The Effects of Drug Exposure and SNPs on Aaptinib-induced Severe Toxicities in Solid Tumors

Youhao Chen1,2, Yaobin Lin1,2, Shaoxing Guan1,2, Zerui Zhao2, Daren Lin3, Jin Guan4, Chengzhi Zhou5, Junling Liu6, Xiaolong Cao7, Zhichao Lin8, Diyao Chen9, Jianbiao Shang10, Weijian Zhang11, Huohui Chen12, Likun Chen6, Shudong Ma13, Lijia Gu14, Jian Zhao15, Min Huang1, Xueding Wang1 & Hao Long2

1 Institute of Clinical Pharmacology, School of Pharmaceutical Sciences, Sun Yat-Sen University, Guangzhou 510006, PR China.

2 Department of Thoracic Oncology, State Key Laboratory of Oncology in South China, Sun Yat-sen University Cancer Center, Guangzhou, Guangdong, 510080, PR China.

3 Department of Medical Oncology, Jiangmen Central Hospital, Jiangmen, Guangdong, 529000, PR China.

4 Department of Oncology, People’s Hospital of Jiangmen, Jiangmen, Guangdong, 529000, PR China.

5 Department of Medical Pneumology, First Affiliated Hospital of Guangzhou Medical University, Guangzhou, Guangdong, 510080, PR China.
6 Department of Medical Oncology, State Key Laboratory of Oncology in South China, Sun Yat-sen University Cancer Center, Guangzhou, Guangdong, 510080, PR China.

7 Department of Medical Oncology, Guangzhou Panyu Central Hospital, Guangzhou, Guangdong, 510080, PR China.

8 Department of Thoracic Surgery, Jiangmen Central Hospital, Jiangmen, Guangdong, 529000, PR China.

9 Department of Targeted Interventional Oncology, First Hospital of Foshan, Foshan, Guangdong, 528000, PR China.

10 Department of Oncology, Wuyi Hospital of Traditional Chinese Medicine, Jiangmen, Guangdong, 529000, PR China.

11 Department of Gynecology, Jiangmen Central Hospital, Jiangmen, Guangdong, 529000, PR China.

12 Department of Medical Oncology, The Second People’s Hospital of Zhaoqing, Zhaoqing, Guangdong, 526000, PR China.

13 Department of Oncology, Nanfang Hospital, Southern Medical University, Guangzhou, Guangdong, 510080, PR China.

14 Department of Cardio-thoracic Surgery, Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, 510080, PR China.
15 Department of Thoracic Surgery, Affiliated Cancer Hospital and Institute of
Guangzhou Medical University, Guangzhou, Guangdong, 510080, PR China.

# These authors contribute to this work equally.
Running Title

Exposure-toxicity relation of apatinib

Correspondence

Hao Long, MD, Professor
E-mail: longhao@sysucc.org.cn
Address: No.651, Dongfeng East Road, Yuexiu District, Guangzhou, Guangdong Province, China.

Xueding Wang, MD, Professor
E-mail: wangxd@mail.sysu.edu.cn
Address: No.132, Waihuan East Road, University City, Panyu District, Guangzhou, Guangdong

Min Huang, PhD, professor
E-mail: huangmin@mail.sysu.edu.cn
Address: No.132, Waihuan East Road, University City, Panyu District, Guangzhou, Guangdong

Number of
Text pages: 18
Tables: 2
Figures: 4
References: 43
Words in the Abstract: 200
Words in Introduction: 572
Words in Discussion: 1386

Abbreviations:
APA, apatinib; AEs, adverse events; C-ANGPTL4, C-terminal fragment of tumor-derived angiopoietin-like 4; GC, gastric cancer; HCC, hepatocellular
carcinoma; HWE, Hardy-Weinberg equilibrium; LC-MS/MS, tandem mass spectrometry; NSCLC, non-small cell lung cancer; PK-PD, pharmacokinetics-pharmacodynamics; RCC, renal cell carcinoma; SNPs, single nucleotide polymorphisms; VEGFR2, vascular endothelial growth factor receptor II.
**Abstract**

Purpose: To investigate the value of drug exposure and host germline genetic factors in predicting apatinib (APA)-related toxicities. Method: In this prospective study, plasma APA concentrations were quantified using liquid chromatography with tandem mass spectrometry, and 57 germline mutations were genotyped in 126 advanced solid tumor patients receiving 250mg daily APA, a vascular endothelial growth factor receptor II inhibitor. The correlation between drug exposure, genetic factors, and the toxicity profile was analyzed. Results: Non-small cell lung cancer (NSCLC) was more prone to APA-related toxicities and plasma concentrations of APA and its main metabolite M1-1 could be associated with high-grade adverse events (AEs) ($P < 0.01$; M1-1: $P < 0.01$) and high-grade anti-angiogenic toxicities (APA: $P = 0.034$; $P < 0.05$), including hypertension, proteinuria and hand-foot syndrome, in the subgroup of NSCLC. Besides, CYP2C9 rs34532201 TT carriers tended to have higher levels of APA ($P < 0.001$) and M1-1 ($P < 0.01$) while CYP2C9 rs1936968 GG carriers were predisposed to higher levels of M1-1 ($P < 0.01$). Conclusion: Plasma APA and M1-1 exposures were able to predict severe AEs in NSCLC patients. Dose optimization and drug exposure monitoring might need considering in NSCLC patients with CYP2C9 rs34532201 TT and rs1936968 GG.
Significance Statement

Apatinib is an anti-VEGFR2 inhibitor for the treatment of multiple cancers. Though substantial in response, apatinib-induced toxicity has been a critical issue that is worth clinical surveillance. Few data on the role of drug exposure and genetic factors in apatinib-induced toxicity are available. Our study demonstrated a distinct drug-exposure relationship in NSCLC but not other tumors and provided invaluable evidence of drug exposure levels and single nucleotide polymorphisms as predictive biomarkers in apatinib-induced severe toxicities.
**Introduction**

Antiangiogenetic agents have revolutionized the treatment of solid tumors through the inhibition of neo-angiogenesis in tumor micro-environment (Ramjiawan et al., 2017). Though substantial in either monotherapy or combination with chemo drugs or immune checkpoint inhibitors (Teleanu et al., 2020), their pharmacological benefits were limited by the common and distinct cardiovascular and dermatological toxicities such as hypertension, proteinuria as well as hand-foot syndrome (Zhu et al., 2007; Poprach et al., 2012; Fuchs et al., 2014). These toxicities pose morbidity to vulnerable cancer patients and require clinical vigilance. Currently, delayed diagnosis and insufficient management strategies for drug-induced toxicity have plagued clinicians for a long time. Therefore, the exploration of predicted biomarkers for toxicity is becoming urgent and necessary.

Apatinib (APA), an oral tyrosine kinase inhibitor targeting vascular endothelial growth factor receptor II (VEGFR2), has been approved in China for the treatment of advanced or metastatic gastric cancer (GC) (Li et al., 2016) and hepatocellular carcinoma (HCC) (Qin et al., 2021). According to the recently published data, treatment was interrupted (60%) or modified (45%) in patients receiving APA as a result of severe and inevitable adverse events (AEs) (Qin et al., 2021). The most frequent grade 3/4 toxicities were hypertension, proteinuria and hand-foot syndrome which are also the main reasons for dose reduction or discontinuation though with varied incidence in different clinical studies (Li et al., 2016; Qin et al., 2021; Lin et al.,...
Clinical trials have initially proved the efficacy of APA in combination with gefitinib or camrelizumab, a programmed death-1 inhibitor, for the treatment of non-small cell cancer (NSCLC) (Zhao et al., 2021) and small cell lung cancer (Fan et al., 2021), esophageal squamous cell carcinoma (Zhang et al., 2020) and HCC (Xu et al., 2021). For a foreseeable future, management of toxicities is particularly challenging with the more complexity in prescription, diversity of cancer types and large individual variation of high patient volume.

Pharmacokinetics-pharmacodynamics (PK-PD) relation and pharmacogenetics are speculated in drug response and adverse reactions for anti-angiogenetic agents (Zhu et al., 2007; Lambrechts et al., 2014). Results from clinical trials showed that the incidence of adverse reactions increased with APA dosage (Song et al., 2020; Yang et al., 2020), making PK a suspicious and relevant biomarker that could be applied to optimize the administration of APA. Notably, the recommended dose of APA varied vastly with cancer types i.e. 750 or 850 mg daily for GC (Li et al., 2016) and HCC (Qin et al., 2021) respectively while only 250 or 500 mg daily for lung cancer (Wu et al., 2018; Duan et al., 2019; Liang et al., 2020), suggesting the exposure-response relationship of APA or its metabolites differs in various types of solid tumors. However, the plasma exposure-response relation of APA remains elusive and the optimal dose for patients needs further investigation. Apart from plasma exposure, pharmacogenetic factors have a great impact on PK as well as the host sensibility to toxicities. Single nucleotide polymorphisms (SNPs) involved VEGFR2 signaling
pathway have been reported to be associated with anti-angiogenic agents’ response and AEs (Jain et al., 2010; Herraez et al., 2013; Qin et al., 2016) while a few studies focus on SNPs in drug metabolism which are indicatives of an exposure-toxicity mechanism. Unfortunately, whether SNPs play an important role in APA-induced toxicity is an uninvestigated area.

Therefore, this study aimed to improve the dosing strategy of APA and identify patients who are at risk of APA-induced toxicity based on the PK-PD analysis and pharmacogenetic study.
Materials and Methods

Patient enrollment
This study was initiated in 2018 (NCT03475589). From March 2018 to May 2020, a total of 126 advanced solid tumor patients were enrolled in this study at Sun Yat-sen University Cancer Center (Guangzhou, China), the Third Affiliated Hospital of Sun Yat-sen University (Guangzhou China), the First Affiliated Hospital of Guangzhou Medical University (Guangzhou, China), Guangzhou Panyu Central Hospital (Guangzhou, China), Affiliated Cancer Hospital and Institute of Guangzhou Medical University (Guangzhou, China), Nanfang Hospital (Guangzhou, China), the People’s Hospital of Jiangmen (Jiangmen, China), Jiangmen Central Hospital (Jiangmen, China) and the First Hospital of Foshan (Foshan, China).

All patients enrolled were above 18 years old and received a single dose of APA daily (250mg) half an hour after a meal for cancer treatment. Patients with uncontrollable hypertension or renal or liver dysfunction before treatment were excluded from this study. Patients who received medication that had potential drug-drug interaction with APA were also excluded from our study. Such medications include substrates, inducers, and inhibitors of CYP3A4 (e.g., barbiturates, rifampin, ketoconazole), warfarin, metoprolol, and drugs that raise the pH of gastric juice persistently (e.g., antacids such as sodium bicarbonate, ranitidine, and omeprazole).

Approximately 4 mL peripheral blood sample of each patient was collected
immediately every 4 weeks during APA treatment. All samples were stored at -80°C until analysis. AEs were recorded and graded according to the Common Terminology Criteria for Adverse Events 4.0. The study was approved by the ethical committee of Sun Yat-sen University Cancer Center. The informed consents were obtained from all patients enrolled in this study.

Quantification of APA and M1-1
APA and its main metabolite M1-1 were quantified by liquid chromatography with tandem mass spectrometry (LC-MS/MS) using our published method (Guan et al., 2019). Briefly, APA and M1-1 were extracted from 50µL plasma with ter-butyl methyl ether at room temperature and separated on a Hypersil GOLD™ aQ C18 Polar Endcapped LC column (50 × 2.1 mm, 1.9 µm, Thermo) with the mobile phase consisting of 5 mmol/L ammonium acetate with 0.1% formic acid:acetonitrile (20:80, v/v). Trough/steady-state concentrations (Cmin/ss) of patients were quantified in plasma samples collected on each follow-up day after at least 4 weeks of treatment. To indicate the total APA or M1-1 exposure over one dosing interval, the average of Cmin/ss of each follow-up was calculated and chosen for further exposure-toxicity relationship and pharmacogenomic study.

DNA extraction and SNP genotyping
The peripheral blood DNA of each patient was extracted by the TIANGEN blood genome extraction kit (TIANGEN, Beijing, China) based on the manufacturer's instructions. A total of 57 tag SNPs (Supp. Table 1), involved in APA transport,
metabolism, and angiogenesis signaling pathway, were selected using HaploView 4.2 and genotyped by a previously published Agena MassARRAY platform (Agena, CA, USA) (Xin et al., 2020). Forward, reverse and extended primers are listed in Supp. Table 1.

Statistical analysis
All statistical analyses were conducted in R 4.0.0 software and GraphPad Prism 9.0 (CA, USA) with appropriate statistical methods. The association between APA/M1-1 and grade≥3 adverse reactions or clinical confounders was analyzed using t test for continuous variables and chi-square test for categorical variables in GraphPad Prism. The difference in the incidence of AEs among tumor types was analyzed by chi-square test in GraphPad Prism. Receiver operating characteristic curve (ROC Curve) analysis was performed in GraphPad Prism to generate the ROC curves of APA/M1-1 and determine the cut-off values accordingly. Hardy-Weinberg equilibrium (HWE) test and the association between SNPs and APA/M1-1 and grade≥3 adverse reactions were analyzed in R language using R package SNPassoc 2.0.2. Multi-variable and multi-linear regression were both performed in GraphPad Prism 9.0 while variable importance evaluation was carried out in R language using the R package randomForest.
Results

Patients’ characteristics

A total of 126 patients with advanced solid tumors were enrolled in this research. The characteristics of enrolled patients at baseline are shown in Table 1. The enrolled patients had a median age of 56 years old with 56% of them being male. AEs and their severity during the treatment were recorded and shown in Table 2. Hand-foot syndrome (51.6%), hypertension (31.0%), and proteinuria (16.7%) were the most frequent AEs after APA treatment, which were also referred to as angiogenetic AEs. Although most AEs were mild and moderate, a number of patients developed grade≥3 AEs including hypertension (11.9%), hand-foot syndrome (5.6%) proteinuria (4.8%), transaminase elevation (1.6%), and diarrhea (1.6%). None of the clinical confounders including age, height, weight, gender, and performance score were associated with AEs (Supp. Table 2). Notably, NSCLC patients were more prone to both overall severe toxicity ($P < 0.01$) and angiogenetic toxicity ($P < 0.01$) relative to other patients under the same dose of 250mg/d (Supp. Table 3).

APA and M1-1 plasma concentration was linear under the daily dose of 250mg/d

A total of 210 plasma samples were collected and quantified. The plasma exposure of APA and M1-1, the main metabolite with 5.42 to 19.3% of APA’s pharmacological effects (Ding et al., 2013), varied vastly in patients receiving APA. Overall, the level of APA was relatively higher than that of M1-1. Under the dose of 250mg/d, the plasma concentration of APA is $185.1 \pm 177.4$ ng/mL while M1-1 is $136.5 \pm 103.7$ ng/mL (Figure 1A, 1B). More importantly, the plasma level of M1-1 had a great linear
relationship with that of APA in the same subjects. Collectively, our results showed notable individual variations in plasma concentration of APA and M1-1 in solid tumor patients receiving APA which were also linear at the dose of 250mg/d (Figure 1C).

**Higher APA and M1-1 plasma exposures predicted severe AEs in NSCLC but not other cancer types**

Since APA-induced adverse were suspected to be dose-related, we sought to investigate the relationship between the plasma level of APA or M1-1 and APA-induced AEs. In all patients enrolled, plasma concentration of APA was relatively higher in patients with severe AEs but without statistical significance (166.2 vs 114.4 ng/mL, \( P > 0.05 \)) while plasma concentration of M1-1 was higher in patients with overall severe AEs (136.3 vs. 99.1 ng/mL, \( P < 0.05 \)) (Figure 2A). Since hypertension, hand-foot syndrome, and proteinuria were the major AEs and pharmacologically related, accounting for 80.6% of the grade≥3 AEs, we next analyzed the correlation between these AEs and APA and M1-1 exposure. Consistently, higher exposure M1-1 was observed in patients with these severe anti-angiogenetic toxicities (138.4 vs 100.2 ng/mL, \( P < 0.05 \)) while APA did not show an association (165.2 vs 119.6 ng/mL, \( P > 0.05 \)) (Figure 2B).

Even though predisposed to toxicity, NSCLC cohort and other cancer type cohorts did not differ in APA and M1-1 exposure levels (Supp. Table 4) suggesting that NSCLC patients were probably less tolerant to APA treatment than other cancer types.
Therefore, we investigated whether APA and M1-1 exposures could discriminate NSCLC patients with and without severe AEs. As expected, the APA and M1-1 exposure levels were higher in NSCLC patients with grade≥3 AEs (APA: 177.3 vs 102.6 ng/mL, \(P < 0.01\); M1-1: 152.3 vs 81.9 ng/mL, \(P < 0.01\)) (Figure 2C) and grade≥3 anti-angiogenetic toxicities (APA: 164.1 vs 104.7 ng/mL, \(P < 0.05\); M1-1: 147.0 vs 91.4 ng/mL, \(P < 0.05\)) (Figure 2D) whereas no associations were found in patients of other cancer types (Supp. Table 5). Collectively, APA and M1-1 exposures were able to predict AEs in NSCLC patients who were more vulnerable to APA toxicity compared with other cancer types.

To generate a more precise therapeutic window, a cut-off value of 85.34 ng/mL for APA and 101.70 ng/mL for M1-1 were determined to discriminate NSCLC patients who were predisposed to develop grade≥3 AEs (AUC\(_{APA}=0.7331,\ 95\%\ CI: 0.5909-0.8752, \(P < 0.01\); AUC\(_{M1-1}=0.7462,\ 95\%\ CI: 0.6070-0.8854, \(P < 0.01\)) or angiogenetic toxicity (AUC\(_{APA}=0.6882,\ 95\%\ CI: 0.5370-0.8395, \(P < 0.05\); AUC\(_{M1-1}=0.7196,\ 95\%\ CI: 0.5755-0.8637, \(P < 0.05\)) with specificity and sensitivity (Figure 2E, 2F). Collectively, plasma levels of APA and M1-1 could be the potential predictors of severe AEs in NSCLC.

**APA and M1-1 plasma exposures outperformed angiogenesis pathway-related SNPs in toxicity prediction in NSCLC**
To further identify biomarkers for APA-induced toxicity, we performed pharmacogenetic analysis in all the patients enrolled. 35 SNPs related to the angiogenesis pathway were genotyped. 5 SNPs including CXCL14 rs11739936, KIT rs2237029, AKT1 rs2498797 and PDGFRB rs3733678, STAT3 rs8071537 were associated with APA-induced severe AE in all the patient groups ($P < 0.05$) (Figure 3A), among which CXCL14 rs11739936, KIT rs2237029, PDGFRB rs3733678 were also correlated with severe AEs in NSCLC subgroup (Figure 3B).

Given that a bunch of factors including APA/M1-1 exposures and SNPs might be associated with severe AEs in NSCLC, we next asked whether these variables should be attached with equal importance or which ones should be prioritized in clinical practice. To evaluate variable importance, we first perform multiple logistic regression between severe AEs and possible predictive factors in NSCLC including APA or M1-1, CXCL14 rs11739936, KIT rs2237029, PDGFRB rs3733678. Results showed that only APA ($P = 0.014$) and M1-1 ($P = 0.0063$) were identified as potent predictors in the model (Figure 3C, 3D). In line with this, random forest variable importance evaluation also showed that APA and M1-1 were both much more effective predictors in NSCLC depicted by the mean decrease gini coefficient (Figure 3E, 3F). Overall, consistent with the PK-PD analysis, these results stressed the predictive values of APA and M1-1 exposures of severe AEs in NSCLC which outperformed SNP related to the angiogenesis pathway.
CYP2C9 rs34532201 and rs1934968 polymorphisms were associated with plasma APA and M1-1 levels

Given the predictive potential and vast individual variation in APA and M1-1 exposures, we sought to identify drug disposition gene SNPs that could confer higher APA or M1-1 exposure. Besides, risk factors of a possible higher level of exposure were also able to guide dose adjustment before initiation of APA and thus avoid the development of severe AEs. As such, 20 SNPs involved in APA disposition genes were genotyped. Among 18 SNPs satisfying HWE, 3 SNPs (CYP2C9 rs1934968, rs1934969 and rs34532201) were associated with both APA plasma exposure levels ($P < 0.05$) (Figure 4A) while 5 SNPs (CYP2D6 rs1135840, CYP2C9 rs1934968, rs1934969, rs34532201 and CYP2E1 rs2515641) were correlated with M1-1 exposure level ($P < 0.05$) (Figure 4B). Further multiple linear regression characterized CYP2C9 rs34532201 as an indicator of APA and M1-1 exposures with higher APA and M1-1 exposures in TT than CC/CT carriers (Figure 4C, 4D, 4E, 4F). Besides, CYP2C9 rs1934968 was also identified as a predictive factor for APA exposure with higher APA exposure in GG than AA/AG carriers (Figure 4C, 4G). The characteristics of patients carrying different rs34532201 and rs1934968 variants were similar between the two compared groups (Supp. Table 6). Collectively, these results indicated that CYP2C9 rs34532201 TT and rs1934968 GG carriers were more prone to higher APA and M1-1 exposures and genotyping of these two alleles might be clinically translated as evidence for dose adjustment.
Discussion

The addition of anti-angiogenetic agents including APA to oncologic therapy has promoted the PFS and OS in a variety of tumors. However, drug-induced toxicities are the main course of dose adjustment or even discontinuation which is worth clinical vigilance. To our best knowledge, this is the first pan-cancer study to explore biomarkers for APA-induced toxicities based on exposure-toxicity relation and pharmacogenetics. In this study, we observed a higher risk of severe AEs in NSCLC patients than in other tumor types where an exposure-toxicity relationship was speculated. Besides, thresholds of 85.34 and 101.70 ng/mL for APA and M1-1 respectively were proposed in NSCLC based on this clinical trial. Lastly, CYP2C9 rs34532201 and rs1934968 were also identified as risk factors in higher APA and M1-1 exposures.

In this study, clinical data of patients demonstrated that APA-associated major toxicities are mostly grade 1 and 2. Hand-foot syndrome, hypertension, and proteinuria were the most frequently observed toxicities ether of all grades or grade≥3. These toxicities were also reported in other angiogenetic agents including bevacizumab (Li and Kroetz, 2018), sorafenib (Wu et al., 2008; Blanchet et al., 2010; Iijima et al., 2011), sunitinib (Zhu et al., 2009), regorafenib (Grothey et al., 2020) with varied incidence in different studies which are typical of drugs in this category. Previous clinical studies have suggested NSCLC were less tolerant to APA than HCC and GC (Li et al., 2016; Wu et al., 2018; Duan et al., 2019; Liang et al., 2020; Qin et
al., 2021). In a randomized placebo-controlled clinical trial, even with a relatively lower dose (500mg/d), 48.4% and 29.3% of NSCLC patients still underwent dose reduction or discontinuation respectively and there was 1 patient in the APA+gefitinib cohort (n=157) suffered severe hypertension and died of cerebral hemorrhage (Zhao et al., 2021). In line with this, our results showed that NSCLC patients were less tolerant to APA with a higher risk of developing severe AEs. Thus the dosage setting in our study (250 mg/d) probably makes it less feasible to observe a significant exposure-toxicity relationship in other cancer types. That being said, our study highlighted the exposure-toxicity relations of other cancer types, especially the well-tolerated ones should be further studied in other dosages (e.g., 400, 500 mg/d).

The mechanisms behind why NSCLC showed a distinct drug exposure relationship largely remain elusive. A PBPK study indicated that patients with advanced gastric cancer who usually received 850 mg apatinib daily exhibited lower bioavailability than other cancer types including colorectal cancer, hepatic cancer, and breast cancer (Yu et al., 2017). Besides, a higher volume of distribution and absorption rate was found in patients with nondigestive system cancers than in digestive system cancers (Yang et al., 2023). Such inter-tumor type variability in PK was not exclusive to apatinib. For example, intravenously administered pertuzumab also had a lower steady-state concentration in gastric cancer patients than in breast cancer patients (Garg et al., 2014). Furthermore, some other antiangiogenetic agents also demonstrate similar inter-malignancy difference in toxicity profile. There was a higher incidence
of sunitinib and sorafenib-associated hypertension in metastatic renal cell carcinoma (RCC) than in non-RCC patients (Zhu et al., 2009; Li et al., 2014). RCC and breast cancer patients also seemed to be at higher risk of high-grade hypertension following bevacizumab treatment than patients with other tumor types (An et al., 2010; Ranpura et al., 2010). Interestingly, the inflammatory response in the tumor microenvironment of lung cancer has been shown to trigger pulmonary hypertension (Pullamsetti et al., 2017). Besides, a recent study provided evidence that the C-terminal fragment of tumor-derived angiopoietin-like 4 (C-ANGPTL4) could inhibit angiogenesis systematically and C-ANGPTL4 was highly enriched in the circulation of lung cancer patients (Hubers et al., 2023), which could exacerbate the toxicity induced by systematic inhibition from APA. These studies suggested pathology of a specific tumor type could distinctly reshape the cardiovascular system which might have an impact on the presentation of toxicity profiles in response to anti-angiogenesis treatment.

Dose-dependent toxicity has been indicated in angiogenetic agents including bevacizumab (Ranpura et al., 2010), sunitinib (Westerdijk et al., 2021), sorafenib (Iijima et al., 2011) with a higher risk of toxicity observed at a higher dose or drug exposure. Consistent with this, we found higher APA and M1-1 exposures in NSCLC patients with high-grade AEs. M1-1 was the main metabolite contributing to 5.42 to 19.3% of APA’s pharmacological effects (Ding et al., 2013). The mechanism under its association with AEs could be attributed to its potential inhibition of VEGFR2 or just
its linear relation to APA as shown in Figure 1C. Currently, dose reduction is required when severe AEs occur while sufficient monitoring and symptomatic therapy such as antihypertensives for hypertension was recommended in terms of moderate AEs (Bellmunt et al., 2011; Plummer et al., 2019). However, no well-recognized therapeutic window was proposed for drugs in this category including APA. In this study, thresholds of 85.34 and 101.70 ng/mL for APA and M1-1 respectively were indicated for NSCLC. Notably, any decision of dose adjustment should be made with caution because it might affect efficacy. Previous studies have indicated that lower dose of bevacizumab showed similar PFS and OS extension compared with the high-dose group but the overall incidence of serious toxicities was higher in the high-dose arm (Reck et al., 2009; Ajlan et al., 2017). Even though no exposure-efficacy relation was observed in the patients with available computed tomography data in our study (Supp. Fig. 1), results in our study should be clinically interpreted cautiously and need further validation in clinical studies with a higher patient volume.

To further discriminate patients predisposed to APA-induced toxicity before the initiation of treatment, we focused on SNPs on angiogenesis pathway-related genes and drug disposition genes which indicated a pharmacokinetic and pharmacological mechanism respectively. On one hand, even if VEGFR2 SNPs were reported as potential biomarkers for toxicities, the results were contradictory in different studies (Jain et al., 2010; Herraez et al., 2013; Qin et al., 2016). In this study, several SNPs
were associated with severe AEs but none of them was comparable to APA or M1-1 exposure in severe AE prediction in NSCLC. Whether or not there are other SNPs affecting patients’ response to APA remains to be investigated.

On the other hand, APA was metabolized mainly by CYP3A4, CYP3A5, and CYP2D6 and partially by CYP2E1, and CYP2C9 (Ding et al., 2013). However, contrary to our expectation, we did not find any significant SNPs in CYP2D6, CYP3A4, and CYP3A5 associated with APA and M1-1 exposures. This might be due to the limited patient number in our study. Indeed, a recent pharmacokinetic study of APA has suggested that CYP3A5 rs776746 (6986A>G) had a significant effect on both oral clearance and volume of distribution of APA (Yang et al., 2023). In silico dose simulation based on the proposed PK model indicated different AUC levels between CYP3A5 rs776746_A and G carriers (Yang et al., 2023). However, whether or not the influence of CYP3A5 rs776746 on PK parameters can be substantially translated into the variability of drug exposure or PD (efficacy or toxicity) remains elusive. In this study, we did not observe significantly different drug exposure levels between CYP3A5 rs776746_A and _G carriers. Instead, we found that patients with rs34532201 T, rs1936968 G alleles, and intron variants in CYP2C9 tended to have a higher level of APA and M1-1 exposure, suggesting a lower initial dose of APA for these patients, especially in the relatively intolerant tumor type like NSCLC. We hypothesized that rs34532201_T and rs1936968_G might affect the function of CYP2C9 and thus contribute to the conversion of APA and M1-1 into other metabolites without pharmacological effects. However, more experimental evidence is
needed to explore the impact of rs34532201_T and rs1936968_G on CYP2C9 function in the future. Further studies are still warranted to study the impact of SNPs in these enzymes on APA and M1-1 exposure.

There are some limitations in this study. Firstly, all the patients recruited in this study received a fixed dose of 250mg/d and whether plasma APA or M1-1 can predict toxicity at a higher dose is unknown. Besides, the frequency of rs34532201_T allele is low with only 5 out of 126 patients being TT carriers. Other reliable genetic biomarkers other than CYP2C9 SNPs remain elusive. Finally, the number of patients of each cancer type is limited and the conclusions should be validated in a large prospective clinical trial.
Conclusion

In conclusion, plasma APA and M1-1 exposures were able to predict severe AEs in NSCLC patients at the dose of 250mg/d. Dose reduction was recommended in NSCLC patients with high APA exposure levels especially in CYP2C9 rs34532201 TT and rs1936968 GG carriers.
Acknowledgments

This work was supported by the National Natural Science Foundation of China (Grant Nos. 81973398, 81730103, 81573507 and 82020108031), The National Key Research and Development Program (Grant Nos. 2017YFC0909300 and 2016YFC0905000), Guangdong Provincial Key Laboratory of Construction Foundation (Grant No. 2017B030314030 and 2020B1212060034), Science and Technology Program of Guangzhou (Grant No.201607020031), National Engineering and Technology Research Center for New drug Druggability Evaluation (Grant No. 2017B090903004), the 111 Project (Grant No. B16047), China Postdoctoral Science Foundation (Grant Nos.2019M66324, 2020M683140 and 2020M683139) and Natural Science Foundation of Guangdong Province (Grant No.2022A1515012549 and No.2023A1515012667).
Data Availability Statement

The authors declare that all the data included in this review are available within the paper and/or are openly available in the NaPDI Center Database (https://repo.napdi.org/).
Author contributions

Participated in research design: Y.H Chen, Y.B Lin, S Guan, M Huang, H Long, and X.D Wang; Conducted experiments: Y.H Chen and S Guan; Contributed new reagents or analytic tools: Y.B Lin, Z.R Zhao, and D.R Lin, J Guan, C.Z Zhou, J.L Liu, X.L Cao, Z.C Lin, D.Y Chen, J.B Shang, W.J Zhang, H.H Chen, L.K Chen, S.D Ma, L.J Gu, J Zhao, and H Long; Performed data analysis: Y.H Chen and S Guan; Wrote or contributed to the writing of the manuscript: Y.H Chen, Y.B Lin, S Guan, M Huang, H Long, and X.D Wang.
References


Footnotes
No author has an actual or perceived conflict of interest with the contents of this article.

This work was supported by the National Natural Science Foundation of China (Grant Nos. 81973398, 81730103, 81573507 and 82020108031), The National Key Research and Development Program (Grant Nos. 2017YFC0909300 and 2016YFC0905000), Guangdong Provincial Key Laboratory of Construction Foundation (Grant No. 2017B030314030 and 2020B1212060034), Science and Technology Program of Guangzhou (Grant No.201607020031), National Engineering and Technology Research Center for New drug Druggability Evaluation (Grant No. 2017B090903004), the 111 Project (Grant No. B16047), China Postdoctoral Science Foundation (Grant Nos.2019M66324, 2020M683140 and 2020M683139) and Natural Science Foundation of Guangdong Province (Grant No.2022A1515012549 and No.2023A1515012667).
Legends for Figures

Figure 1. APA and M1-1 plasma concentration was linear under the daily dose of 250mg. A, B: Distribution APA (A) and M1 (B) exposure levels in all patients; C: Linear correlation between APA and M1-1 exposure.

Figure 2. APA and M1-1 plasma concentration predict severe overall and angiogenetic adverse events in NSCLC. A, B: all patients; C, D: NSCLC subgroup; E, F: ROC curves of APA and M1-1 for severe overall adverse events (E) and severe anti-angiogenetic adverse events (F). *: P < 0.05, **: P < 0.01, ns: no significance.

Figure 3. APA and M1-1 were more potent predictors of severe AEs than angiogenesis pathway-related SNPs identified. A, B: Association between angiogenesis pathway-related gene SNPs and severe AEs in all patients(A) and NSCLC subgroup(B); C, D: Multiple logistic regression of angiogenesis pathway-related SNPs and APA (C) or M1-1 (D) exposure in NSCLC; E, F: Random forest to identify key variables in predicting severe AEs. E: ROC curves of the prediction model. F: Variable importance defined by mean decrease in Gini coefficient.

Figure 4. CYP2C9 rs34532201 and rs1934968 polymorphisms were associated with plasma APA and M1-1 levels. A, B: Association between drug disposition gene SNPs and APA (A) and M1-1 (B ) exposure level; C, D: Multiple linear regression of between drug disposition gene SNPs and APA (C) and M1-1 (D )exposure level; E, F: Association between CYP2C9 rs34532201 genotypes and APA (E) and M1-1 (F)
exposure level; G: Association between CYP2C9 rs1934968 genotypes and APA (G)
exposure level. *P < 0.05, **P < 0.01, ***P < 0.001.

Table 1. Patients' characteristics at baseline

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>N of patients</th>
<th>Gender (% male)</th>
<th>Age (median; SD)</th>
<th>Height (median; SD)</th>
<th>Weight (median; SD)</th>
<th>PS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-small cell lung cancer</td>
<td>53</td>
<td>64</td>
<td>58 (12)</td>
<td>163 (8)</td>
<td>58 (13)</td>
<td>4 94  2</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>11</td>
<td>73</td>
<td>57 (10)</td>
<td>165 (10)</td>
<td>60 (6)</td>
<td>0 82 18</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>10</td>
<td>100</td>
<td>58 (10)</td>
<td>160 (4)</td>
<td>49 (5)</td>
<td>30 70 0</td>
</tr>
<tr>
<td>Small cell lung cancer</td>
<td>6</td>
<td>100</td>
<td>71 (10)</td>
<td>165 (2)</td>
<td>70 (11)</td>
<td>7 83 0</td>
</tr>
<tr>
<td>Pulmonary lymphoepithelioma-like carcinoma</td>
<td>6</td>
<td>33</td>
<td>46 (12)</td>
<td>161 (14)</td>
<td>62 (18)</td>
<td>7 83 0</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>5</td>
<td>0</td>
<td>55 (9)</td>
<td>158 (2)</td>
<td>60 (4)</td>
<td>20 80 0</td>
</tr>
<tr>
<td>Thymic Cancer</td>
<td>5</td>
<td>60</td>
<td>37 (10)</td>
<td>161 (6)</td>
<td>55 (7)</td>
<td>0 100 0</td>
</tr>
<tr>
<td>Nasopharyngeal carcinoma</td>
<td>4</td>
<td>50</td>
<td>56 (8)</td>
<td>155 (3)</td>
<td>56 (3)</td>
<td>50 50 0</td>
</tr>
<tr>
<td>Others*</td>
<td>26</td>
<td>62</td>
<td>63 (16)</td>
<td>157 (7)</td>
<td>55 (9)</td>
<td>15 81 4</td>
</tr>
<tr>
<td>Total</td>
<td>126</td>
<td>56</td>
<td>57 (13)</td>
<td>161 (8)</td>
<td>56 (11)</td>
<td>11 86 3</td>
</tr>
</tbody>
</table>

Abbreviation: PS, performance score;

*Cervical cancer, colorectal cancer, cecal adenocarcinoma, esophageal cancer, pancreatic cancer, gallbladder cancer, gastric cancer, gastrointestinal stromal, perihemangioma, pulmonary pleomorphic carcinoma, renal pelvis cancer, synovial sarcoma, Ewing’s sarcoma.
Table 2. Incidence and severity of treatment-related adverse events

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>Grade 1 (%)</th>
<th>Grade 2 (%)</th>
<th>Grade 3 (%)</th>
<th>Any grade (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hand-foot syndrome</td>
<td>32 (25.4)</td>
<td>16 (12.7)</td>
<td>7 (5.6)</td>
<td>55 (43.7)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>13 (10.3)</td>
<td>11 (8.7)</td>
<td>15 (11.9)</td>
<td>39 (31.0)</td>
</tr>
<tr>
<td>Proteinuria</td>
<td>11 (8.7)</td>
<td>4 (3.2)</td>
<td>6 (4.8)</td>
<td>21 (16.7)</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>11 (8.7)</td>
<td>2 (1.6)</td>
<td>1 (0.8)</td>
<td>13 (10.3)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>8 (6.3)</td>
<td>3 (2.4)</td>
<td>1 (0.8)</td>
<td>12 (9.5)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>5 (4)</td>
<td>3 (2.4)</td>
<td>2 (1.6)</td>
<td>10 (7.9)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>8 (6.3)</td>
<td>1 (0.8)</td>
<td>1 (0.8)</td>
<td>10 (7.9)</td>
</tr>
<tr>
<td>Abdominal distension</td>
<td>6 (4.8)</td>
<td>1 (0.8)</td>
<td>0 (0)</td>
<td>7 (5.6)</td>
</tr>
<tr>
<td>Edema</td>
<td>3 (2.4)</td>
<td>2 (1.6)</td>
<td>0 (0)</td>
<td>5 (4.0)</td>
</tr>
<tr>
<td>Rash</td>
<td>5 (4)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>5 (4.0)</td>
</tr>
<tr>
<td>Transaminase elevation</td>
<td>1 (0.8)</td>
<td>0 (0)</td>
<td>2 (1.6)</td>
<td>3 (2.4)</td>
</tr>
<tr>
<td>Gastralgia</td>
<td>1 (0.8)</td>
<td>2 (1.6)</td>
<td>0 (0)</td>
<td>3 (2.4)</td>
</tr>
<tr>
<td>Pulmonitis</td>
<td>0 (0)</td>
<td>1 (0.8)</td>
<td>1 (0.8)</td>
<td>2 (1.6)</td>
</tr>
<tr>
<td>Periodontitis</td>
<td>1 (0.8)</td>
<td>0 (0)</td>
<td>1 (0.8)</td>
<td>2 (1.6)</td>
</tr>
<tr>
<td>Angioatelectasis</td>
<td>1 (0.8)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (0.8)</td>
</tr>
<tr>
<td>Odontalgia</td>
<td>0 (0)</td>
<td>1 (0.8)</td>
<td>0 (0)</td>
<td>1 (0.8)</td>
</tr>
<tr>
<td>Foot pain</td>
<td>1 (0.8)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (0.8)</td>
</tr>
<tr>
<td>Waist pain</td>
<td>1 (0.8)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (0.8)</td>
</tr>
<tr>
<td>Neck pain</td>
<td>1 (0.8)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (0.8)</td>
</tr>
<tr>
<td>Back pain</td>
<td>1 (0.8)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (0.8)</td>
</tr>
<tr>
<td>Pericardial effusion</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (0.8)</td>
<td>1 (0.8)</td>
</tr>
</tbody>
</table>
Fig. 1
Fig. 2
Fig. 3
Fig. 1

A. Number of patients (n) vs. APA concentration (ng/mL)

B. Number of patients (n) vs. M1-1 concentration (ng/mL)

C. Scatter plot of APA concentration (ng/mL) vs. M1-1 concentration (ng/mL)

$r = 0.7967$

$P < 0.0001$
Fig. 3

A

B

C

D

E

F

Variables | OR (95% CI) | p value
--- | --- | ---
rs11739936 | 2.17 (0.3310-14.28) | 0.40
rs2237029 | 1.825 (0.4750-7.856) | 0.39
rs3733678 | 0.4794 (0.1160-1.887) | 0.29
APA | 1.007 (1.002-1.014) | 0.014

Variables | OR (95% CI) | p value
--- | --- | ---
rs11739936 | 2.549 (0.3806-18.10) | 0.35
rs2237029 | 1.974 (0.4949-8.950) | 0.35
rs3733678 | 2.883 (0.6938-13.35) | 0.15
M1-1 | 1.012 (1.004-1.022) | 0.0063

1.500 (0.778, 0.500)
AUC: 0.639