohort

All but 2 (2.4%) patients were cirrhotic. A total of 54 (65.9%) patients had HCV and 10 (12.2%) had HBV. Ninety-three percent of patients were asymptomatic (ECOG-PS 0) and 40 (48.8%) were BCLC B that failed or presented contraindication to loco-regional treatment, 70 (85.4%) were Child-Pugh class A. Twenty-two (26.8%) presented vascular invasion, 24 (29.3%) had extra-hepatic spread. AHT was present in 45.1% of patients and diabetes in 26.8%. Seventy-seven patients (93.9%) started sorafenib treatment at 800mg.

BCLC-2 cohort

All but 5 (6.3%) patients were cirrhotic. A total of 38 (48.1%) patients had HCV and 6 (7.6%) had HBV. Ninety-three percent of patients were

asymptomatic (ECOG-PS 0) and 36 (45.6%) were BCLC B that failed or presented contraindication to loco-regional treatment, 63 (79.8%) were Child-Pugh class A. Twenty-six (32.9%) presented vascular invasion, 27 (34.2%) had extra-hepatic spread. AHT was present in 45.6% of patients and diabetes in 35.4%. Seventy-seven patients (97.4%) started sorafenib treatment at 800mg.

Northern Italy cohort

All patients were cirrhotic. A total of 111 (50.2%) patients had HCV and 46 (20.8%) had HBV. Seventy percent of patients were asymptomatic (ECOG-PS 0) and 76 (34.4%) were BCLC B that failed or presented contraindication to loco-regional treatment, 207 (93.7%) were Child-Pugh class A. Sixty-one (27.6%) presented vascular invasion, 79 (35.8%) had extra-hepatic spread. AHT was present in 29.4% of patients and diabetes in 27.6%. One hundred ninety-seven patients (89.1%) started sorafenib treatment at 800mg.

Naples cohort

All but 1 (1.5%) patient were cirrhotic. A total of 44 (63.7%) patients had HCV and 12 (17.4%) had HBV. All patients were asymptomatic (ECOG-PS 0) and 20 (29%) were BCLC B that failed or presented contraindication to loco-regional treatment, 58 (84.1%) were Child-Pugh class A. Thirty-

one (44.9%) presented vascular invasion, 23 (33.3%) had extra-hepatic spread. AHT was present in 65.2% of patients and diabetes in 33.3%. All patients started sorafenib treatment at 800mg.

Adverse events

The rate of DAEs at any time point in the BCLC-1, BCLC-2, Northern Italy and Naples cohorts were 51.2 %, 35.4%, 14.5% and 39.1%; respectively (Table 3). The incidence of eDAEs in the BCLC-1cohort was 40.2% and 27.8%, 12.7% and 36.2% in the BCLC2, Northern Italy and Naples cohorts, respectively.

The association between DAEs and a history of AHT was statistically significant in the BCLC-1 cohort, with a HR=1.96 (95%CI: 1.05 – 3.65; p-value=0.04) and confirmed when all patients are analyzed as a unique cohort with a HR=1.61 (95%CI:1.14 - 2.28; p-value=0.007).

Follow-up and Overall survival

BCLC-1 cohort

The median follow-up was 18.6 months [IQR: 10.3 – 34.2] and 75 (91.5%) patients died. Ninety-eight percent of deaths were due to HCC-related causes. The median treatment duration and OS were 9.1 [IQR: 4.1 – 17.5] and 18.8 months (95%CI: 14.7 – 23.6), respectively.

BCLC-2 cohort

The median follow-up was 13.1 months [IQR: 6.6 – 22.4] and 47 (59.5%) patients died. Ninety-seven percent of deaths were due to HCC-related causes. The median treatment duration and OS were 5.9 [IQR: 2.1 – 13.5] and 18.3 months (95%CI: 13.1 – 26.4), respectively.

Northern Italy cohort

The median follow-up was 12.7 months [IQR: 6.1 – 25.9] and 180 (81.4%) patients died. Sixty-five percent of deaths were due to HCC-related causes. The median treatment duration and OS were 8.5 [IQR: 2.6 – 20.8] and 14.3 months (95%CI: 11.8 – 18), respectively.

Naples cohort

The median follow-up was 9.9 months [IQR: 4.5 – 18.3] and 57 (82.6%) patients died. Eighty-four percent of deaths were due to HCC-related causes. The median treatment duration and OS were 8.1 [IQR: 3.7 – 17] and 9.9 months (95%CI: 7.7 – 12.8), respectively.

Overall survival according to eDAE

Using a landmark time point of 60 (+7) days and excluding 17 patients with less than 60 (+7) days of follow-up, the median OS in eDAE and in non-eDAE patients was 21.6 (95%CI: 12.7 – 28.2) and 14.8 (95%CI: 9.9

– 17.6) in BCLC-1,19.5 (95%CI: 8 – 24.2) and 14.2 (95%CI: 8.9 – 30.5) in BCLC-2,15.9 (95%CI: 8.3 – 40.6) and 12.1 (95%CI: 9.6 – 16.6) in the Northern Italy cohort, 12.4 (95%CI: 7.86 – 21.14) and 6.8 (95%CI: 2.7 – 8.7) in the Naples cohort, respectively.

Single-nucleotide polymorphisms (SNPs)

BCLC-1 cohort

Of all SNPs analyzed, only the AGT1 (rs699) AA genotype had a significant estimated increase of probability of eDAE with a HR=2.31 (95%CI:1.03 - 5.14; p-value=0.04; AA vs AG) in the univariate model and a HR=2.3 (95%CI: 1.02-5.16; p-value=0.04; AA vs AG) in the multivariate model (Table 4). For DAEs at any time point, AGT1 (rs699) AA genotype showed a significant estimated increase of probability of DAEs with a HR=2.7 (95%CI:1.27 - 5.75; p-value=0.01; AA vs AG) in the univariate model and a HR=2.68 (95%CI: 1.25-5.77; p-value=0.01; AA vs AG) in the multivariate model. No other polymorphism showed a significant association with general AEs or specifically DAE or eDAE development in the BCLC-1 cohort.

Allele distribution of Single-nucleotide polymorphisms (SNPs) AGT1 (rs699) and AGT2 (rs4762)

Allele distributions of AGT1 (rs699) and AGT2 (rs4762) are summarized in Table 1. There were no significant differences between all the included cohorts (p-value 0.5 and 0.2 for AGT1 rs699 and AGT2 rs4762, respectively). Thus, the present cohorts are comparable in terms of genetic variants.

AGT1 (rs699) and AGT2 (rs4762) influence in the development of DAE and eDAE

Tables 4 and 5 describe the Cox regression models for eDAE and DAE development by AGT1 (rs699) and AGT2 (rs4762), respectively. The results of the BCLC-1 cohort have been commented above.

BCLC-2 cohort

The AGT1 (rs699) did not show a significant association with DAEs. By contrast, the AGT2 (rs4762) AA genotype was associated to a significant increased risk of eDAE with a HR=4.43 (95%CI:1.01 - 19.39; p-value=0.048; AA vs GG) in the univariate analysis, and showed a trend in the multivariate model with a HR=4.24 (95%CI:0.95-19.06]; p-value=0.06; AA vs GG), Table 5.

Northern Italy cohort

In this cohort, the AGT2 (rs4762) AA genotype showed a statistically significant increased probability of eDAE both in the univariate analysis (HR=4.54 [95%CI:1.05 - 19.64]; p-value=0.04; AA vs GG) and in the multivariate analysis (HR=5.15 [1.17-22.63]; p-value=0.03; AA vs GG).

Naples cohort

In the Naples cohort, none of the SNPs showed a significant effect on DAE nor eDAE development.

Validation of the AGT2 (rs4762) value identified in the Northern Italy cohort in the large cohort combining all cohorts (without the Northern Italy one).

The results in the individual cohorts suggested that the inconclusive results obtained in the BCLC and Naples cohorts could be due to a limited sample size. Thus, we combined these cohorts into a single one that would match the Northern Italy sample size.

This analysis shows that AGT2 (rs4762) is significantly associated to DAE development with a HR=2.94 (95%CI:1.14 - 7.6; p-value=0.03; AA vs AG) and HR=2.49 (95%CI:1.08 - 5.73; p-value=0.03; AA vs GG) in univariate models, and HR=2.85 (95%CI:1.1 - 7.39; p-value=0.03; AA vs

AG) and HR=2.48 (95%CI:1.08 - 5.72; p-value=0.03; AA vs GG) in multivariate models (Table 5).

Influence of AGT2 (rs4762) in DAE and eDAE development after adjusting for baseline tumor burden, liver function, performance status and comorbidities

Table 5 shows the multivariate analyses adjusted for baseline BCLC stage, ECOG-PS, diabetes and AHT in the same model, considering diabetes and AHT together and each one separately. The multivariate analysis adjusted for baseline BCLC stage, ECOG-PS, diabetes and AHT shows a statistically significant increased risk of probability of eDAE in patients harboring AGT2 (rs4762) AA genotype in the Northern Italy cohort (HR=8.51, 95%CI: 1.78- 40.54; p-value=0.007; AA vs GG; and HR= 5.61,95%CI: 1.01- 31.12; p-value=0.048; AA vs AG).

The same analysis was done for AGT2 (rs4762) AA genotype and DAE development. A statistically significant increased risk of probability of DAE was observed in the Northern Italy cohort (HR= 5.97, 95%CI: 1.32-27.01; p-value=0.02; AA vs GG) and also when considering all but the Northern Italy cohort altogether as a unique cohort (HR=3.12, 95%CI: 1.2 -8.14; p-value=0.02; AA vs AG, and HR=2.73, 95%CI: 1.18- 6.32: p-value=0.02; AA vs GG).

AGT1 (rs699) and AGT2 (rs4762) influence on survival

No statistically significant effect on survival was found in AGT1 (rs699) nor AGT2 (rs4762) using univariate or multivariate models in any cohort or combination thereof (data not shown).

DISCUSSION

The aim of Precision Oncology is to decide the treatment to be recommended to a specific patient according to the individualized evaluation of the clinical, biochemical and hopefully, molecular profile. It is common to focus all the attention on the genomic abnormalities of cancer to define the best intervention, but as is well known, patients' genetic background, irrespective of the tumor, is involved in the efficacy and safety of any therapeutic intervention. The best example is the clearance related to the glucuronidation activity resulting in fast and slow elimination of drugs and their metabolites.[18] Response to inflammation or tolerance to antiangiogenic agents is also influenced by such genetic background and most cancer treatments have targets affecting several of these separate domains. In some instances, these noncancer effects may become a surrogate of drug activity and even be correlated with improved outcomes as already described in the introduction.

This multicenter and international study explores whether specific genetic variants, as identified by SNP analysis, may be linked to the development of adverse events that have been associated with improved outcome. This is not only the case of DAEs in patients with HCC treated with sorafenib[12,19],as has been extensively proven, but also when using other TKIs such as regorafenib.[20] Furthermore, the association of DAEs with improved outcome is also being reported when using chemotherapy

or immunotherapy not only in liver cancer but also in other tumor types.[3–5]

The results of our multicenter study confirm that the genetic background of patients plays a key role in the emergence of specific events that are linked to a distinct outcome under HCC treatment. Previously, different SNPs were reported to be potentially associated with survival outcomes[16,17] while others were identified as significantly associated with a higher likelihood of DAEs affecting the angiotensin gene and its AGT2 (rs4762) variant.

Our results confirmed that the distribution of the AGT genetic variants studied, AGT1 (rs699)and AGT2 (rs4762), was comparable across patients from Northern and Southern Italy and those from Barcelona, and also confirmed that the frequency of reference and alternative alleles follow the reported distribution for the European population. [21,22]

Although rs699 and rs4762 could not be associated with AHT events in our patients, the most relevant finding is the identification of AGT2 (rs4762) AA genotype as a predictor of DAE development [HR= 5.97; p-value=0.0201] in the Northern Italy cohort and its validation in the remaining 3 cohorts when they were considered as one unique cohort

[HR=3.12 (95%CI: 1.2 -8.14); p-value=0.02 and HR=2.73 (95%CI: 1.18-6.32); p-value=0.02].

AGT2 (rs4762) is a missense variant that codes for the replacement of threonine for methionine with no reported clear association with blood AGT protein levels.[23]AGT2 (rs4762) has been associated with renal dysplasia, a potentially likely-benign disease.[22] However, published data suggest that rs4762 may be associated with increased risk of mortality in patients with heart failure[23] and also with the development of intracranial hemorrhage in stroke patients.[24]Available data at this moment do not allow to unequivocally associate an increase in blood AGT levels with rs4762 polymorphism, but it is speculated that it could induce Renin-Angiotensin System (RAS) activation. RAS is a key regulator of systemic homeostasis by controlling salt-water balance and consequently, blood pressure. Interestingly, several studies have unveiled the activation of this system also in several peripheral tissues (tRAS)[25] and organs including skin and liver.[26] Since activation of tRAS is associated with tissue regeneration, inflammation and fibrosis[27], all of these key components of tumor development, tRAS activation is likely to play a role in carcinogenesis. A review by Ager EI and collaborators[28] describes the potential contribution of tRAS activation in cancer development and progression putting the emphasis not only on tumor angiogenesis, but also

on inflammation and fibrosis. Considering that the components of tRAS pathway are also participating in physiological and pathological wound healing and fibrosis processes that are particularly important in skin homeostasis[29,30], DAE development in our patients with rs4762 AA genotype may be envisioned as a consequence of tRAS activation at skin level.

The role of genetic variants in components of the RAS pathway has been reported extensively in the past years and some of these roles involve response to anti-neoplastic treatments, disease prognosis and patient survival. In that sense, it is already known that ACE I/D rs4646994, a variant of the Angiotensin-Converting Enzyme (ACE), has been associated with prediction of response to bevacizumab in metastatic breast and colorectal cancer patients.[31] AGT rs5050 GG genotype[32] is reported to be linked to poor prognosis in patients with astrocytoma. A very interesting in silico study by Goswami and colleagues analyses 354 SNPs in AGT gene[33] in order to predict those variants that are pathogenic and how amino acid substitutions would impact protein function. In this study, AGT2rs4762 is categorized mainly as a damaging AGT SNP with controversial results on its pathogenicity or disease identity. Thus, the importance of genetic variants is determined by the levels and/or functionality of the protein they code for. Along these lines,

Feng et al.[34] proposed that cancer tissue levels of ACE2 correlates with immune infiltrates and these would affect the prognosis of cancer patients. In another study, Urupet al.[35] suggested that low expression of AGT gene and high expression of an HLA-class II gene (HLADQA1) were independent predictors associated with response in glioblastoma patients treated with bevacizumab.

AGT2 (rs4762) has been associated with increased risk of AHT in several studies[36,37] but this association remains controversial since the results could not been confirmed in other series of individuals analyzed[38].We were not able to identify an association between AGT2 rs4762 and AHT in our patients not even when analyzing the impact of concomitant medication that the BCLC-1 and BCLC-2 cohort patients received for AHT that included IECA. This could be related to the low frequency of AGT2 rs4762 in patients who developed this AE [0 (0%) in the BCLC1 and Northern Italy cohorts, 1 (1.27%) in the BCLC2 cohort and 2 (2.9%) in the Naples cohort].

However, in our cohort, the impact of AGT2 (rs4762) was maintained when the multivariate was adjusted for history of AHT.

To the best of our knowledge, the relationship between AGT2 rs4762AA genotype and DAE development in HCC patients under sorafenib

treatment has not been previously reported. This is a ‘proof-of-concept’ study to identify a novel genetic marker to screen for patients with good outcome. It would be interesting for our results to be validated in other cancer types besides HCC or even in different therapeutic approaches. If this were to be the case, AGT2 (rs4762) should be considered a good prognosis marker instead of being only a predictor of DAE development. In previous studies of the BCLC group, it has been demonstrated that the occurrence of DAE is unequivocally associated with a longer OS[12,19]. This, added to the fact that patients included in the study are patients with advanced disease under systemic treatment and radiological response is a suboptimal surrogate of OS, we consider that rs4762 association with DAE development is a significant finding.

CONCLUSION

In summary, our finding paves the way to explore individual genetic susceptibility as prognostic factors or predictors of treatment outcome,

and to unveil novel mechanisms triggered by oncological treatment and their potential link to tumor response and patient survival.

REFERENCES

1. Gordon KB, Tajuddin A, Guitart J, Kuzel TM, Eramo LR, Vonroenn J. Hand-foot syndrome associated with liposome-encapsulated doxorubicin therapy. Cancer 1995;75:2169–73 [PMID: 7697608 DOI: 10.1002/1097-0142(19950415)75:8<2169::AID-CNCR2820750822>3.0.CO;2-H]

2. Nagore E, Insa A, Sanmartín O. Antineoplastic therapy-induced palmar plantar erythrodysesthesia ('hand-foot') syndrome: Incidence, recognition and management. Am. J. Clin. Dermatol.2000;1:225–34 [PMID: 11702367 DOI: 10.2165/00128071-200001040-00004]

3. LB Saltz, M Kies, JL Abbruzzesse, N Azarnia, M Needle, L Saltz JA. The presence and intensity of the cetuximab-induced acne-like rash predicts increased survival in studies across multiple malignancies. Proc Am Soc Clin Oncol 2003;22:204 (Abstract #817)

4. Naidoo J, Page DB, Li BT, Connell LC, Schindler K, Lacouture ME, Postow MA, Wolchok JD. Toxicities of the anti-PD-1 and anti-PD-L1 immune checkpoint antibodies. Ann Oncol 2015;26:2375–91 [PMID: 26371282 DOI: 10.1093/annonc/mdv383]

5. Freeman-Keller M, Kim Y, Cronin H, Richards A, Gibney G, Weber JS. Nivolumab in resected and unresectable metastatic melanoma: Characteristics of immune-related adverse events and association with

outcomes. Clin Cancer Res 2016;22:886–94 [PMID: 26446948 DOI: 10.1158/1078-0432.CCR-15-1136]

6. Li Y, Gao ZH, Qu XJ. The adverse effects of sorafenib in patients with advanced cancers. Basic Clin. Pharmacol. Toxicol.2015;116:216–21 [PMID: 25495944 DOI: 10.1111/bcpt.12365]

7. Lai SE, Kuzel T, Lacouture ME. Hand-foot and stump syndrome to sorafenib. J Clin Oncol 2007;25:341–2 [PMID: 17235051 DOI: 10.1200/JCO.2006.08.9565]

8. Jacobi U, Waibler E, Schulze P, Sehouli J, Oskay-Özcelik G, Schmook T, Sterry W, Lademann J. Release of doxorubicin in sweat: First step to induce the palmar-plantar erythrodysesthesia syndrome? [4]. Ann. Oncol.2005;16:1210–1 [PMID: 15857845 DOI: 10.1093/annonc/mdi204]

9. Robert C, Soria JC, Spatz A, Le Cesne A, Malka D, Pautier P, Wechsler J, Lhomme C, Escudier B, Boige V, Armand JP, Le Chevalier T. Cutaneous side-effects of kinase inhibitors and blocking antibodies. Lancet Oncol.2005;6:491–500 [PMID: 15992698 DOI: 10.1016/S1470-2045(05)70243-6]

10. Flatz L, Berner F, Bomze D, Diem S, Ali OH, Fässler M, Ring S, Niederer R, Ackermann CJ, Baumgaertner P, Pikor N, Cruz CG, Van De

Veen W, Akdis M, Nikolaev S, Läubli H, Zippelius A, Hartmann F, Cheng HW, Hönger G, Recher M, Goldman J, Cozzio A, Früh M, Neefjes J, Driessen C, Ludewig B, Hegazy AN, Jochum W, Speiser DE. Association of Checkpoint Inhibitor-Induced Toxic Effects with Shared Cancer and Tissue Antigens in Non-Small Cell Lung Cancer. JAMA Oncol 2019;5:1043–7 [PMID: 31021392 DOI: 10.1001/jamaoncol.2019.0402]

11. Ruiz-Pinto S, Pita G, Martín M, Nuñez-Torres R, Cuadrado A, Shahbazi MN, Caronia D, Kojic A, Moreno LT, de la Torre-Montero JC, Lozano M, López-Fernández LA, Ribelles N, García-Saenz JA, Alba E, Milne RL, Losada A, Pérez-Moreno M, Benítez J, González-Neira A. Regulatory CDH4 Genetic Variants Associate With Risk to Develop Capecitabine-Induced Hand-Foot Syndrome. Clin Pharmacol Ther 2021;109:462–70 [PMID: 32757270 DOI: 10.1002/cpt.2013]

12. Reig M, Torres F, Rodriguez-Lope C, Forner A, LLarch N, Rimola J, Darnell A, Ríos J, Ayuso C, Bruix J. Early dermatologic adverse events predict better outcome in HCC patients treated with sorafenib. J Hepatol 2014;61:318–24 [PMID: 24703956 DOI: 10.1016/j.jhep.2014.03.030]

13. Rimola J, Díaz-González Á, Darnell A, Varela M, Pons F, Hernandez-Guerra M, Delgado M, Castroagudin J, Matilla A, Sangro B, Rodriguez de Lope C, Sala M, Gonzalez C, Huertas C, Minguez B, Ayuso

C, Bruix J, Reig M. Complete response under sorafenib in patients with hepatocellular carcinoma: Relationship with dermatologic adverse events. Hepatology 2018;67:612–22 [PMID: 28898447 DOI: 10.1002/hep.29515]

14. Corominas J, Sapena V, Sanduzzi-Zamparelli M, Millán C, Samper E, Llarch N, Iserte G, Torres F, Da Fonseca LG, Muñoz-Martínez S, Forner A, Bruix J, Boix L, Reig M. Activated lymphocytes and increased risk of dermatologic adverse events during sorafenib therapy for hepatocellular carcinoma. Cancers (Basel) 2021;13:1–20 [DOI: 10.3390/cancers13030426]

15. Llovet JM, Ducreux M, Lencioni R, Di Bisceglie AM, Galle PR, Dufour JF, Greten TF, Raymond E, Roskams T, De Baere T, Ducreux M, Mazzaferro V, Bernardi M, Bruix J, Colombo M, Zhu A. EASL-EORTC Clinical Practice Guidelines: Management of hepatocellular carcinoma. J Hepatol 2012;56:908–43 [PMID: 22424438 DOI: 10.1016/j.jhep.2011.12.001]

16. Casadei Gardini A, Marisi G, Faloppi L, Scarpi E, Foschi FG, Iavarone M, Lauletta G, Corbelli J, Valgiusti M, Facchetti F, della Corte C, Neri LM, Tamberi S, Cascinu S, Scartozzi M, Amadori D, Nanni O, Tenti E, Ulivi P, Frassineti GL. eNOS polymorphisms and clinical

outcome in advanced HCC patients receiving sorafenib: Final results of the ePHAS study. Oncotarget 2016;7:27988–99 [PMID: 27058899 DOI: 10.18632/oncotarget.8569]

17. Marisi G, Petracci E, Raimondi F, Faloppi L, Foschi FG, Lauletta G, Iavarone M, Canale M, Valgiusti M, Neri LM, Ulivi P, Orsi G, Rovesti G, Vukotic R, Conti F, Cucchetti A, Ercolani G, Andrikou K, Cascinu S, Scartozzi M, Casadei-Gardini A. ANGPT2 and NOS3 polymorphisms and clinical outcome in advanced hepatocellular carcinoma patients receiving sorafenib. Cancers (Basel) 2019;11 [DOI: 10.3390/cancers11071023]

18. Wilkinson GR. Drug Metabolism and Variability among Patients in Drug Response. 2005.

19. Díaz-González Á, Sanduzzi-Zamparelli M, Sapena V, Torres F, LLarch N, Iserte G, Forner A, da Fonseca L, Ríos J, Bruix J, Reig M. Systematic review with meta-analysis: the critical role of dermatological events in patients with hepatocellular carcinoma treated with sorafenib. Aliment Pharmacol Ther 2019;49:482–91 [PMID: 30695819 DOI: 10.1111/apt.15088]

20. Bruix J, Merle P, Granito A, Huang Y-H, Bodoky G, Yokosuka O, Rosmorduc O, Breder VV, Gerolami R, Masi G, Ross PJ, Qin S, Song T, Bronowicki J-P, Ollivier-Hourmand I, Kudo M, Xu L, Baumhauer A,

Meinhardt G, Han G. Hand-foot skin reaction (HFSR) and overall survival (OS) in the phase 3 RESORCE trial of regorafenib for treatment of hepatocellular carcinoma (HCC) progressing on sorafenib. J Clin Oncol 2018;36:412–412 [DOI: 10.1200/JCO.2018.36.4_suppl.412]

21. rs699 RefSNP Report - dbSNP - NCBI [Internet]. [cited 2021 Mar 3];Available from: https://www.ncbi.nlm.nih.gov/snp/rs699#frequency_tab, https://www.ncbi.nlm.nih.gov/snp/rs4762%23frequency_tab

22. rs4762 RefSNP Report - dbSNP - NCBI [Internet]. [cited 2021 Mar 3];Available from: https://www.ncbi.nlm.nih.gov/snp/rs4762#clinical_significance

23. Pilbrow AP, Palmer BR, Frampton CM, Yandle TG, Troughton RW, Campbell E, Skelton L, Lainchbury JG, Richards AM, Cameron VA. Angiotensinogen M235T and T174M gene polymorphisms in combination doubles the risk of mortality in heart failure. Hypertension 2007;49:322–7 [PMID: 17145981 DOI: 10.1161/01.HYP.0000253061.30170.68]

24. Park HK, Kim MC, Kim SM, Jo DJ. Assessment of two missense polymorphisms (rs4762 and rs699) of the angiotensinogen gene and stroke. Exp Ther Med 2013;5:343–9 [DOI: 10.3892/etm.2012.790]

25. Bader M. Tissue renin-angiotensin-aldosterone systems: Targets for pharmacological therapy. Annu. Rev. Pharmacol. Toxicol.2010;50:439–65 [PMID: 20055710 DOI: 10.1146/annurev.pharmtox.010909.105610]

26. Hunyady L, Catt KJ. Pleiotropic AT1 receptor signaling pathways mediating physiological and pathogenic actions of angiotensin II. Mol. Endocrinol.2006;20:953–70 [PMID: 16141358 DOI: 10.1210/me.2004-0536]

27. Deshayes F, Nahmias C. Angiotensin receptors: A new role in cancer? Trends Endocrinol Metab 2005;16:293–9 [PMID: 16061390 DOI: 10.1016/j.tem.2005.07.009]

28. Ager EI, Neo J, Christophi C. The renin-angiotensin system and malignancy. Carcinogenesis2008;29:1675–84 [PMID: 18632755 DOI: 10.1093/carcin/bgn171]

29. Bernasconi R, Nyström A. Balance and circumstance: The renin angiotensin system in wound healing and fibrosis. Cell. Signal.2018;51:34–46 [PMID: 30071289 DOI: 10.1016/j.cellsig.2018.07.011]

30. Steckelings UM, Wollschläger T, Peters J, Henz BM, Hermes B, Artuc M. Human skin: Source of and target organ for angiotensin II. Exp Dermatol 2004;13:148–54 [PMID: 14987254 DOI: 10.1111/j.0906-6705.2004.0139.x]

31. Moreno-Muñoz D, de la Haba-Rodríguez JR, Conde F, López-Sánchez LM, Valverde A, Hernández V, Martínez A, Villar C, Gómez-España A, Porras I, Rodríguez-Ariza A, Aranda E. Genetic variants in the renin-angiotensin system predict response to bevacizumab in cancer patients. Eur J Clin Invest 2015;45:1325–32 [PMID: 26509357 DOI: 10.1111/eci.12557]

32. Perdomo-Pantoja A, Mejía-Pérez SI, Reynoso-Noverón N, Gómez-Flores-Ramos L, Soto-Reyes E, Sánchez-Correa TE, Guerra-Calderas L, Castro-Hernandez C, Vidal-Millán S, Sánchez-Corona J, Taja-Chayeb L, Gutiérrez O, Cacho-Diaz B, Alvarez-Gomez RM, Gómez-Amador JL, Ostrosky-Wegman P, Corona T, Herrera-Montalvo LA, Wegman-Ostrosky T. Angiotensinogen rs5050 germline genetic variant as potential biomarker of poor prognosis in astrocytoma. PLoS One 2018;13 [PMID: 30383794 DOI: 10.1371/journal.pone.0206590]

33. Goswami AM. Computational analyses prioritize and reveal the deleterious nsSNPs in human angiotensinogen gene. Comput Biol Chem 2020;84 [PMID: 31931433 DOI: 10.1016/j.compbiolchem.2019.107199]

34. Feng H, Wei X, Pang L, Wu Y, Hu B, Ruan Y, Liu Z, Liu J, Wang T. Prognostic and Immunological Value of Angiotensin-Converting Enzyme 2 in Pan-Cancer. Front Mol Biosci 2020;7 [DOI: 10.3389/fmolb.2020.00189]

35. Urup T, Michaelsen SR, Olsen LR, Toft A, Christensen IJ, Grunnet K, Winther O, Broholm H, Kosteljanetz M, Issazadeh-Navikas S, Poulsen HS, Lassen U. Angiotensinogen and HLA class II predict bevacizumab response in recurrent glioblastoma patients. Mol Oncol 2016;10:1160–8 [PMID: 27262894 DOI: 10.1016/j.molonc.2016.05.005]

36. Jeunemaitre X, Gimenez-Roqueplo AP, Célérier J, Corvol P. Angiotensinogen variants and human hypertension. Curr Hypertens Rep 1999;1:31–41 [PMID: 10981040 DOI: 10.1007/s11906-999-0071-0]

37. Ehret GB, Munroe PB, Rice KM, Bochud M, Johnson AD, Chasman DI, Smith A V., Tobin MD, Verwoert GC, Hwang SJ, Pihur V, Vollenweider P, O'Reilly PF, Amin N, Bragg-Gresham JL, Teumer A, Glazer NL, Launer L, Zhao JH, Aulchenko Y, Heath S, Sober S, Parsa A, Luan J, Arora P, Dehghan A, Zhang F, Lucas G, Hicks AA, Jackson AU,

Peden JF, Tanaka T, Wild SH, Rudan I, Igl W, Milaneschi Y, Parker AN, Fava C, Chambers JC, Fox ER, Kumari M, Go MJ, van der Harst P, Kao WHL, Sjögren M, Vinay DG, Alexander M, Tabara Y, Shaw-Hawkins S, Whincup PH, Liu Y, Shi G, Kuusisto J, Tayo B, Seielstad M, Sim X, Hoang Nguyen KD, Lehtimäki T, Matullo G, Wu Y, Gaunt TR, Charlotte Onland-Moret N, Cooper MN, Platou CGP, Org E, Hardy R, Dahgam S, Palmen J, Vitart V, Braund PS, Kuznetsova T, Uiterwaal CSPM, Adeyemo A, Palmas W, Campbell H, Ludwig B, Tomaszewski M, Tzoulaki I, Palmer ND, Aspelund T, Garcia M, Chang YPC, O'Connell JR, Steinle NI, Grobbee DE, Arking DE, Kardia SL, Morrison AC, Hernandez D, Najjar S, McArdle WL, Hadley D, Brown MJ, Connell JM, Hingorani AD, Day INM, Lawlor DA, Beilby JP, Lawrence RW, Clarke R, Hopewell JC, Ongen H, Dreisbach AW, Li Y, Hunter Young J, Bis JC, Kähönen M, Viikari J, Adair LS, Lee NR, Chen MH, Olden M, Pattaro C, Bolton JAH, Köttgen A, Bergmann S, Mooser V, Chaturvedi N, Frayling TM, Islam M, Jafar TH, Erdmann J, Kulkarni SR, Bornstein SR, Grässler J, Groop L, Voight BF, Kettunen J, Howard P, Taylor A, Guarrera S, Ricceri F, Emilsson V, Plump A, Barroso I, Khaw KT, Weder AB, Hunt SC, Sun Y V., Bergman RN, Collins FS, Bonnycastle LL, Scott LJ, Stringham HM, Peltonen L, Perola M, Vartiainen E, Brand SM, Staessen JA, Wang TJ, Burton PR, Artigas MS, Dong Y, Snieder H, Wang X, Zhu

H, Lohman KK, Rudock ME, Heckbert SR, Smith NL, Wiggins KL, Doumatey A, Shriner D, Veldre G, Viigimaa M, Kinra S, Prabhakaran D, Tripathy V, Langefeld CD, Rosengren A, Thelle DS, Corsi AM, Singleton A, Forrester T, Hilton G, McKenzie CA, Salako T, Iwai N, Kita Y, Ogihara T, Ohkubo T, Okamura T, Ueshima H, Umemura S, Eyheramendy S, Meitinger T, Wichmann HE, Cho YS, Kim HL, Lee JY, Scott J, Sehmi JS, Zhang W, Hedblad B, Nilsson P, Smith GD, Wong A, Narisu N, Stančáková A, Raffel LJ, Yao J, Kathiresan S, O'Donnell CJ, Schwartz SM, Arfan Ikram M, Longstreth WT, Mosley TH, Seshadri S, Shrine NRG, Wain L V., Morken MA, Swift AJ, Laitinen J, Prokopenko I, Zitting P, Cooper JA, Humphries SE, Danesh J, Rasheed A, Goel A, Hamsten A, Watkins H, Bakker SJL, van Gilst WH, Janipalli CS, Radha Mani K, Yajnik CS, Hofman A, Mattace-Raso FUS, Oostra BA, Demirkan A, Isaacs A, Rivadeneira F, Lakatta EG, Orru M, Scuteri A, Ala-Korpela M, Kangas AJ, Lyytikäinen LP, Soininen P, Tukiainen T, Würtz P, Twee-HeeOng R, Dörr M, Kroemer HK, Völker U, Völzke H, Galan P, Hercberg S, Lathrop M, Zelenika D, Deloukas P, Mangino M, Spector TD, Zhai G, Meschia JF, Nalls MA, Sharma P, Terzic J, Kranthi Kumar M V., Denniff M, Zukowska-Szczechowska E, Wagenknecht LE, Fowkes FGR, Charchar FJ, Schwarz PEH, Hayward C, Guo X, Rotimi C, Bots ML, Brand E, Samani NJ, Polasek O, Talmud PJ, Nyberg F, Kuh D,

Laan M, Hveem K, Palmer LJ, van der Schouw YT, Casas JP, Mohlke KL, Vineis P, Raitakari O, Ganesh SK, Wong TY, Shyong Tai E, Cooper RS, Laakso M, Rao DC, Harris TB, Morris RW, Dominiczak AF, Kivimaki M, Marmot MG, Miki T, Saleheen D, Chandak GR, Coresh J, Navis G, Salomaa V, Han BG, Zhu X, Kooner JS, Melander O, Ridker PM, Bandinelli S, Gyllensten UB, Wright AF, Wilson JF, Ferrucci L, Farrall M, Tuomilehto J, Pramstaller PP, Elosua R, Soranzo N, Sijbrands EJG, Altshuler D, Loos RJF, Shuldiner AR, Gieger C, Meneton P, Uitterlinden AG, Wareham NJ, Gudnason V, Rotter JI, Rettig R, Uda M, Strachan DP, Witteman JCM, Hartikainen AL, Beckmann JS, Boerwinkle E, Vasan RS, Boehnke M, Larson MG, Järvelin MR, Psaty BM, Abecasis GR, Chakravarti A, Elliott P, van Duijn CM, Newton-Cheh C, Levy D, Caulfield MJ, Johnson T. Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. Nature 2011;478:103–9 [PMID: 21909115 DOI: 10.1038/nature10405]

38. Charita B, Padma G, Sushma P, Deepak P, Padma T. Estimation of risk and interaction of single nucleotide polymorphisms at angiotensinogen locus causing susceptibility to essential hypertension: A case control study. JRAAS - J Renin-Angiotensin-Aldosterone Syst 2012;13:461–71 [PMID: 22570327 DOI: 10.1177/1470320312444650]

Tables

Table 1. Baseline characteristics of patients included in each cohort.

	BCLC1 cohort	**BCLC2 cohort**	**Northern Italy cohort**	**Naples cohort**
Patients, n	82	79	221	69
Gender (Male)	73 (89.02)	67 (84.81)	184 (83.26)	60 (86.96)
Age (Years)	63 [56 – 71]	63 [56 – 72]	69 [60 – 74]	70 [60 – 74]
AGT1 (rs699)				
AA	26 (31.71)	25 (31.65)	72 (32.58)	22 (31.88)
AG	34 (41.46)	35 (44.3)	101 (45.7)	38 (55.07)
GG	22 (26.83)	19 (24.05)	47 (21.27)	9 (13.04)
NA	0 (0)	0 (0)	1 (0.45)	0 (0)

AGT2 (rs4762)				
AA	5 (6.1)	3 (3.8)	5 (2.26)	0 (0)
AG	16 (19.51)	10 (12.66)	44 (19.91)	15 (21.74)
GG	61 (74.39)	66 (83.54)	172 (77.83)	54 (78.26)
AHT (Yes)	37 (45.12)	36 (45.57)	65 (29.41)	45 (65.22)
Diabetes (Yes)	22 (26.83)	28 (35.44)	61 (27.6)	23 (33.33)
HBV (Yes)	10 (12.2)	6 (7.59)	46 (20.81)	12 (17.39)
HCV (Yes)	54 (65.85)	38 (48.1)	111 (50.23)	44 (63.77)
HIV (Yes)	2 (2.44)	1 (1.27)	3 (1.36)	0 (0)
Child-Pugh				
A: 5-6	70 (85.37)	63 (79.75)	207 (93.67)	58 (84.06)
B: 7-9	10 (12.2)	11 (13.93)	14 (6.33)	10 (14.49)
Not applicable	2 (2.44)	5 (6.33)	0 (0)	1 (1.45)
ECOG-PS (0)	77 (93.9)	74 (93.67)	155 (70.14)	69 (100)
Ascites (Yes)	11 (13.41)	9 (11.39)	25 (11.31)	14 (20.29)

Encephalopathy (Yes)	0 (0)	0 (0)	11 (4.98)	0 (0)
Extrahepatic spread (Yes)	24 (29.27)	27 (34.18)	79 (35.75)	23 (33.33)
Vascular Invasion (Yes)	22 (26.83)	26 (32.91)	61 (27.6)	31 (44.93)
BCLC (A† or B / C)	42 (51.22) / 40 (48.78)	36 (45.57) / 43 (54.43)	76 (34.39) / 145 (65.61)	20 (28.99) / 49 (71.01)
Alpha-fetoprotein (ng/ml)	20.5 (7 - 212.5)	25 (8 - 228)	100.5 (10 - 869)	98 (5 - 1903)
Hemoglobin basal (g/dl)	13.8 [12.95 - 14.95]	13.1 [11.9 - 14.5]	12.5 [11.2 - 14]	13 [11.9 - 13.9]
prothrombin time (%)	88.3 [76.5 - 95.6]	76 [65 - 88]	NA	84.5 [76 - 100]
International Normalized Ratio	NA	NA	1.1 [1 - 1.22]	1.13 [1.03 - 1.24]

Total bilirubin (mg/dl)	1 [0.8 - 1.6]	1.1 [0.6 - 1.7]	0.9 [0.72 - 1.3]	0.95 [0.7 - 1.4]
AST (UI/L)	78 [46 - 119]	54 [34 - 84]	NA	52 [35 - 80]
ALT (UI/L)	72 [35 - 106.5]	44 [25 - 65]	43 [23 - 56]	42 [32 - 55]
GGT (UI/L)	134.5 [93.5 - 285.5]	143 [83 - 264]	NA	96 [48 - 204]
Albumin (mg/L)	38.5 [35 - 43]	40 [35 - 43]	38 [35 - 40]	3.6 [3.3 - 4]
Initial dosage of sorafenib (mg)				
400	5 (6.1)	2 (2.6)	19 (8.6)	0 (0)
600	0 (0)	0 (0)	5 (2.26)	0 (0)
800	77 (93.9)	77 (97.4)	197 (89.14)	69 (100)

Descriptive statistics are frequencies (%) or median [IQR: Interquartile range], as appropriate.

AHT: Arterial Hypertension; HCV: Hepatitis C Virus; HBV: Hepatitis B Virus; HIV: human immunodeficiency virus; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: Gamma glutamyl transpeptidase;

IQR: Interquartile range; ECOG-PS: Eastern Cooperative Oncology Group Performance Status; BCLC: Barcelona Clinic Liver Cancer; INR: International Normalized Ratio.

†5 BCLC A patients; NA: Not available.

Table 2. Overall survival of each cohort by SNPs.

		SNP alleles (A/G)	Patients at risk	Events	Median OS (95%CI), months	P-value (log-rank)
BCLC- 1 cohort			82	75	18.81 (14.76-23.58)	
BCLC- 2 cohort			79	47	18.32 (13.05-26.44)	
Northern Italy cohort			221	180	14.3 (11.84 - 17.99)	
Naples cohort			69	57	9.9 (7.69-12.82)	
BCLC- 1 cohort	*AGT1* (rs699)		82	75		0.16
		AA	26	23	18.73 (11.84-41.4)	

		AG	34	33	18.43 (10.75-22.76)	
		GG	22	19	18.81 (9.67-30.42)	
	AGT2 (rs4762)		82	75		0.4
		AA	5	5	41.34 (0.39-74.12)	
		AG	16	15	13.95 (7.3-23.87)	
		GG	61	55	19.11 (14.86-24.47)	
BCLC- 2 cohort	*AGT1* (rs699)		79	47		0.15
		AA	25	15	23.74 (7.46-26.5)	

		AG	35	19	21.74 (11.15-33.77)	
		GG	19	13	6.64 (3.42-30.29)	
	AGT2 (rs4762)		79	47		0.3
		AA	3	1	NE (13.61-NE)	
		AG	10	5	30.29 (3.88-32.69)	
		GG	66	41	16.41 (8.78-23.74)	
Northern Italy cohort	*AGT1* (rs699)		220	179		0.5
		AA	72	58	13.58 (10.92 - 19.2)	

		AG	101	83	17.59 (10.85 - 20.68)	
		GG	47	38	12.43 (8.81 - 20.68)	
	AGT2 (rs4762)		221	180		0.7
		AA	5	2	NE (1.94 - NE)	
		AG	44	36	14.3 (7.46 - 20.68)	
		GG	172	142	14.9 (11.25 - 18.09)	
Naples cohort	*AGT1* (rs699)		69	57		0.7
		AA	22	19	12.66 (6.15-18.25)	

		AG	38	31	8.32 (4.9-11.71)	
		GG	9	7	10.95 (2.6-21.83)	
	AGT2 (rs4762)		69	57		0.6
		AG	15	11	9.8 (2.89-24.93)	
		GG	54	46	10.1 (7.14-12.82)	

NE: Not estimable; OS: Overall Survival; 95%CI: 95% confidence interval; SNP: Single-Nucleotide Polymorphisms; BCLC: Barcelona Clinic Liver Cancer.

Table 3. Follow-up and evolutionary events in included patients of each cohort.

	BCLC1 cohort	**BCLC2 cohort**	**Northern Italy cohort**	**Naples cohort**
Patients, n	82	79	221	69
Follow-up (months)	18.58 [10.33 - 34.17]	13.05 [6.64 - 22.36]	12.73 [6.05 - 25.88]	9.87 [4.51 - 18.25]
Treatment duration (months)	9.06 [4.11 - 17.46]	5.95 [2.14 - 13.52]	8.52 [2.56 - 20.78]	8.06 [3.72 - 16.97]
Adverse Events				
Gastrointestinal (Yes)	35 (42.68)	27 (34.18)	23 (10.41)	38 (55.07)
Dermatologic (Yes)	42 (51.22)	28 (35.44)	32 (14.48)	27 (39.13)
Early Dermatologic (Yes)	33 (40.24)	22 (27.85)	28 (12.67)	25 (36.23)

Performance status deterioration (Yes)	44 (53.66)	46 (58.23)	53 (23.98)	0 (0)
Cardiovascular (Yes)	18 (21.95)	14 (17.72)	16 (7.24)	16 (23.19)
Dermatologic and Cardiovascular simultaneously (Yes)	7 (8.54)	5 (6.33)	0 (0)	10 (14.49)
Other (Yes)	48 (58.54)	34 (43.04)	45 (20.36)	65 (94.2)
Death (Yes)	75 (91.46)	47 (59.49)	180 (81.44)	57 (82.61)
Cause of death				
HCC	74 (98.67)	46 (97.87)	118 (65.56)	48 (84.21)
Not HCC related	0 (0)	1 (2.13)	58 (32.22)	9 (15.79)
Others†	1 (1.33)	0 (0)	4 (2.22)	0 (0)

Descriptive statistics are frequencies (%) or median [IQR: Interquartile range], as appropriate.

AE: Adverse events; DAE: Dermatological Adverse Events; eDAE: early Dermatological Adverse Events; IQR: Interquartile range.

†Other causes of Exitus are: 1 Sudden death, 4 unknown.

Table 4. Cox regression models for eDAE and DAE by AGT1 (rs699).

Event	Centre	*AGT1* (rs699)	HR (95% CI)	p-value	HR (95%CI) adjusted by BCLC + ECOG-PS	p-value	HR (95%CI) adjusted by BCLC + ECOG-PS + AHT + DM	p-value	HR (95%CI) adjusted for AHT + DM	p-value	HR (95%CI) adjusted forDM	p-value	HR (95% CI) adjusted for AHT	p-value
eDAE	BCLC1cohort	AA vs AG	2.31 (1.03-5.14)	0.04	2.3 (1.02-5.16)	0.04	2.34 (1.02-5.37)	0.04	2.33 (1.03-5.24)	0.04	2.45 (1.1-5.5)	0.03	2.24 (1-5.03)	0.049
		AA vs GG	1.68 (0.71-3.97)	0.2	1.69 (0.71-4)	0.2	1.64 (0.69-3.93)	0.3	1.65 (0.69-3.92)	0.3	1.75 (0.74-4.13)	0.2	1.62 (0.68-3.87)	0.3
		AG vs GG	0.73 (0.29-1.85)	0.5	0.73 (0.29-1.89)	0.5	0.7 (0.27 - 1.82)	0.5	0.71 (0.28-1.79)	0.5	0.71 (0.28-1.8)	0.5	0.72 (0.29-1.84)	0.5
	BCLC2 cohort	AA vs AG	0.66 (0.25-1.76)	0.4	0.63 (0.24-1.7)	0.4	0.71 (0.26-1.93)	0.5	0.72 (0.27-1.93)	0.5	0.72 (0.27-1.91)	0.5	0.68 (0.25-1.83)	0.5
		AA vs GG	1.13 (0.32-4.01)	0.9	1.08 (0.3-3.84)	0.9	1.35 (0.37-4.95)	0.7	1.36 (0.38-4.9)	0.7	1.32 (0.37-4.72)	0.7	1.13 (0.32-4)	0.9
		AG vs GG	1.71 (0.55-5.3)	0.4	1.7 (0.55-5.28)	0.4	1.89 (0.6 - 5.91)	0.3	1.89 (0.6 -5.9)	0.3	1.85 (0.6 -5.74)	0.3	1.66 (0.53-5.17)	0.4
	Northern Italy cohort	AA vs AG	0.8 (0.33-1.95)	0.6	0.75 (0.3-1.86)	0.5	1.02 (0.4 - 2.61)	0.9	0.96 (0.39-2.36)	0.9	0.83 (0.34-2.02)	0.7	0.91 (0.37-2.23)	0.8

		AA vs GG	0.9 (0.31-2.6)	0.8	0.71 (0.24-2.1)	0.5	0.96 (0.31-2.98)	0.9	1.22 (0.4-3.73)	0.7	0.96 (0.33-2.8)	0.9	1.12 (0.37-3.36)	0.8
		AG vs GG	1.12 (0.42-3.01)	0.8	0.95 (0.35-2.58)	0.9	0.94 (0.33-2.69)	0.9	1.27 (0.46-3.49)	0.7	1.15 (0.43-3.12)	0.8	1.23 (0.45-3.34)	0.7
	Naples cohort	AA vs AG	1.26 (0.54-2.95)	0.6	1.21 (0.51-2.86)	0.7	1.35 (0.56-3.27)	0.5	1.36 (0.57-3.25)	0.5	1.23 (0.52-2.93)	0.6	1.44 (0.61-3.39)	0.4
		AA vs GG	1.26 (0.34-4.66)	0.7	1.18 (0.31-4.43)	0.8	1.33 (0.35- 5)	0.7	1.34 (0.36-4.96)	0.7	1.27 (0.34-4.68)	0.7	1.35 (0.37-5)	0.7
		AG vs GG	1 (0.28-3.51)	0.9	0.97 (0.28-3.43)	0.9	0.98 (0.28-3.49)	0.9	0.99 (0.28-3.49)	0.9	1.03 (0.29-3.65)	0.9	0.94 (0.27-3.3)	0.9
	BCLC2 cohort + Naples cohort + Northern Italy cohort	AA vs AG	0.87 (0.52-1.47)	0.6	0.85 (0.51-1.43)	0.5	0.84 (0.5 - 1.41)	0.5	0.85 (0.51-1.43)	0.6	0.87 (0.52-1.47)	0.6	0.85 (0.51-1.43)	0.6
		AA vs GG	1.05 (0.54-2.04)	0.9	0.95 (0.49-1.86)	0.9	0.92 (0.47-1.81)	0.8	1.01 (0.52-1.97)	0.9	1.05 (0.54-2.04)	0.9	1.01 (0.52-1.97)	0.9
		AG vs GG	1.2 (0.65-2.22)	0.6	1.12 (0.61-2.08)	0.7	1.1 (0.59 - 2.05)	0.8	1.18 (0.64-2.18)	0.6	1.2 (0.65 -2.22)	0.6	1.18 (0.64-2.18)	0.6
	BCLC1 cohort + Naples cohort + Northern	AA vs AG	1.35 (0.84-2.17)	0.2	1.35 (0.84-2.18)	0.2	1.33 (0.82-2.15)	0.2	1.31 (0.81-2.11)	0.3	1.35 (0.84-2.18)	0.2	1.3 (0.81 - 2.1)	0.3
		AA vs GG	1.19 (0.67-2.12)	0.6	1.13 (0.6-2.01)	0.7	1.08 (0.6 - 1.93)	0.8	1.1 (0.61 -1.97)	0.8	1.19 (0.67-2.12)	0.6	1.09 (0.61-1.96)	0.8

	Italy cohort	AG vs GG	0.88 (0.5-1.55)	0.7	0.83 (0.47-1.48)	0.5	0.81 (0.46-1.43)	0.5	0.84 (0.48-1.48)	0.6	0.88 (0.5 -1.55)	0.7	0.84 (0.48-1.48)	0.6
	BCLC1c ohort + BCLC2 cohort + Naples cohort	AA vs AG	1.32 (0.81-2.15)	0.3	1.29 (0.79-2.11)	0.3	1.3 (0.79 - 2.12)	0.3	1.31 (0.81-2.14)	0.3	1.33 (0.82-2.17)	0.3	1.31 (0.8 - 2.13)	0.3
		AA vs GG	1.4 (0.75-2.6)	0.3	1.38 (0.7-2.57)	0.3	1.44 (0.77-2.69)	0.3	1.45 (0.78-2.7)	0.2	1.46 (0.79-2.72)	0.2	1.38 (0.74-2.57)	0.3
		AG vs GG	1.06 (0.58-1.94)	0.9	1.06 (0.58-1.95)	0.9	1.11 (0.6 - 2.03)	0.8	1.1 (0.6 - 2.02)	0.8	1.1 (0.6 - 2.01)	0.8	1.06 (0.58-1.94)	0.9
DAE	BCLC1c ohort	AA vs AG	2.7 (1.27-5.75)	0.01	2.68 (1.25-5.77)	0.01	2.52 (1.16-5.47)	0.02	2.6 (1.21 -5.57)	0.01	2.82 (1.32-6.06)	0.008	2.5 (1.17 - 5.35)	0.02
		AA vs GG	1.26 (0.62-2.58)	0.5	1.24 (0.61-2.55)	0.6	1.11 (0.53-2.31)	0.8	1.13 (0.55-2.35)	0.8	1.3 (0.63 -2.66)	0.5	1.12 (0.54-2.32)	0.8
		AG vs GG	0.47 (0.21-1.05)	0.06	0.46 (0.2-1.06)	0.07	0.44 (0.19-1.01)	0.053	0.44 (0.19 - 0.98)	0.045	0.46 (0.2 -1.03)	0.06	0.45 (0.2 - 1.01)	0.052
	BCLC2 cohort	AA vs AG	0.98 (0.43-2.2)	0.9	0.94 (0.42-2.13)	0.9	0.99 (0.43-2.26)	0.9	1.01 (0.45-2.3)	0.9	1.03 (0.45-2.32)	0.9	0.95 (0.42-2.16)	0.9
		AA vs GG	1.89 (0.59-6.04)	0.3	1.78 (0.55-5.76)	0.3	2.08 (0.63-6.85)	0.2	2.18 (0.67-7.03)	0.19	2.08 (0.65-6.66)	0.2	1.88 (0.59-6.01)	0.3
		AG vs GG	1.94 (0.64-5.9)	0.2	1.89 (0.62-5.77)	0.3	2.12 (0.69-6.49)	0.19	2.15 (0.7 -6.57)	0.18	2.02 (0.66-6.15)	0.2	1.98 (0.65-6.05)	0.2

	Northern Italy cohort	AA vs AG	0.89 (0.39-2.06)	0.8	0.85 (0.37-1.98)	0.7	1.01 (0.42-2.41)	0.9	1 (0.42 - 2.33)	0.9	0.91 (0.39-2.11)	0.8	0.95 (0.41-2.22)	0.9
		AA vs GG	0.62 (0.25-1.57)	0.3	0.54 (0.21-1.37)	0.2	0.6 (0.23 - 1.6)	0.3	0.74 (0.28-1.92)	0.5	0.64 (0.25-1.62)	0.4	0.7 (0.27 - 1.79)	0.5
		AG vs GG	0.7 (0.3-1.62)	0.3	0.63 (0.27-1.48)	0.2	0.6 (0.25 - 1.43)	0.2	0.74 (0.32-1.72)	0.5	0.71 (0.3 -1.63)	0.4	0.73 (0.31-1.69)	0.5
	Naples cohort	AA vs AG	1.29 (0.57-2.92)	0.5	1.23 (0.54-2.81)	0.6	1.35 (0.58-3.15)	0.5	1.38 (0.6 -3.17)	0.5	1.23 (0.54-2.81)	0.6	1.49 (0.66-3.4)	0.3
		AA vs GG	1.38 (0.38-5.03)	0.6	1.28 (0.35-4.73)	0.7	1.45 (0.39-5.36)	0.6	1.49 (0.41-5.41)	0.6	1.39 (0.38-5.05)	0.6	1.51 (0.41-5.51)	0.5
		AG vs GG	1.07 (0.31-3.72)	0.9	1.04 (0.3-3.62)	0.9	1.08 (0.31-3.77)	0.9	1.08 (0.31-3.79)	0.9	1.13 (0.32-3.96)	0.9	1.01 (0.29-3.52)	0.9
	BCLC2 cohort + Naples cohort + Northern Italy cohort	AA vs AG	1 (0.62-1.61)	0.9	0.98 (0.61-1.57)	0.9	0.95 (0.59-1.54)	0.9	0.97 (0.6 -1.56)	0.9	1.01 (0.63-1.62)	0.9	0.96 (0.6 - 1.55)	0.9
		AA vs GG	1.13 (0.61-2.08)	0.7	1.04 (0.56-1.92)	0.9	0.98 (0.53-1.81)	0.9	1.05 (0.57-1.95)	0.9	1.12 (0.61-2.07)	0.7	1.05 (0.57-1.95)	0.9
		AG vs GG	1.13 (0.63-2)	0.7	1.06 (0.59-1.89)	0.8	1.02 (0.57-1.83)	0.9	1.09 (0.61-1.94)	0.8	1.12 (0.63-1.99)	0.7	1.09 (0.61-1.95)	0.8
	BCLC1c ohort + Naples	AA vs AG	1.43 (0.91-2.24)	0.12	1.43 (0.91-2.24)	0.12	1.39 (0.88-2.19)	0.15	1.36 (0.87-2.14)	0.18	1.44 (0.92-2.26)	0.11	1.35 (0.86-2.12)	0.19

	cohort + Northern Italy cohort	AA vs GG	0.94 (0.57-1.56)	0.8	0.9 (0.54-1.51)	0.7	0.82 (0.49-1.38)	0.5	0.83 (0.49-1.39)	0.5	0.94 (0.57-1.57)	0.8	0.82 (0.49-1.38)	0.5
		AG vs GG	0.66 (0.4-1.09)	0.1	0.63 (0.38-1.05	0.08	0.59 (0.36-0.99)	0.04	0.61 (0.37-1.01)	0.052	0.66 (0.4 -1.08)	0.1	0.61 (0.37-1.01)	0.053
	BCLC1c ohort + BCLC2 cohort + Naples cohort	AA vs AG	1.54 (0.98-2.41)	0.06	1.49 (0.95-2.34)	0.08	1.48 (0.94-2.32)	0.09	1.52 (0.97-2.37)	0.07	1.55 (0.99-2.43)	0.055	1.5 (0.96 - 2.35)	0.07
		AA vs GG	1.35 (0.78-2.32)	0.3	1.3 (0.75-2.25)	0.3	1.32 (0.76-2.28)	0.3	1.35 (0.78-2.33)	0.3	1.39 (0.81-2.4)	0.2	1.3 (0.76 - 2.24)	0.3
		AG vs GG	0.88 (0.51-1.51)	0.6	0.87 (0.51-1.5)	0.6	0.89 (0.52-1.54)	0.7	0.89 (0.52-1.54)	0.7	0.9 (0.52 -1.54)	0.7	0.87 (0.5 - 1.49)	0.6

AE: Adverse events; DAE: Dermatological Adverse Events; eDAE: early Dermatological Adverse Events; HR: Hazard Ratio; AHT: Arterial Hypertension; DM: Diabetes Mellitus; ECOG: Eastern Cooperative Oncology Group; BCLC: Barcelona Clinic Liver Cancer

Table 5. Cox regression models for eDAE and DAE by AGT2 (rs4762).

Event	Center	*AGT2*(rs4762)	HR (95%CI)	p-value	HR (95%CI) adjusted for BCLC + ECOG-PS	p-value	HR (95%CI) adjusted for BCLC + ECOG-PS + AHT + DM	p-value	HR (95%CI) adjusted for AHT + DM	p-value	HR (95%CI) adjusted for DM	p-value	HR (95% CI) adjusted for AHT	p-value
eDAE	BCLC1c ohort	AA vs AG	1.14 (0.22-5.89)	0.9	0.98 (0.19-5.12)	0.9	0.97 (0.18-5.04)	0.9	1.09 (0.21-5.64)	0.9	1.15 (0.22-5.95)	0.9	1.11 (0.21-5.72)	0.9
		AA vs GG	0.84 (0.2-3.53)	0.8	0.73 (0.17-3.15)	0.7	0.71 (0.16-3.1)	0.7	0.81 (0.19-3.4)	0.8	0.8 (0.19-3.39)	0.8	0.84 (0.2-3.54)	0.8
		AG vs GG	0.73 (0.28-1.91)	0.5	0.74 (0.28-1.94)	0.5	0.74 (0.28-1.97)	0.6	0.74 (0.28-1.94)	0.6	0.7 (0.27-1.82)	0.5	0.76 (0.29-1.98)	0.6
	BCLC2 cohort	AA vs AG	3.71 (0.62-22.39)	0.2	3.52 (0.58-21.5)	0.2	4.8 (0.74-31.28)	0.1	4.81 (0.74-31.24)	0.1	4.78 (0.76-29.88)	0.09	4.46 (0.7-28.35)	0.11
		AA vs GG	4.43 (1.01-19.39)	0.048	4.24 (0.95-19.06)	0.06	6.14 (1.28-29.55)	0.02	6.28 (1.32-29.95)	0.02	6.25 (1.35-28.89)	0.02	5.34 (1.15-24.86)	0.03
		AG vs GG	1.19 (0.35-4.08)	0.8	1.21 (0.35-4.15)	0.8	1.28 (0.37-4.45)	0.7	1.31 (0.38-4.47)	0.7	1.31 (0.38-4.47)	0.7	1.2 (0.35-4.08)	0.8
	Northern Italy cohort	AA vs AG	2.72 (0.57-13.1)	0.2	3.21 (0.64-15.99)	0.15	5.61 (1.01-31.12)	0.048	3.43 (0.69-16.96)	0.13	2.69 (0.56-12.97)	0.2	3.2 (0.66-15.6)	0.15
		AA vs GG	4.54 (1.05-19.64)	0.04	5.15 (1.17-22.63)	0.03	8.51 (1.78-40.54)	0.007	5.51 (1.25-24.33)	0.02	4.72 (1.09-20.48)	0.04	4.93 (1.13-21.41)	0.03

		AG vs GG	1.67 (0.69-4.02)	0.3	1.6 (0.66-3.9)	0.3	1.52 (0.6-3.82)	0.4	1.61 (0.66-3.9)	0.3	1.75 (0.73-4.24)	0.2	1.54 (0.63-3.73)	0.3
	Naples cohort	AG vs GG	1.2 (0.48-3.01)	0.7	1.2 (0.48-3.02)	0.7	1.25 (0.5-3.15)	0.6	1.26 (0.5-3.16)	0.6	1.2 (0.48-3)	0.7	1.29 (0.51-3.23)	0.6
	BCLC2 cohort + Naples cohort + Northern Italy cohort	AA vs AG	2.76 (0.92-8.27)	0.07	2.95 (0.97-9.84)	0.06	2.78 (0.9-8.56)	0.07	2.61 (0.87-7.86)	0.09	2.75 (0.92-8.25)	0.07	2.61 (0.87-7.86)	0.09
		AA vs GG	3.5 (1.27-9.67)	0.02	3.8 (1.36-10.58)	0.01	3.67 (1.31-10.3)	0.01	3.39 (1.22-9.37)	0.02	3.5 (1.27-9.66)	0.02	3.38 (1.22-9.37)	0.02
		AG vs GG	1.27 (0.73-9.67)	0.4	1.29 (0.74-2.25)	0.4	1.32 (0.75-2.32)	0.3	1.3 (0.74-2.27)	0.4	1.27 (0.73-2.22)	0.4	1.3 (0.74-2.27)	0.4
	BCLC1cohort + Naples cohort + Northern Italy cohort	AA vs AG	1.66 (0.65-4.9)	0.4	1.63 (0.55-4.85)	0.4	1.53 (0.51-4.57)	0.5	1.54 (0.52-4.57)	0.4	1.66 (0.56-4.9)	0.4	1.54 (0.52-4.57)	0.4
		AA vs GG	1.83 (0.67-5.03)	0.2	1.73 (0.63-4.77)	0.3	1.7 (0.62-4.69)	0.3	1.8 (0.65-4.94)	0.3	1.85 (0.67-5.08)	0.2	1.79 (0.65-4.93)	0.3
		AG vs GG	1.1 (0.65-1.86)	0.7	1.06 (0.63-1.81)	0.8	1.11 (0.65-1.9)	0.7	1.17 (0.69-1.97)	0.6	1.11 (0.66-1.88)	0.7	1.16 (0.69-1.97)	0.6
	BCLC1cohort + BCLC2 cohort + Naples cohort	AA vs AG	1.67 (0.55-5.09)	0.4	1.6 (0.53-4.87)	0.4	1.61 (0.53-4.92)	0.4	1.66 (0.55-5.06)	0.4	1.71 (0.56-5.19)	0.4	1.63 (0.54-4.95)	0.4
		AA vs GG	1.7 (0.62-4.67)	0.3	1.67 (0.61-4.59)	0.3	1.68 (0.61-4.63)	0.3	1.7 (0.62-4.67)	0.3	1.7 (0.62-4.67)	0.3	1.68 (0.61-4.62)	0.3

		AG vs GG	1.01 (0.57-1.81)	0.9	1.04 (0.58-1.86)	0.9	1.04 (0.58-1.86)	0.9	1.02 (0.57-1.82)	0.9	0.99 (0.56-1.78)	0.9	1.03 (0.58-1.84)	0.9
DAE	BCLC1c ohort	AA vs AG	2.8 (0.78-10.01)	0.1	2.45 (0.68-8.81)	0.2	2.73 (0.74-9.99)	0.13	3.09 (0.85-11.2)	0.09	2.85 (0.79-10.22)	0.11	2.86 (0.8-10.28)	0.11
		AA vs GG	1.82 (0.64-5.16)	0.3	1.61 (0.56-4.64)	0.4	1.89 (0.64-5.57)	0.2	2.12 (0.74-6.1)	0.16	1.79 (0.63-5.08)	0.3	2.03 (0.71-5.78)	0.19
		AG vs GG	0.65 (0.27-1.56)	0.3	0.66 (0.27-1.59)	0.4	0.69 (0.28-1.72)	0.4	0.69 (0.28-1.68)	0.4	0.63 (0.26-1.52)	0.3	0.71 (0.29-1.71)	0.4
	BCLC2 cohort	AA vs AG	3.83 (0.64-23.05)	0.1	3.71 (0.61-22.68)	0.2	3.91 (0.62-24.73)	0.15	4.05 (0.65-25.33)	0.14	4.49 (0.73-27.55)	0.1	3.79 (0.61-23.44)	0.15
		AA vs GG	3.22 (0.75-13.76)	0.1	3.27 (0.74-14.38)	0.1	3.74 (0.82-17.15)	0.09	3.7 (0.82-16.76)	0.09	4.04 (0.91-18)	0.07	3.18 (0.72-14.13)	0.13
		AG vs GG	0.84 (0.25-2.8)	0.8	0.88 (0.26-2.96)	0.8	0.96 (0.28-3.24)	0.9	0.92 (0.27-3.06)	0.9	0.9 (0.27-3.01)	0.9	0.84 (0.25-2.8)	0.8
	Northern Italy cohort	AA vs AG	2.85 (0.59-13.73)	0.2	3.28 (0.66-16.21)	0.1	4.71 (0.89-24.91)	0.07	3.4 (0.69-16.77)	0.13	2.83 (0.59-13.64)	0.2	3.13 (0.65-15.21)	0.16
		AA vs GG	3.68 (0.86-15.63)	0.08	4.15 (0.96-17.87)	0.06	5.97 (1.32-27.01)	0.02	4.41 (1.02-19.03)	0.046	3.97 (0.93-16.94)	0.06	3.8 (0.89-16.16)	0.07
		AG vs GG	1.29 (0.55-3.01)	0.6	1.26 (0.54-2.96)	0.6	1.27 (0.53-3.05)	0.6	1.3 (0.55-3.05)	0.6	1.4 (0.6-3.29)	0.4	1.21 (0.52-2.84)	0.7

	Naples cohort	AG vs GG	1.12 (0.45-2.77)	0.8	1.11 (0.45-2.76)	0.8	1.12 (0.45-2.79)	0.8	1.13 (0.46-2.82)	0.8	1.12 (0.45-2.77)	0.9	1.16 (0.47-2.88)	0.8
	BCCL2 cohort + Naples cohort + Northern Italy cohort	AA vs AG	2.79 (0.93-8.35)	0.07	3.04 (1-9.21)	0.049	2.72 (0.88-8.34)	0.08	2.54 (0.84-7.63)	0.1	2.74 (0.92-8.21)	0.07	2.56 (0.85-7.7)	0.09
		AA vs GG	2.96 (1.08-8.13)	0.03	3.27 (1.18-9.05)	0.02	3.07 (1.1-8.56)	0.03	2.81 (1.02-7.73)	0.045	2.94 (1.07-8.07)	0.04	2.83 (1.03-7.78)	0.04
		AG vs GG	1.06 (0.62-1.83)	0.8	1.07 (0.62-1.86)	0.8	1.13 (0.65-1.96)	0.7	1.11 (0.64-1.92)	0.7	1.07 (0.62-1.85)	0.8	1.11 (0.64-1.91)	0.7
	BCLC1c ohort + Naples cohort + Northern Italy cohort	AA vs AG	2.82 (1.13-7.07)	0.03	2.9 (1.15-7.32)	0.02	2.7 (1.06-6.84)	0.04	2.66 (1.06-6.69)	0.04	2.81 (1.12-7.05)	0.03	2.68 (1.07-6.74)	0.04
		AA vs GG	2.86 (1.24-6.58)	0.01	2.84 (1.23-6.54)	0.01	2.85 (1.24-6.57)	0.01	2.94 (1.28-6.77)	0.01	2.91 (1.27-6.7)	0.01	2.94 (1.28-6.77)	0.01
		AG vs GG	1.01 (0.61-1.68)	0.9	0.98 (0.59-1.63)	0.9	1.06 (0.63-1.77)	0.8	1.1 (0.67-1.83)	0.7	1.04 (0.63-1.71)	0.9	1.1 (0.66-1.82)	0.7
	BCLC1c ohort + BCLC2 cohort + Naples cohort	AA vs AG	2.94 (1.14-7.6)	0.03	2.85 (1.1-7.39)	0.03	3.12 (1.2-8.14)	0.02	3.21 (1.23-8.34)	0.02	3.05 (1.18-7.9)	0.02	2.9 (1.12-7.5)	0.03
		AA vs GG	2.49 (1.08-5.73)	0.03	2.48 (1.08-5.72)	0.03	2.73 (1.18-6.32)	0.02	2.75 (1.19-6.34)	0.02	2.54 (1.1-5.85)	0.03	2.51 (1.09-5.77)	0.03
		AG vs GG	0.85 (0.49-1.48)	0.6	0.87 (0.5-1.52)	0.6	0.87 (0.5-1.53)	0.7	0.86 (0.49-1.5)	0.6	0.83 (0.48-1.45)	0.5	0.87 (0.5-1.51)	0.6

AE: Adverse events; DAE: Dermatological Adverse Events; eDAE: early Dermatological Adverse Events; HR: Hazard Ratio; AHT: Arterial Hypertension; DM: Diabetes Mellitus; ECOG: Eastern Cooperative Oncology Group; BCLC: Barcelona Clinic Liver Cancer

Figure 1.