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Protective role of 1-methylnicotinamide against voriconazole - induced hepatotoxicity through Kupffer cells polarization and PXR activation in acute myeloid leukemia

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ABSTRACT: 1-Methylnicotinamide (1-MNA), a ubiquitous endogenous metabolite present across animal, plant and microbial systems, demonstrates significant protective efficacy against voriconazole (VRC)-induced hepatotoxicity. VRC serves as first-line antifungal prophylaxis in acute myeloid leukemia (AML) patients, but its clinical utility is frequently compromised by dose-limiting hepatotoxicity. This investigation elucidates the molecular mechanisms underpinning 1-MNA-mediated hepatic protection while concurrently assessing its biomarker utility for predicting VRC-induced liver injury in this patient population. Utilizing integrated analysis of clinical specimens and AML murine models, we established an inverse correlation between circulating 1-MNA concentrations and established biomarkers of VRC hepatotoxicity. The protective mechanism of 1-MNA stimulates IL-10 secretion from Kupffer cells in the liver. Subsequently, IL-10 mediates the transcriptional activation of PXR by promoting its interaction with Hsp90 β , thereby enhancing the expression of the drug-metabolizing enzymes CYP2C19 and CYP3A4, ultimately improving hepatic metabolism of VRC and reducing drug accumulation. This pathway underscores the potential of 1-MNA as both a predictive biomarker and an intervention strategy for mitigating VRC-induced hepatotoxicity in AML management.

Keywords: Fresh pork; 1-Methylnicotinamide, Voriconazole, Metabolism, Acute myeloid leukemia, Anti-inflammatory responses

1. Introduction

Acute myeloid leukemia (AML) represents a clinically challenging hematological malignancy characterized by substantial morbidity and mortality, even though its population incidence remains relatively low. The standard therapeutic paradigm involves intensive induction chemotherapy, notably the “7 + 3”

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protocol, which combines seven-day administration of cytarabine with three days of anthracycline treatment^[1]. This regimen achieves complete remission in approximately 55% of treated patients^[2]. Immunocompromised AML patients receiving such chemotherapy exhibit heightened susceptibility to systemic fungal infections, particularly invasive *Aspergillosis* and *Candidiasis*, with the latter being more prevalent in intensive care settings^[3].

Voriconazole (VRC), a broad-spectrum triazole antifungal, is a first-line agent for preventing and treating invasive *Aspergillosis* in immunologically compromised individuals^[4]. Its clinical utility, however, is complicated by non-linear pharmacokinetics and drug-related toxicity. Hepatic accumulation of VRC, primarily due to diminished metabolic capacity, is a key factor driving associated liver injury^[5]. Moreover, the substantial incidence of breakthrough fungal infections necessitates extensive VRC utilization, consequently augmenting patient irreversible liver damage, thereby increasing the treatment burden on patients^[6]. Although traditional determinants such as gender, age and genetic polymorphisms in drug-metabolizing enzymes (*CYP2C19*, *CYP2C9*, *CYP3A4*), remains inadequate to account for the profound interindividual heterogeneity in VRC treatment response^[7]. Consequently, comprehensive investigation of VRC metabolic variations across divergent disease states emerges as an imperative prerequisite for optimizing therapeutic efficacy while mitigating adverse drug reactions.

Contemporary scientific literature reflects a conspicuous absence of systematically documented evidence concerning chemotherapy-induced hepatotoxicity, while emerging investigations suggest cytarabine may significantly disrupt intestinal mucosal barrier homeostasis^[8]. This pathophysiological cascade facilitates trans-epithelial translocation of microbiota-derived metabolites via enterohepatic circulation, subsequently modulating hepatic immune-inflammatory pathways^[9]. Previous experimental investigations have established that hepatic proinflammatory cytokine IL-1 β can substantially impair the expression and functional capacity of VRC-metabolizing enzymes in hepatocytes subsequent to lipopolysaccharide challenge, thus establishing predisposition to VRC-associated hepatic injury^[6]. Furthermore, the mechanistic interplay between intensive induction chemotherapy and subsequent intestinal-immune responses remains largely uncharacterized within current AML experimental models.

This mechanistic investigation of voriconazole (VRC)-induced hepatotoxicity in AML demonstrates substantial translational potential. Recent studies indicate that elevated levels of inflammatory markers (e.g., C-reactive protein) correlate with impaired VRC metabolism, likely through cytokine-mediated downregulation of hepatic enzymes and transporters^[10]. Moreover, emerging work highlights the role of gut microbiota-derived metabolites in liver pathology. For instance, Gong and colleagues established that granisetron, a bacterial metabolite, exhibits therapeutic efficacy in sepsis management and modulates host response mechanisms in resistant murine models^[11]. Additional microbial metabolites, including 3,4-dihydroxyphenylpropionic acid, demonstrate hepatoprotective properties through modulating macrophage pro-inflammatory responses in hepatic ischemia-reperfusion injury models^[12]. Parallel investigations demonstrate that β -galactosidase derived from *Lactobacillus* species releases bioactive daidzein, which

subsequently suppresses Fdps-triggered ferroptosis in hepatocytes and mitigates acetaminophen-induced hepatocellular damage^[13].

Notably, few studies examine the effects of AML chemotherapy agents on VRC-metabolizing enzymes (CYP2C19 and CYP3A4). Our preliminary clinical data reveal significant heterogeneity in VRC clearance among chemotherapy recipients, underscoring the need to understand how hepatic anti-inflammatory cytokine IL-10 influences VRC metabolism^[14]. Furthermore, the precise mechanisms underlying the interplay between gut-liver immune homeostasis in AML remain to be fully elucidated. This study aims to address these gaps, investigating the mechanisms of VRC-induced hepatotoxicity in the context of AML and gut-liver immune interactions.

1-Methylnicotinamide (1-MNA), a novel food ingredient recognized by the European Union, exhibits multifaceted biological activities including inhibition of reactive oxygen species and the NLRP3 inflammasome. The crosstalk between hepatocytes and Kupffer cells underlies its mechanism, while emerging evidence demonstrates that gut-derived 1-MNA mitigates dextran sulfate sodium (DSS)-induced liver injury by stimulating IL-10 secretion from liver macrophages^[15]. This study provides new perspectives on how 1-MNA modulates drug-metabolizing enzyme activity within the AML microenvironment. Given the established correlation between VRC accumulation and hepatotoxicity, elucidating 1-MNA's regulatory effects on drug-metabolizing enzymes—including cytochrome P450 isoforms and P-glycoprotein transporters—has become imperative. Consequently, this study seeks to clarify the mechanistic basis of 1-MNA-mediated regulation of VRC-metabolizing enzyme activity, with a focus on its immunomodulatory role in hepatic macrophages and its protective efficacy against hepatotoxicity in AML.

2. Materials and methods

2.1. Human sample collection

The study protocols were granted approval by the Ethical Committee of Shenzhen Hospital of Southern Medical University (No. NYSZYYEC20180017) as well as the Ethical Committee of Shenzhen Longhua District Central Hospital (No. 2022-040-02). Furthermore, the study was conducted in adherence to the principles outlined in the Helsinki Declaration. The subjects included in the study were selected from the Hematology Department of Shenzhen Hospital, Southern Medical University, specifically among patients diagnosed with acute myeloid leukemia (AML) who received VRC for antifungal prophylaxis (China Clinical Trial Registry Nos. ChiCTR210043331 and ChiCTR2200065027). The diagnosis of AML was made based on the criteria established in the NCCN Guideline: Acute Myeloid Leukemia^[16]. Prior to the study, all adult patients underwent CYP2C19 genotype testing. Sixty five (65) AML patients with normal CYP2C19 metabolic activity who received a conventional “7 + 3” regimen combining high-dose aracytine (cytarabine 200 mg/m²) for seven days and an anthracycline (idarubicin 12 mg/m² or daunorubicin 60 mg/m²) for three days were included in this study.

The duration of VRC treatment extended for a minimum of five days. Each patient was administered two doses per day in strict adherence to the instructions provided on the drug label. The exclusion criteria encompassed the following conditions: (1) patients who had been pregnant within the previous year or had received antibiotics or probiotics within the preceding two months. (2) individuals below the age of 16. (3) patients for whom combined chemotherapy was contraindicated due to renal/hepatic or cardiac insufficiency. (4) individuals concurrently taking potent CYP2C19 or CYP3A4 modulators such as rifampicin, ritonavir, carbamazepine, long-acting barbiturates or macrolide antibiotics. (5) individuals with hypokalemia (serum potassium < 3.5 mmol/L), those undergoing hemodialysis or peritoneal dialysis. (6) individuals with visual impairment. (7) individuals with hepatopathy, and (8) individuals with poor medication adherence as indicated by electronic medical records.

Clinical data was collected and adverse drug reactions were documented in a previous study [6]. Blood samples were obtained using a 5 mL sodium heparin tube. CRP and TNF- α concentrations were measured in discarded blood samples as markers of inflammation. Serum total bilirubin (TB > 26 μ mol/L), aspartate transaminase (AST > 40 IU/L) and alanine transaminase (ALT > 50 IU/L) levels were also assessed to diagnose hepatotoxicity during VRC treatment. As a result, we determined the metabolic ratio as follows:

$$\text{Metabolic ratio} = \frac{C(\text{Voriconazole N-oxide})}{C(\text{Voriconazole})} \times 100\%$$

2.2. Animal experimental design

In this experiment, two animal models were used to explore the metabolic regulation of voriconazole by 1-MNA. The leukemia mouse model was used to confirm the metabolic promotion effect of 1-MNA on VRC in non-hepatotoxicity mice, and the intestinal sterility model was used to clarify the metabolic mechanism of 1-MNA on VRC. On the last day of the experiment, all mice were euthanized using carbon dioxide. Specifically, mice were placed in batches into a chamber and carbon dioxide was introduced at a filling rate of 50%–70% of the chamber volume per minute until the mice stopped breathing and had dilated pupils. After the carbon dioxide was turned off, the mice were observed for another 3 minutes to confirm their death [17]. A portion of blood samples were then collected into EDTA vacutainer tubes via cardiac puncture and centrifuged at 3,000 rpm at 4°C for 5 minutes to obtain plasma samples. Other blood samples were then collected and centrifuged at 3,000 rpm at 4°C for 5 minutes to obtain serum samples. Serum was used to determine liver function indexes (AST, ALT and total bilirubin). Stool, liver and intestinal were also collected for further analysis. The protocol for the animal study was granted approval by the Nanfang Hospital of Southern Medical University (NFYY-2020-73), in accordance with the animal welfare guidelines of Southern Medical University and the National Research Council Guide for the Care and Use of Laboratory Animals. The specific method for establishing these animal models is as follows:

2.2.1 Retroviral production, cell transduction and mouse models of leukemia

A cohort of 72 SPF-grade male C57BL/6J mice weighing 22–28 g was procured from the Experimental Animal Center of Southern Medical University under production license SCXK 2018-0095. Donor cells were isolated from two distinct sources: fetal hepatocytes harvested from E14.5 embryos and bone marrow cells collected from 6–8 week-old mice preconditioned with 5-fluorouracil administered at 100 mg/kg for five consecutive days.

For retroviral production, 293T cells were transfected with the MSCV-MLL-AF9-IRES-GFP plasmid. The culture medium was refreshed 8 hours post-transfection, with virus-laden supernatant systematically collected at 48- and 72-hour intervals. Viral concentrates were prepared through polyacrylamide treatment at 8 µg/mL followed by centrifugation at 500 rcf for 2 hours.

The cell transplantation protocol comprised three critical phases. First, hematopoietic stem/progenitor cells from fetal liver and bone marrow were sorted and expanded in X-vivo medium enriched with interleukin-3, interleukin-6 and stem cell factor. Subsequently, cells underwent rotational inoculation with MLL-AF9 retrovirus, achieving 16% transduction efficiency as quantified by GFP expression via flow cytometry. Finally, recipient C57BL/6 mice aged 6–8 weeks received lethal irradiation at 900 cGy (dose rate: 900 cGy/min) followed by immediate intravenous transplantation of 4×10^5 preleukemic cells suspended in 400 µL combined with 100 µL protective cell mixture.

Model validation was conducted through multi-level assessment. GFP-positive leukemia cells were isolated in quantities of 3,000 from primary, secondary and tertiary recipients. Secondary transplantations incorporated co-administration of 2×10^5 CD45.1 bone marrow cells. Disease progression parameters included survival kinetics, body weight dynamics, flow cytometric detection of GFP⁺ populations in hematopoietic organs, histopathological evaluation of formalin-fixed specimens, and cytomorphological analysis using Wright-Giemsa staining.

The comprehensive experimental workflow is visually depicted in **Fig. 1F**^[18], illustrating the systematic approach employed to establish this clinically relevant AML model that faithfully recapitulates human disease progression. In this study, a modified intensive induction chemotherapy (IC) regimen for AML was implemented, consisting of cytarabine (aracytine) administered at 100 mg/kg/day and daunorubicin at 3 mg/kg/day. Daunorubicin was delivered via impregnated feed granules formulated with 5% sucrose solution, as previously documented in reference^[19]. Mice were intravenously injected with PBS control and IC regimen for 5 days.

Comprehensive analytical characterization included desorption electrospray ionization-mass spectrometry imaging (DESI-MSI) spatial profiling of liver tissues, UPLC-MS/MS quantification of VRC metabolic pathways, and metabolomic screening of hepatotoxicity biomarkers.

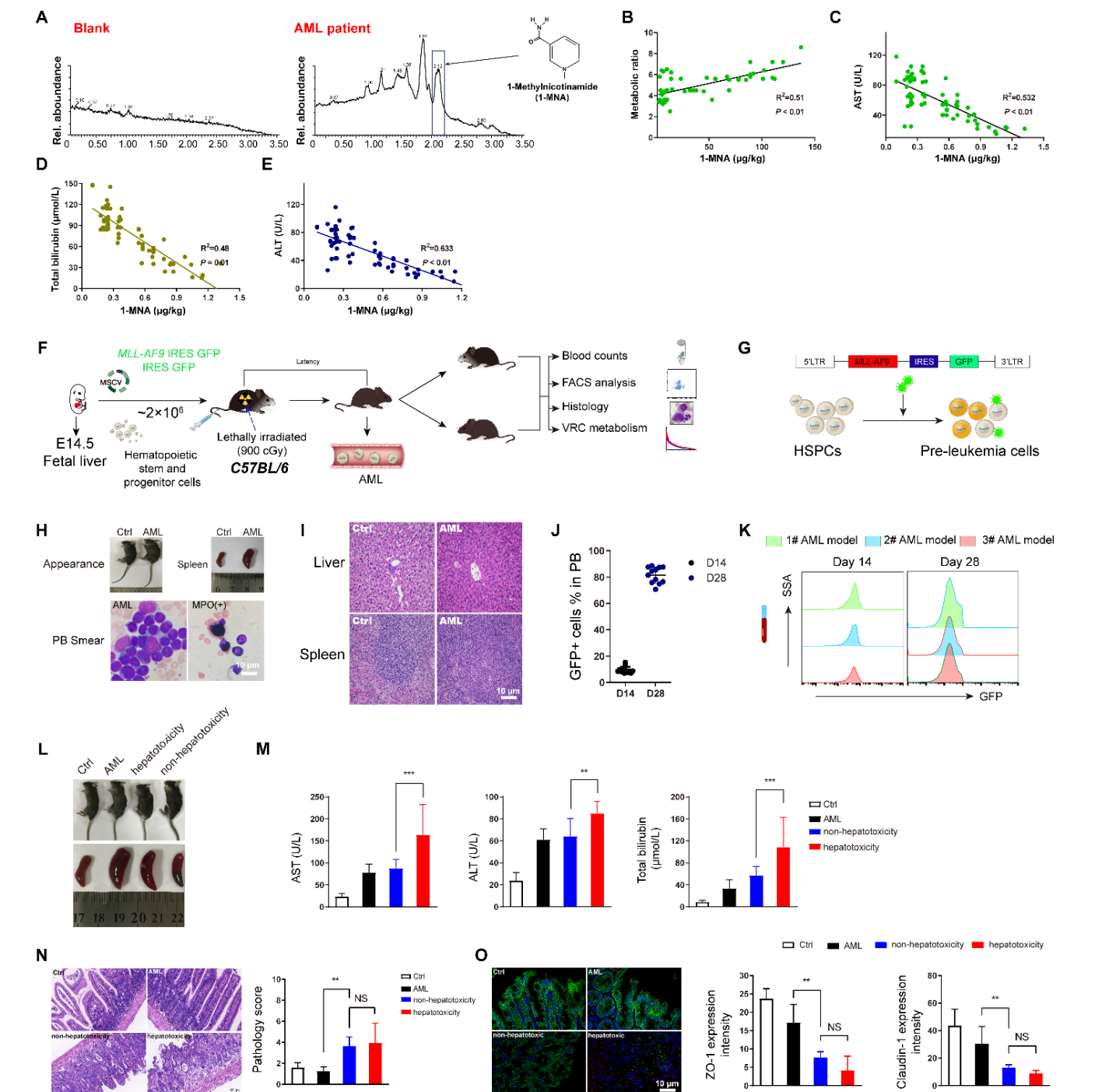


Fig. 1. The occurrence of VRC hepatotoxicity may be due to varying degrees of damage to the intestinal mucosal barrier after intensive induction chemotherapy.

A, Chromatogram of 1-MNA in peripheral blood of AML patients. B, The relationship between the 1-MNA content in feces and the metabolic ratio of VRC. The effective treatment interval of VRC is $C_{ssmin}=1.5\sim5.5$ mg/L ($n=60$). C, The relationship between peripheral blood 1-MNA and AST. D, The relationship between peripheral blood 1-MNA and total bilirubin. E, The relationship between peripheral blood 1-MNA and ALT. Line: linear regression analysis. F, Process of construction of the experimental animal model. G, Information on the MLL-AF9 plasmid. H, Comparison of the appearance, spleen and peripheral blood (PB) smear of normal mice and AML model mice, Bar = 10 μm. I, Comparison of H&E sections of liver and spleen from normal mice and AML model mice. J, Statistic results of GFP⁺ AML cells after transplantation. K, Representative cytometric flow plots of the percentage of GFP⁺ AML cells in peripheral blood at days 14 and 28 after transplantation. L, Comparison of the appearance of mice in each group after IC treatment. IC treatment, intensive induction chemotherapy regimen. M, Comparison of liver function indices after administration of VRC. N, H&E image of the colon (left), pathological section score statistics (right), Bar = 50 μm. O, IF image of the colon (left), expression of ZO-1 protein (red) + expression of occluding-1 protein (green) (right). Bar = 10 μm, * $P<0.05$, ** $P<0.01$, *** $P<0.001$, calculated by Student's t-test with Welch's correction.

2.2.2. Intestinal sterility model

Following acclimatization for one week, the SPF mice were administered three doses of an antibiotic cocktail daily (0.5 mL streptomycin, 1 mL ampicillin, 1 g/L gentamicin, and 0.5 mL vancomycin) in a nasogastric tube (500 mL each) for 2 weeks. As a result of oral gavage stress, antibiotics were administered in drinking water in order to avoid confusing effects. The Meilun Bio (Dalian, China) supplied all antibiotics in antibiotic cocktail (ABX) treatment [6]. The specific experimental design is shown in Fig. 4A.

2.3. Agents

Primary antibodies against P-glycoprotein (P-gp) were provided by Cell Signaling Technology (Danvers, MA, USA). LPS, rifampin and triptolide were obtained from MCE (Monmouth Junction, NJ, USA). Lamin B and other antibodies were purchased from Invitrogen (Carlsbad, CA, USA). FITC-, PE- and APC- labeled antibodies against Ly6C, CD206 and F4/80 were purchased from Biolegend, San Diego, USA. The FITC-labeled Donkey anti-Goat IgG polyclonal secondary antibody was purchased from Invitrogen. Red lysis buffer was purchased from Solarbio company (Beijing, China). Background fluorescence was determined with the irrelevant anti-mouse isotype (Biolegend, San Diego, CA, USA). For intracellular cytokine staining, *Kupffer* cells were stimulated with 1-MNA (Sigma-Aldrich, St. Louis, MO, USA) for 4 hours prior to surface staining, then permeabilized and intracellularly stained with IL-10 (JES5-16E3) mAb (BD Pharmingen).

2.4. DESI-MSI analysis

As in 2.2.1, analysis of livers from leukemic animals, fresh liver tissue were rapidly frozen in liquid nitrogen for 10 minutes. The frozen tissues were then transferred to cryogenic vials and stored at a temperature of -80°C until they were cut into 10 mm pieces using a microtome equipped with a UV cryostat (Leica CM 1860). The tissue sections were then thawed and mounted on microscope slides. Prior to DESI-MSI analysis, the slides were dried under vacuum for 15 minutes.

In DESI-MSI analysis, colorless stained frozen tissue sections were analyzed using a Q-exactive mass spectrometer with a nominal mass resolution of 70,000 in the m/z range of 70 to 1,000 in both positive and negative ion modes. The spray solvent used was a mixture of acetonitrile and water (8:2, vol/vol) at a flow rate of 5 μ L/min. The voltage settings for the spray and delivery pipe were set to 7,500 and 2,000 V in positive ion mode and -5,500 and -1,500 V in negative ion mode. The flow rate of the extraction gas was kept at 45 L/min and the capillary temperature was set at 350°C. MSI experiments were performed by scanning the tissue surface continuously at a constant rate in the X direction, with vertical steps separating adjacent row directions in Y.

2.5. UPLC-MS/MS analysis

For non-targeted metabolomics experiments, samples were taken from each tissue and metabolites were first extracted from the samples using the organic reagent precipitation protein method. Quality control samples were prepared at the same time. ESI-QTOF/MS (Xevo G2-SQ-TOF, Waters) and UPLC-QTOF/MS

(A CQUITY UPLCI-Class, Waters) mass spectrometers were used for metabolomics detection in mice. The sample was precipitated with methanol and extracted, filtered and injected into an ACQUITY UPLC BEHC18 chromatography column (130 Å, 17 µm, 2.1 mm × 50 mm). Mobile phase A contained 0.1% mass spectrometry-grade methane acid water and mobile phase B contained 0.1 % mass spectrometry-grade methane acid acetonitrile. Quality control samples were included before, during and after the samples as experimental techniques for repeated evaluation. Samples were detected using UPLC-QTOF/MS platform mass spectrometry in both positive and negative ion modes. Differential metabolites were quantified and screened using Mass Lynx software.

UPLC-QTOF/MS was used to determine plasma VRC and VRC N-oxide. Quantitative analysis of ion pairs: VRC m/z 350.1 → m/z 127.0, N-oxide VRC m/z 366.1 → m/z 224.0, ketoconazole (internal standard) m/z 531.0 → m/z 82.1. Ion source voltage: 5,500v, temperature: 550.0°C, de-clustering voltage: 13.5 V/49.0 V/15.6 V, collision energy: 46 V/13.8 V/78.8 V. Details are described in the Supplemental Methods.

2.6. 1-MNA determination

Three plasma samples (patients or mice) in each group were randomly selected after treatment and received VRC (10 mg/kg) for 2 hours, and liver tissues were harvested. 1-MNA was detected by UPLC-MS/MS. The isocratic mobile phase was a mixture of 0.1% formic acid and methanol (v/v) and was introduced into the electrospray ionization chamber of the mass spectrometer at a flow rate of 1 ml/min. The retention time of 1-MNA was 2.12 min in each case. Details are referred to the previously report [20].

2.7. Acetaminophen determination

The acetaminophen assay was used to determine the activity of P-gp in the intestinal tract after chemotherapy. The maximum absorption time of acetaminophen in mice was 30~60 minutes. The peripheral blood of mice was collected 30 minutes after administration of paracetamol by gavage. The plasma samples were allowed to rest for 1 hour and then centrifuged at $4,500 \times g$ for 10 minutes. The sample was measured using full-measurement spectrophotometry [21,22].

2.8. Mouse liver microsomes, intestinal microsomes and enzyme activity assays

On the last day of the experiment, the mice in each group fasted for 12 hours. Details of mouse liver microsomes, intestinal microsomes preparation are described in the Supplemental Methods. The liver microsome was then suspended in a solution containing 1% Tris HCl buffer (pH 7.4) at a concentration of 10 mg / mL. and the concentration of protein in the microsomes was determined using the BCA method.

In order to assess the function of CYPs in liver microsome samples, a mixture of 75 µL of microsomes (with a final concentration of 0.5 mg/mL), 5 µL of substance at varying concentrations, and 20 µL of NADPH regeneration system consisting of 2 mmol/L NADPH, 10 mmol/L G-6-P, and 1 U/mL PDH, and 10 mmol/L MgCl₂ were mixed and then incubated in a water bath at 37°C for 5 min. After incubation, the samples were quickly placed on ice and the reaction was stopped by adding 300 µL of methanol containing tamsulosin (100

ng/mL) as an internal standard. Mixtures were then vigorously extracted for 5 min and then centrifuged twice at $18,000 \times g$ for 5 min before injection for HPLC–MS/MS analysis. The data were fitted to the Michaelis-Menten model defined by the following equation:

$$V = V_{\max} \times S / (K_m + S)$$

Where V is the reaction rate, S is the substrate concentration, $V_{\max}$ is the maximum enzyme velocity and K_m is the Michaelis constant, which is equal to the concentration of substrate for half-maximal velocity.

The purpose of this assay was to ensure that the selected animals had a uniform metabolic capacity for VRC. Prior to the experiment, mice were randomly assigned to the pre-experiment 1 group or the pre-experiment 2 group. They were administered an oral cocktail of 5 ml/kg containing 15 mg/kg omeprazole in CMC-Na solution. Blood samples were collected in heparinized test tubes at 0 h, 0.08 h, 0.25 h, 0.5 h, 1 h, 1.5 h, 2 h, 4 h, 6 h, 8 h, 12 h and 24 h after administration. The plasma was separated by centrifugation at 13,000 rpm for 10 minutes. The concentrations of omeprazole and VRC were determined by lipid-LC-MS/MS.

2.9. Intestinal biodiversity in AML patients

In order to conduct a microbial diversity analysis, fecal samples were collected from patients with AML at Shenzhen Hospital, Southern Medical University and Shenzhen Longhua District Central Hospital. In accordance with the manufacturer's instructions, fecal samples were collected in labeled stool tubes, flash-frozen, and stored at -80°C . Microbial DNA was extracted from all samples using an E.Z.N.A.® DNA kit. The primers are listed in Supplementary Table 1 and the sequencing steps are the same as previously reported [6].

2.10. Real time PCR

As in 2.2, liver tissues were extracted from mice. RNA was extracted from mouse liver tissue using the TRIpure kit according to the manufacturer's instructions. Real time PCR was then performed. The primer sequences of the target genes are shown in Supplementary Table 1-2.

2.11. Metabolome analysis

The vein plasma metabolome of mice was detected using ESI-QTOF/MS and UPLC-QTOF/MS mass spectrometers. Samples were subjected to methanol precipitation and extraction, filtered and injected into the Acquity UPLC BEHC18 column (130 Å, 17 µm, 2.1 mm × 50 mm). The mobile phase A contained 0.1% mass spectrometry-grade formic acid water and the mobile phase B contained 0.1% mass spectrometry-grade formic acid acetonitrile. Elution was performed according to the following gradient procedures: 0~2 min, 99% A and 1% B; 2~9 min, 40% A and 60% B; 9 ~ 9.1 min, 1% A and 99% B; 9.1 ~ 10 min, 99% A and 1% B. The injection volume was 10 µL. The flow rate was 0.4 mL/min. The capillary voltage was set to 3 kV and the voltage was set to 30 V. The data acquisition rate was set to 0.2 s and scanning was performed every 0.01 s. The atomizing gas was set to 800 L/h, the temperature was 500°C, the conical gas was set to 60 L/h and the

temperature was set to 120°C. Peak detection and pre-processing of the data was carried out using Mass Lynx software. The self-contained software was used for biometric analysis.

The pretreated samples were analyzed in positive ion mode under mass spectrometry conditions. The dual ESI source was used. The dry gas flow rate was 10 L/min, the dry gas temperature was 350°C and the scan mode was full scanning mode. The scanning range was m/z 50~1,200, the atomization chamber pressure was 35 psig, the fragment voltage was 120 V, and the cone hole voltage was 60 V. The reference solution matched to the instrument was calibrated to produce 5 $\mu\text{L}/\text{min}$ in real time via the automatic transfer injection port to ensure the stability and reproducibility of the instrument. In deionization mode (3,500 V), the conditions were the same as in positive ion mode. All samples were taken at the same volume and quality control samples were prepared to validate the method. Samples were injected every six samples to monitor the stability of the overall instrument operation.

2.12. Co-culture of Kupffer cells and hepatocytes

Cell isolation and digestion conditions. Hepatocytes and Kupffer cells were isolated using a two-step enzymatic digestion protocol. Initially, in situ liver digestion was performed through portal vein perfusion with type IV collagenase (0.05% concentration dissolved in Hanks' balanced salt solution, pre-warmed to 37°C) at a perfusion flow rate of 10-15 mL/min for 15 minutes until the tissue reached a semi-viscous consistency. Following excised liver fragmentation, the tissue underwent digestion with 0.25% trypsin-EDTA solution in a 37°C shaking incubator at 120 oscillations per minute for 20 minutes. Digestion was terminated by filtration through a 200-mesh stainless steel sieve to collect the cell suspension. Kupffer cells were further purified using Percoll density gradient centrifugation (25% and 50% concentration gradients in a 3:2 volume ratio) with centrifugation parameters set at 120g for 20 minutes.

Co-culture system establishment. The culture medium consisted of DMEM/F12 basal medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin antibiotic solution. To maintain hepatocyte functionality, 10 mM HEPES buffer and non-essential amino acids were added to the medium. For Kupffer cell culture, an additional 10 ng/mL macrophage colony-stimulating factor (M-CSF) was incorporated to promote attachment and growth. Cells were seeded at a density of 5×10^5 cells/cm² and cultured using the Transwell co-culture system (0.4 μm pore size membrane) to enable indirect co-culture. The co-culture system was maintained in a 37°C incubator with 5% CO₂ atmosphere, with two-thirds of the medium replaced every 48 hours. Cell culture inserts (0.3 μm pore size) were used to co-culture Kupffer cells with hepatocytes to determine the effect of 1-MNA on hepatocytes. In brief, the upper chamber contained Kupffer cells, and the lower chamber contained hepatocytes isolated from normal or AML livers (Fig. 5A).

IL-10 or 1-MNA treatment. To determine the effect of IL-10 or 1-MNA on the biological properties of hepatocytes, hepatocytes from mouse liver were grown in a culture plate coated with IL-10 or 1-MNA. The experimental group received IL-10 treatment at a dose of 20 $\mu\text{g}/\text{mL}$ (dissolved in sterile PBS) or 10 $\mu\text{mol}/\text{L}$ 1-MNA, while the control group was administered an equivalent volume of PBS. The drug treatment duration was set at 24 hours, with the treatment solution added through the Transwell chamber to avoid direct cell

contact interference. To assess drug metabolism effects, the co-culture system was simultaneously exposed to voriconazole at concentration gradients of 50 μ M (dissolved in DMSO, final concentration < 0.1%). The treatment duration lasted 72 hours, during which cell viability and cytokine secretion were monitored. All experiments were conducted in triplicate, and cell viability was confirmed to exceed 95% using trypan blue staining before proceeding with subsequent analyses.

2.13. Dual-luciferase reporter gene assay

The plasmids CYP2C19-luc was used to analyse CYP2C19 activity. The pRL-TK Renilla luciferase (an internal control, Promega, USA) plasmid was used to control for transfection efficiency. Gene-specific primer sequences used for CYP2C19 promoter prediction refer to the Supplementary Table 2. In order to perform the dual-luciferase reporter gene assay, hepatocytes were cultured in 10 cm cell culture dishes at 70 to 80% density. At 24 h posttransfection, cells were treated with or without rifampin (5 μ g/mL), and firefly and renilla luciferase reporter signals were determined immediately using the Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA).

2.14. Gene knockdown by small interfering RNA (siRNA)

Inhibition of Hsp90 β expression in hepatocytes was performed using directed siRNA reagents. Hsp90 β -specific siRNA (siHsp90 β) and negative control siRNAs (siNC) used as controls, were purchased from Ribo BIO (GuangZhou, China). Several siRNA fragments were synthesised based on the mouse Hsp90 β mRNA transcript sequence from the NCBI PDB database. The siRNA was transfected into cells using Lipofectamine™ 3000 (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. The fragment was selected for further study when its effectiveness was over 50%, which indicates no off-target effects. Cells were rinsed and harvested for immunoblot analysis 24–48 h after transfection.

2.15. Flow cytometry analysis

The analytical procedure for flow cytometry was rigorously executed following the established protocols outlined in section 2.2.1. Liver tissue samples underwent enzymatic dissociation utilizing collagenase solution at 37°C for 30 minutes. The enzymatic reaction was terminated by adding 10% fetal bovine serum (FBS) to the homogenate, followed by one or two systematic washing cycles with phosphate-buffered saline (PBS). Hepatic single-cell suspensions were subsequently treated with 1 $\times$ red blood cell lysis buffer for 2–3 minutes to ensure complete erythrocyte removal.

Kupffer cells were effectively isolated from liver specimens through implementation of the three-step methodology described in reference [23]. The prepared cellular suspensions were subjected to staining procedures employing fluorescent-conjugated antibodies specific for surface markers, as comprehensively documented in Supplementary Table 3. All cytometric measurements were systematically performed using either the FACS Aria instrumentation manufactured by Becton Dickinson (New Jersey, USA). Immunofluorescence processing involved initial incubation with primary antibodies targeting CD45, CD3,

CD11b, F4/80, CD206 and iNOS, each at 1:200 dilution, maintained overnight at 4°C under light-protected conditions.

Following PBS rinsing at ambient temperature for 5 minutes, the cells were incubated with 1:1,000 diluted secondary antibodies for one hour under light-protected conditions. Nuclear staining was subsequently performed using DAPI at 37°C for 30 minutes. After application of fluorescence quenching mounting medium, immunostaining patterns of Kupffer cells were comprehensively assessed using fluorescence microscopy. Subsequent detailed analytical evaluation was performed through computational processing with FlowJo software version. Cellular subpopulations were methodologically classified according to established immunophenotypic criteria: the M1 macrophage subpopulation was identified by its characteristic F4/80⁺iNOS⁺CD206⁻ marker expression profile, while the M2 subpopulation displayed F4/80⁺iNOS⁻CD206⁺ phenotypic signature. CD45⁺ and CD3⁻ staining is used to identify liver macrophages, while F4/80⁺ and CD11b⁺ staining are used to identify Kupffer cells. The specific gating strategy can be found in Figure S6.

2.17. Histologic analysis

To stain liver tissue with H&E, a biopsy sample was inserted in 10% buffered formalin, sliced into 5 mm squares, and embedded in 10% buffered formalin. Slices of liver tissue from 2.2.1 section were cut into 5 mm thick sections after fixing in 10% buffered formalin. It was determined that lipolysis, inflammation, and coagulation were all measured in the liver. Immunofluorescence was performed on frozen sections of intestinal tissue as described in 2.2.1. On the other hand, liver tissue from ABX/ ABX+1-MNA treatment mice as described in section 2.2.2, monoclonal IL-10 antibody (1:1,000) was used to immunohistochemical (IHC) analysis.

2.18. Enzyme-linked immunosorbent assay (ELISA)

Liver, plasma or stool was collected from section 2.2 mice. Il1r1, Il1r12, Il6, Ccl7, Cxcl5, Tnfrsf11b, Tnfrsf10b, Il10 and other cytokines were analyzed using ELISA immunoassay kit (R&D System, Inc., Minneapolis, USA) according to the instructions of the manufacturer.

2.19. Western blotting analysis

Liver tissues from mice as described in section 2.2 were treated with RIPA lysate. The primary antibodies such as Hsp90 β (83.21 kDa, 1:500), CYP2C19 (57 kDa, 1:500), β -actin (43 kDa, 1:500), CYP3A4 (21.6 kDa, 1:500), β -Tubulin (55 kDa, 1:500), Lamin B (60 kDa, 1:500), PXR (48.7 kDa, 1:500), CYP2C (57 kDa, 1:1,000) and GAPDH (37 kDa, 1:1,000) were incubated overnight at 4°C and the corresponding goat anti-rabbit and goat anti-mouse IgG antibodies (1:2,000) were incubated for 1 hour.

2.20. Immunofluorescence

Immunofluorescence was used to assess the expression of key proteins in the intestinal mucosa. Fresh intestinal tissue was immediately sliced on a consistently cold slicer, with a thickness of approximately 5-6

μmol/L. If the tissue was not immediately stained and sealed, it was stored at -20°C. Prior to staining, the tissue was fixed with cold propionic acid at 4°C for 15 minutes, followed by two 5-minute washes with PBS. The endogenous peroxidase was then inactivated with 3% H₂O₂ for 20 minutes. To minimize light exposure, samples were washed twice with PBS for approximately 5 minutes. Normal serum was blocked and prepared with PBS at a ratio of 1:20. The serum was removed with filter paper and an antibody against the tight junction proteins Claudin-1 (1:1,000), ZO-1 (1:1,000), Hsp90β (1:500) and PXR (1:500) was added and incubated at 37°C for 2 hours. The samples were then rinsed twice with PBS for 5 minutes each time on a shaking table. A fluorescent secondary antibody was then incubated at 37°C for 40 minutes. The samples were rinsed twice with PBS for 5 minutes each time. DAPI (P-36931, Life Technologies Ltd., Australia) was then added dropwise to facilitate staining of the nucleus at the recommended duration of 40 minutes at 37°C. The samples were then washed three times with PBS (pH 7.4) for 5 minutes each time. To attenuate spontaneous fluorescence, a circle was drawn and a fluorescence quenching agent was added for approximately 5 minutes. The samples were then rinsed under running water and immediately sealed with a fluorescence quenching sealing film. Finally, the samples were observed and photographed under a microscope.

2.21. Cell Treatment and Luciferase Reporter Assay

Hepa1-6 cells were seeded in 48-well plates and co-transfected with a rifampin-responsive luciferase reporter plasmid (200 ng), a pRL-TK vector (10 ng) serving as an internal control for transfection efficiency, and a PXR expression plasmid. After 24 hours of incubation, the cells were treated with IL-10 at concentrations of 10 ng/mL, 50 ng/mL, or 100 ng/mL for an additional 24 hours. Negative control groups included untreated cells, while positive controls were treated with rifampicin (a known activator of CYP enzymes). Following treatment, cells were lysed, and luciferase activities were measured using the Dual-Luciferase Reporter Assay System (Promega) with a GloMax 20/20 Luminometer [24]. Firefly luciferase activity was normalized to Renilla luciferase activity to assess the effect of IL-10 on CYP2C and CYP3A4 promoter activity.

To evaluate the regulatory effect of IL-10 on CYP2C and CYP3A4 promoter activity, Hepa1-6 cells were transfected with specific promoter-driven luciferase reporter constructs, e.g., pGL4.25 (CYP2C9 promoter) or pGL4.26 (CYP3A4 promoter). Transfected cells were treated with IL-10 at the same concentration gradients (10 ng/mL) for 24 hours. Cell lysates were prepared, and luciferase activity was measured as described above. Data were expressed as relative light units after normalization to Renilla luciferase activity. This approach allowed quantification of IL-10-induced changes in CYP enzyme expression at the transcriptional level.

2.22. Chromatin Immunoprecipitation (ChIP) - quantitative polymerase chain reaction (qPCR)

ChIP assays were conducted using a SimpleChIP plus Enzymatic ChIP kit [25]. The ChIP-qPCR experiment utilized a PXR-specific antibody (Abcam, ab176774) and an IgG control antibody. Briefly, mouse liver tissues were fixed with formaldehyde. After lysing the samples, the chromatin was fragmented and subjected to immunoprecipitation with either anti-PXR antibodies or normal IgG as a control. The resulting

DNA fragments were subsequently purified and quantified using qPCR with primers designed to target specific regions (see Supplementary Table 2 for primer details).

2.23. Statistical analysis

The data were analyzed in GraphPad Prism version 8.0. An analysis of linear regression was conducted using R 3.5.3 (R Core Team, Vienna, Austria) using the *lm* function. One-way or two-way analysis of variance (ANOVA) was performed to compare the means of more than two groups followed by the post-hoc Bonferroni test for further group comparisons. Data were presented as means $\pm$ standard deviations (SD). The means of two groups were compared using Student's *t*-test. Statistically significant differences were considered at $P < 0.05$ for all two-sided tests.

3. Results and discussion

3.1. Demographic information

The clinical characteristics of the 65 AML patients during induction chemotherapy are shown in **Supplementary Table 4**. All patients were divided into a hepatotoxicity group and a non-hepatotoxicity group, according to whether VRC hepatotoxicity occurred.

The feces of the 65 AML patients above were collected and the content of 1-MNA in the feces was detected using the HPLC-MS/MS method. Compared with the blank control, 1-MNA components were detected in the feces of AML patients (**Fig. 1A**). A total of 65 steady-state trough concentrations (C_{ssmin}) of VRC were included in the analysis. To determine whether 1-MNA promotes hepatic metabolism of VRC, we measured the metabolites of VRC, calculated metabolic capacity, and established a correlation between 1-MNA and metabolic capacity. There was a significant positive linear correlation between the level of 1-MNA and the VRC metabolic ratio ($R^2=0.51$, $P < 0.01$; **Fig. 1B**). Meanwhile, the correlation between serum levels of AST, ALT, total bilirubin and peripheral blood 1-MNA was also analyzed in AML patients. As shown in **Fig. 1C-E**, a negative correlation was found between serum AST level and peripheral blood 1-MNA level in AML patients, with a correlation coefficient of -0.73 ($R^2=0.532$), indicating statistically significant differences. Similarly, there was a negative correlation between serum ALT level and peripheral blood 1-MNA level in AML patients, with a correlation coefficient of -0.69 ($R^2=0.48$), indicating a statistically significant difference. Therefore, by detecting the 1-MNA content in the feces of AML patients during chemotherapy, the degree of hepatotoxicity of VRC intake in AML patients can be preliminarily determined. Meanwhile, our finding providing a theoretical foundation for the use of 1-MNA in clinical AML patients to predict VRC induced hepatotoxicity.

3.2. Intestinal mucosal barrier damage and liver immune responses following intensive induction chemotherapy

An AML mouse model was established to explore the mechanism of action on VRC metabolism after intensive induction chemotherapy (**Fig. 1F-G&2A**). The mice were presented with enlarged body and spleen, positive MPO detection in peripheral blood smears, H&E-stained sections of liver and spleen (**Fig. 1H-I**), and the percentage of positive cells in GFP transfected MLL-9 reached approximately 80% after 28 days (**Fig. 1J-K**), indicating that the AML model was successfully established.

In a state of physiological equilibrium, the liver is subject to constant exposure to enterogenic antigens through the portal vein, while the liver's innate and adaptive immune responses are influenced by the bacterial breakdown of metabolites produced by commensal bacteria^[26]. Following the disruption of the intestinal barrier, viable bacteria and bacterial products migrate from the gastrointestinal tract into the portal vein or systemic circulation, subsequently stimulating immune cells in the liver and initiating an immune response^[27]. In this study, based on the results of liver function indicators (AST, ALT and total bilirubin) after chemotherapy, the animals were divided into the hepatotoxicity group and the non-hepatotoxicity group (**Fig. 1L-M**). The substances or microenvironments in the intestine and liver between the two groups were compared to explore the factors that might influence the differences in VRC metabolism. We harvested the colon tissues of mice from each group for H&E staining and two-color immunofluorescence analysis to observe the expression and distribution of tight junction proteins claudin-1 and ZO-1 in the colon tissues of mice from each group. The results of H&E staining are shown in **Fig. 1N**. The surface of the basal tissue of the control group and the AML model group was smooth and well-structured. The mice in the non-hepatotoxicity group and the hepatotoxicity group had incomplete mucosa and uneven folds after induction chemotherapy. Pathological score statistics showed that the damage to the colon tissue of the mice in the hepatotoxicity group was more pronounced than in the non-hepatotoxicity group, with a significant difference ($P < 0.05$). Further validation of the changes in expression and distribution of tight junction proteins was performed by immunofluorescence staining. Claudin-1 and ZO-1 proteins were significantly downregulated in the colon of AML mice after induction chemotherapy (**Fig. 1O**). It is worth noting that there was no significant difference in mucosal protein expression between the non-hepatotoxicity group and the hepatotoxicity group. The difference suggests that the intestinal mucosal barrier degrades significantly after chemotherapy, but the final difference in VRC metabolism may not be related to the degradation of the mucosal barrier.

3.3. Correlation between *IL-10* levels and VRC-induced hepatotoxicity mitigation

Previous studies have shown that the hepatic metabolism of drugs is closely related to the hepatic inflammatory response^[28]. In this study, the gene expression of inflammatory factors in liver tissue was examined. The results of mRNA expression levels of mice in each group are shown in **Fig. 2B**. The mRNA expression levels of *TNF* and *IL6* were lower in mice in the non-hepatotoxicity group than those in hepatotoxicity group ($P < 0.05$). On the contrary, the mRNA expression of *IL10* in the hepatotoxicity group was significantly lower than that of the non-hepatotoxicity group ($P < 0.01$). The liver microsomes of mice in each group were extracted and the enzyme activity in liver tissue was detected by the metabolic enzyme

substrate probe method. As shown in **Fig. 2C**, omeprazole is a specific substrate of CYP2C19 and is metabolized by CYP2C19 to 5'-OH omeprazole. In the non-hepatotoxicity groups, 5'-OH omeprazole production was significantly higher than in the hepatotoxicity group, suggesting that there was a significant difference in the activity of CYP2C19 in the liver between the groups with and without hepatotoxicity. On the other hand, intestinal P-gp is the transporter responsible for the efflux of acetaminophen, therefore, the concentration of acetaminophen in the blood was used to detect the activity of P-gp in the intestine. As shown in **Fig. 2D**, there was no significant change in the concentration of acetaminophen in plasma between the hepatotoxicity and non-hepatotoxicity groups, indicating that there was no significant difference in intestinal P-gp activity between the groups.

Xenosensors or drug-responsive transcription factors involved in regulating CYP enzymes involved in VRC metabolism. An analysis of mRNA expression of these factors involved in VRC metabolism was performed to investigate the mechanism of metabolic differences between patients with hepatotoxicity and those without hepatotoxicity (**Fig. 2E**). In hepatocytes obtained from the mice, there was no difference in the expression of *CAR* and *AhR* mRNA between the hepatotoxicity and non-hepatotoxicity groups. Contrary to this, PXR expression in the hepatotoxicity group was significantly lower than in the non-hepatotoxicity group ($P < 0.001$).

Further mRNA expression analysis was conducted on liver cell metabolic enzyme-related genes and their potential upstream and downstream targets. The results revealed significant differential expression patterns of the IL-10 receptor (*IL10R*) and *CYP2C19/CYP3A11* genes, while no significant differences were detected in *P-gp* gene and protein expression (**Fig. 2F-G**). In contrast to P-gp expression, CYP enzymes involved in voriconazole metabolism—CYP2C29, CYP2C38, CYP2C50, CYP2C65, and CYP3A11 — exhibited significant differential expression between the hepatotoxicity and non-hepatotoxicity groups (**Figure 2H**). These findings suggest that hepatotoxicity is associated with the expression of voriconazole-metabolizing enzymes, but not with P-gp expression.

To evaluate hepatic VRC metabolism, a metabolic ratio was calculated four hours post-VRC administration. Our analysis revealed a positive correlation between IL-10 levels and this metabolic ratio (**Fig. 2I**). Concurrently, we observed a positive association between liver 1-MNA content and hepatic IL-10 expression (**Fig. 2J**). To further investigate the molecular basis of hepatotoxicity, we employed DESI-MSI to quantify VRC accumulation (**Fig. 2K**). Mass spectrometry imaging, which visualizes spatial distribution through mass-to-charge ratio (m/z) analysis, demonstrated significantly elevated voriconazole fluorescence intensity in the liver periphery of hepatotoxicity group animals (m/z 350.1228) compared to metabolically normal controls. Statistical analysis of three liver lobe samples per group confirmed substantially higher voriconazole accumulation concentrations in hepatotoxicity group tissues (**Fig. 2K**). The hepatotoxicity group exhibited significantly greater VRC residues in liver tissue versus the non-hepatotoxicity group. Correlation analysis in seven randomly selected animals further revealed a significant association between elevated AST levels and hepatic VRC accumulation following chronic administration (**Fig. 2L**).

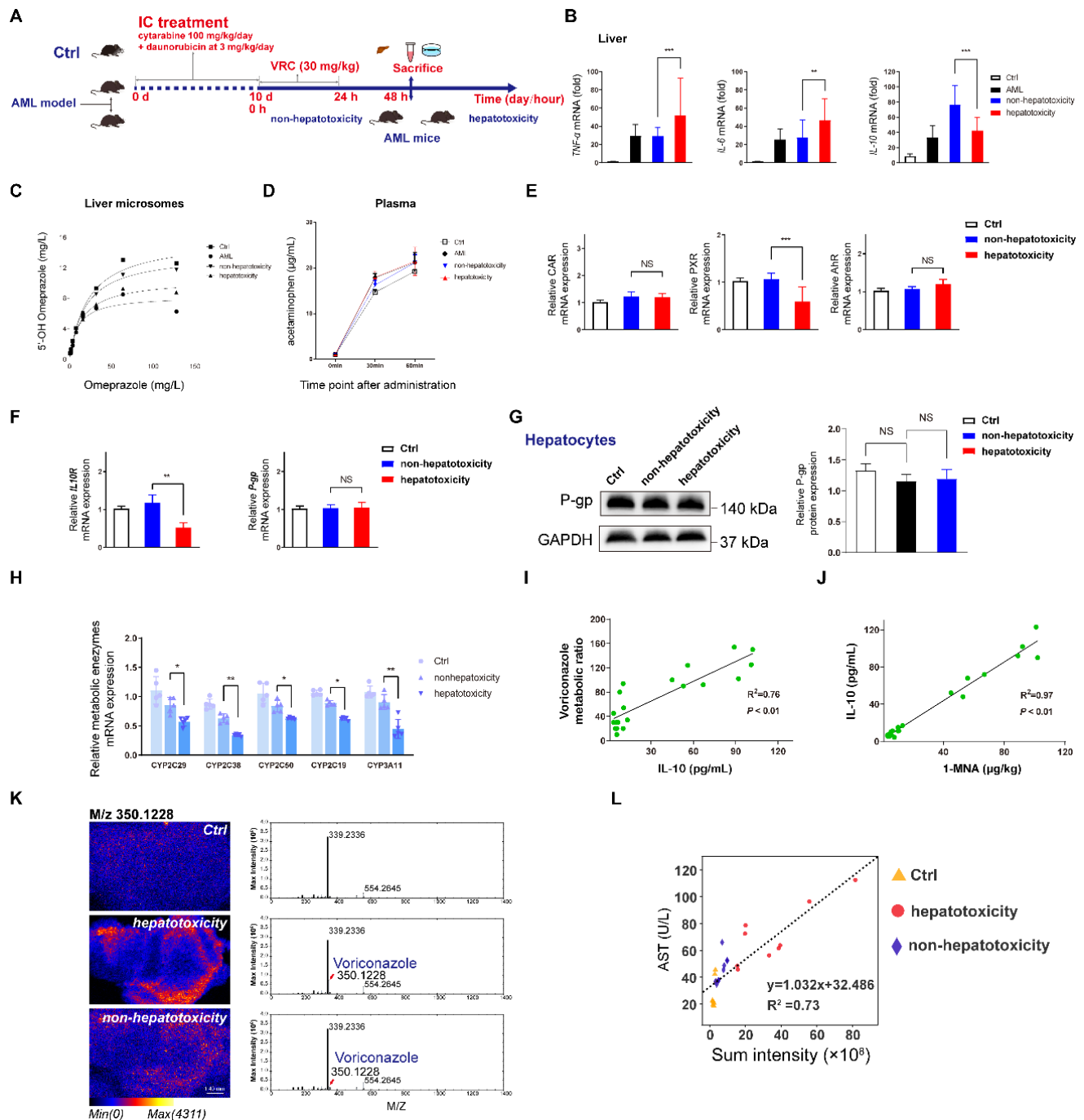


Fig. 2. The secretion of anti-inflammatory factor (IL-10) after intensive induction chemotherapy contributes to the metabolism of VRC in the liver.

A, Schematic diagram illustrating the comprehensive experimental workflow implemented in this study. AML, acute myeloid leukemia. IC treatment, intensive induction chemotherapy regimen. B, Statistical analysis of the relative expression of inflammatory factor genes in the liver of experimental mice in each group. C, Difference in the ability of liver microsomes to metabolize omeprazole. D, Concentration of acetaminophen in peripheral blood 30 and 60 minutes after administration. E, Analysis of the differential expression of key nuclear transcription factors that regulate drug metabolism enzymes in the hepatocytes of each group. F, Analysis of the differential expression of IL-10 receptor (*IL10R*) and *P-gp* in the hepatocytes of each group. G, Western blots of P-gp protein in hepatocytes (left) and intergroup statistical analysis (right). NS indicates no significant difference. H, Analysis of the differential expression of VRC-related drug metabolizing enzyme genes in the hepatocytes of each group. I, Correlation of IL-10 level and the VRC metabolic ratio in each group ($n = 25$). J, Correlation of 1-MNA level and the IL-10 level in each group ($n = 25$). K, Representative diagram of quantitative analysis of VRC DESI-MSI localization ($n = 3$). Bar = 1.4 mm. L, AST-VRC correlation analysis ($n = 9$). The equation of the line and R^2 are shown in the panel. * $P < 0.05$, ** $P < 0.01$. one-way ANOVA with Dunnett's or Bonferroni's multiple comparisons post hoc tests. Bar = 100 μm .

3.4. IL-10 secretion and liver macrophage M2 polarization regulation by intestinal-derived 1-MNA

It is known that the immune response of the liver is strongly influenced by the intestine. Genomic and metabolomic analysis of the intestinal microbiota in each animal group allows the examination of specific substances that could potentially influence the immune response of the liver. The Spearman correlation method was used to evaluate the relationship between the microbial flora and the biochemical indicators. **Fig. 3A** shows a heatmap illustrating the environmental biochemical indicators and the relative abundance of microbiota at the species level in each group. Consistent with these results, examination of the 16S composition of the intestinal microecology in the cohorts revealed notable differences in bacterial community structure between the hepatotoxicity and non-hepatotoxicity groups, particularly with respect to the abundance of *Bacteroidetes* (**Fig. 3B**). The gut microbiota is involved in host metabolism by interacting with host signaling pathways [29]. Analysis performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) demonstrated that alterations in the gut microbiota were associated with significant perturbations in the nicotinate and nicotinamide metabolic pathway, which was found to be a crucial metabolic pathway affected in hepatotoxicity and non-hepatotoxicity groups (**Fig. 3C**). We determined the metabolites in the plasma of the hepatotoxicity and non-hepatotoxicity groups by capillary electrophoresis TOF mass spectrometry (CE-TOFMS)-based metabolomic analysis (**Fig. 3D**). Several metabolites in the plasma of non-hepatotoxicity mice had higher levels compared to those in the plasma of hepatotoxicity mice, including 1-MNA, a terminal product of nicotinamide clearance. Spearman analysis revealed no significant association between the levels of other two metabolites (Threonate and Leu) and hepatic inflammatory factor expression in AML. The elevation of Threonate and Leu does not appear to modulate voriconazole-induced hepatotoxicity through inflammatory pathways (**Figure S2**). In order to determine the influence of intestinal-derived 1-MNA on the induction of IL-10 secretion in liver macrophages, we performed a correlation analysis of 1-MNA levels and gene expression of immune and inflammatory factors in feces, plasma and liver. The results of the analysis showed a significant negative correlation between 1-MNA levels and the expression of pro-inflammatory factors, while a significant positive correlation was observed between 1-MNA levels and the secretion of the anti-inflammatory factor IL-10 (**Fig. 3E**). Accordingly, mouse-derived liver macrophages from hepatotoxicity or non-hepatotoxicity mice were cultured *in vitro* for 6 hours, followed by analysis of M2 polarization in each group. The proportion of M2 polarization in the non-hepatotoxicity group was significantly higher than that in the hepatotoxicity mice (**Fig. 3F and Figure S7A**). In addition, the expression of 1-MNA in the liver had a remarkable positive correlation with the secretion of IL-10 (**Fig. 3G**). As shown in **Fig. 3H**, the experimental results of administering IL-10 neutralizing antibodies in mice revealed that 1-MNA specifically promotes IL-10 secretion in the liver and upregulates the gene expression of the anti-inflammatory cytokine (IL-10) while downregulating the gene expression of pro-inflammatory cytokines (TNF- α and IL-1 β) (**Fig. 3I-J**). These results suggest that the increased expression of IL-10 in the liver of the non-hepatotoxicity group may be due to the modulation of M2 polarization in Kupffer cells by the intestinal flora metabolite 1-MNA (**Figure S7B**).

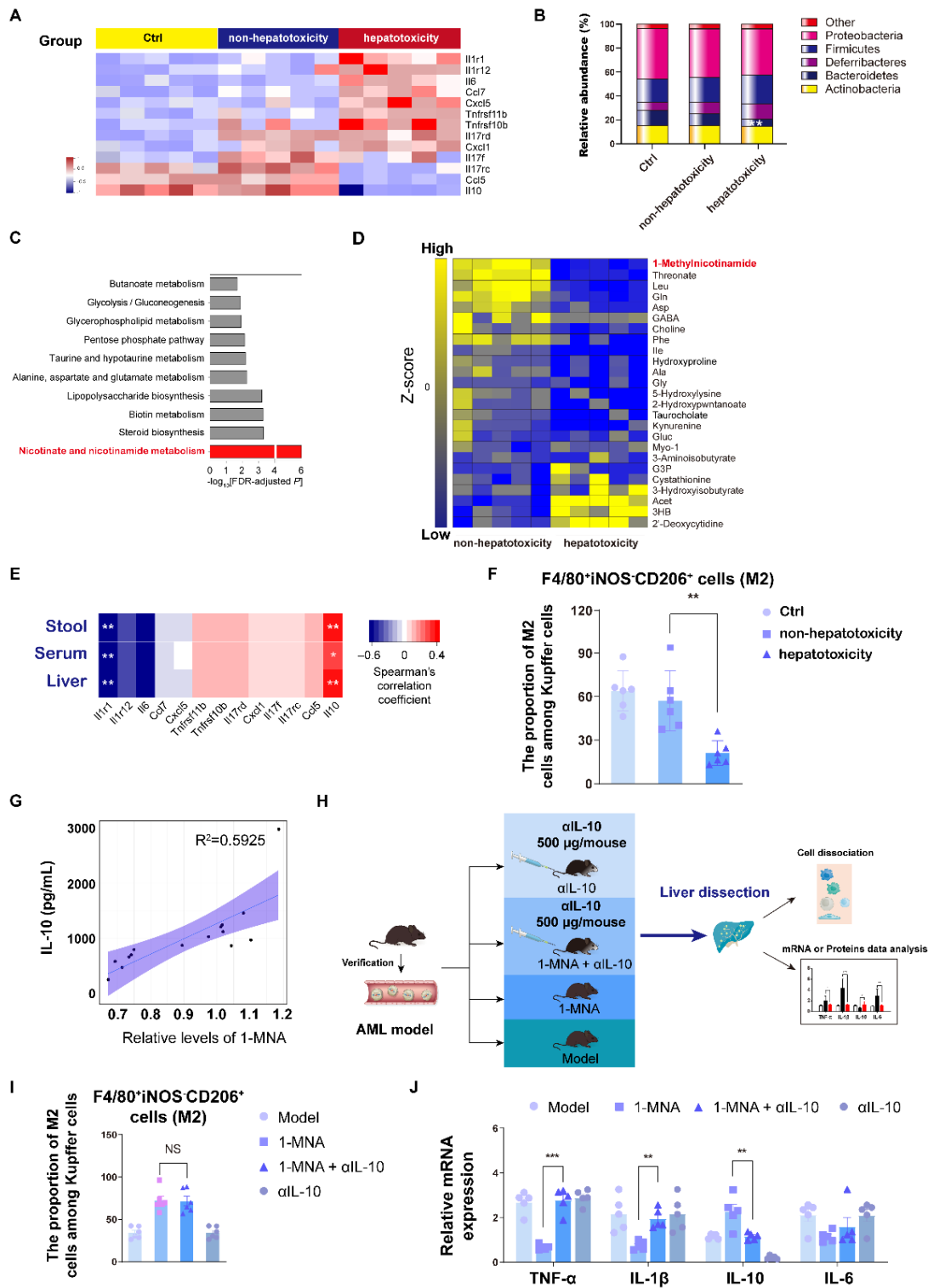


Fig. 3. Differences between hepatotoxicity and non-hepatotoxicity in terms of hepatic inflammatory response caused by intestinal-derived substances.

A, Heatmap analysis of the correlation between the abundance of microbiota and environmental factors (e.g. inflammatory factors). B, The relative abundance of bacteria at the phylum level in the intestinal. C, KEGG annotation of key altered metabolic pathways in individuals. D, Differential analysis of metabolomics in peripheral vein plasma between the groups.

E, Correlations of 1-MNA level with the levels of immune and inflammatory factors in the stool, serum and liver. F, Quantitative statistical analysis of M2-like macrophages (F480⁺iNOS⁺CD206⁺ cells) in the Kupffer cells, as analyzed by flow cytometry (n = 6). G, The correlation analysis between the relative levels of 1-MNA and the quantitative expression of IL-10 in the tissues (n=30). H, Flowchart of detection of hepatic tissue cell flow cytometry and inflammatory factor expression in AML mice administered with 1-MNA (100 mg/kg) and/or IL-10 neutralizing antibody (αIL-10) groups. I, Quantitative statistical analysis of M2-like macrophages in the Kupffer cells. J, Intergroup difference analysis of inflammatory factor gene expression levels. NS, no significance, **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *t*-test.

3.5. Intestinal sterilization exacerbates liver pro-inflammatory response and VRC hepatotoxicity

Based on the aforementioned human samples and animal experimental findings, a series of *in vivo* verification experiments were conducted to investigate the impact of intestinal sterilization on the severity of VRC-induced hepatotoxicity, with the aim of elucidating the role of the intestinal microbiota in these mechanisms. For this purpose, mice were orally administered a broad-spectrum antibiotic cocktail and induction chemotherapy, followed by VRC treatment. The specific process path can be seen in **Fig. 4A**.

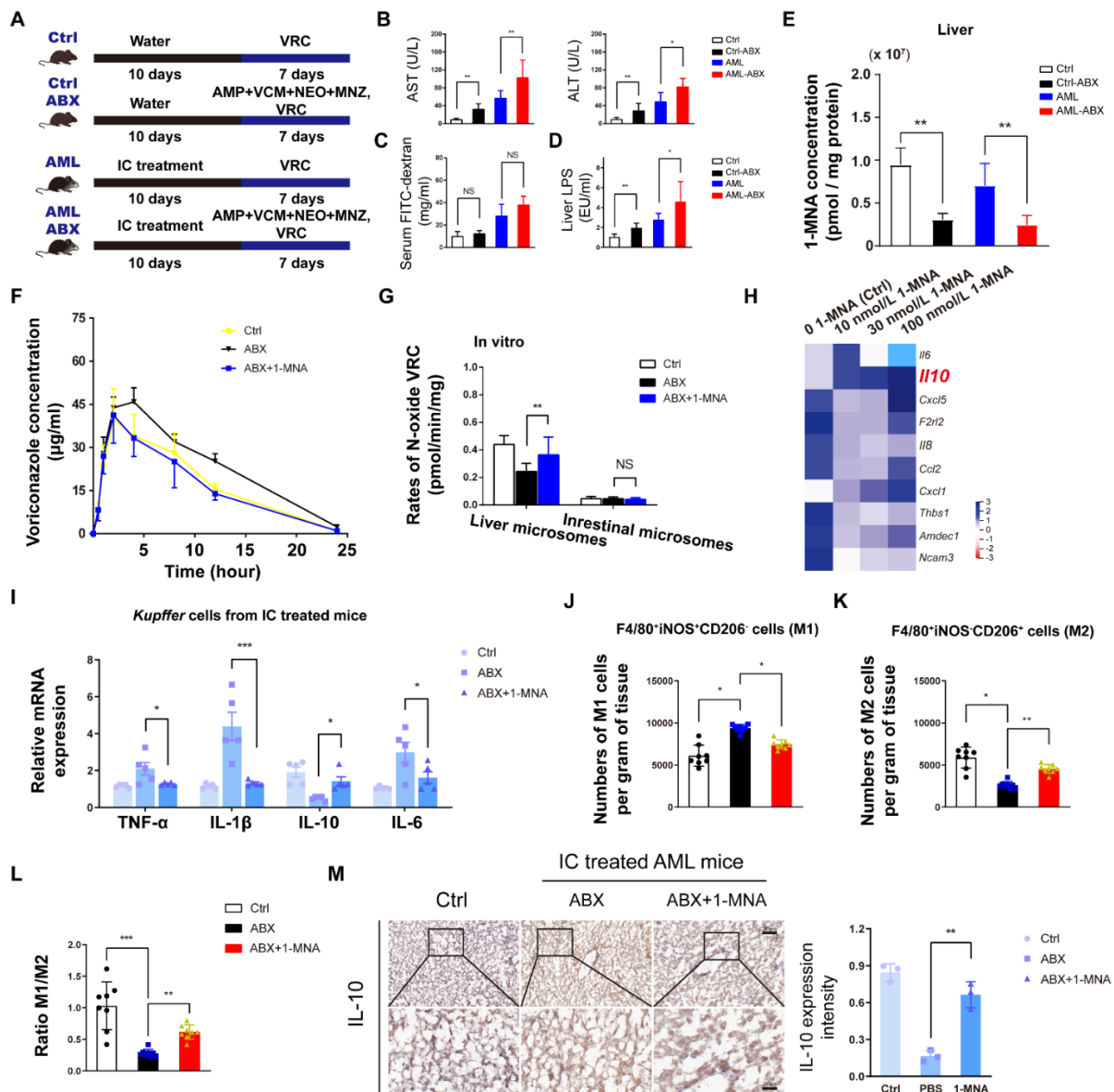


Fig. 4. Intestinal metabolite 1-MNA promotes the secretion of the anti-inflammatory factor IL-10 in Kupffer cells.

A, Model diagram of the animal experiment verification. B, Effects of intestinal clearance on liver function in normal and AML model mice after chemotherapy. C, FITC-dextran serum levels of mice in each experimental group after 4-hour intragastric administration. D, LPS levels in the liver of each experimental group. E, Levels of 1-MNA in liver tissue are shown. F, Effects of intestinal sterilization and 1-MNA administration on VRC metabolism. G, Analysis of differences in microsomal metabolism of VRC after intestinal sterilization and 1-MNA administration. H, Heatmap analysis of the correlation between administered 1-MNA concentration and gene expression of inflammatory factors in hepatocytes, nM=nmol/L. I, Effect of 1-MNA on the secretion of inflammatory factors in Kupffer cells in the liver after chemotherapy. J, Quantification of M1 macrophage polarization across liver tissue groups. K, Quantification of M2 macrophage polarization across liver tissue groups. L, Statistical analysis of M1/M2 ratio differences in liver tissues post-1-MNA administration. M, Immunohistochemistry to verify the effect of 1-MNA on IL-10 secretion (brown color) in the liver. $n = 3$. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, *t*-test.

Following a 27-day dosing experiment, the detection of serum indicators of liver function revealed that the liver function indicators of the animals in the intestinal sterile group were significantly elevated compared to those in the non-sterile group (**Fig. 4B**). The results of the FITC-dextran assay confirmed previous experimental results (**Fig. 4C**), indicating a substantial increase in intestinal mucosal permeability after chemotherapy compared to the normal animals (specific differential values were not reported). No significant difference in intestinal mucosal barrier damage was observed between the mice in the normal group and the AML group following intestinal sterilization. Interestingly, the LPS content in the liver was significantly higher after intestinal sterilization than in the group without intestinal sterilization (**Fig. 4D**). These results suggest that the metabolites produced by the intestinal microbiota may inhibit the hepatic inflammatory response and attenuate the hepatotoxicity resulting from the accumulation of VRC. Additionally, 1-MNA content in the liver was significantly reduced after intestinal sterilization than in the group without intestinal sterilization (**Fig. 4E**). The result further verified that 1-MNA in the liver was likely derived from intestinal metabolites.

3.6. 1-MNA regulates hepatic inflammatory factor secretion and enhances VRC metabolism in hepatocytes

To elucidate the mechanism by which intestinal-derived 1-MNA modulates hepatic inflammation and affects VRC metabolism, mice were administered 1-MNA and subjected to intestinal sterilization. The model was divided into two groups: an ABX control group and a 1-MNA-treated group, both undergoing 10 days of induction chemotherapy. The results demonstrated that 1-MNA administration significantly improved VRC metabolic capacity compared to ABX (**Fig. 4F**). Hepatic microsomes isolated four hours post-VRC administration revealed significantly higher VRC N-oxide production in the 1-MNA group, indicating enhanced hepatic VRC metabolism. In contrast, no significant difference in intestinal microsomal VRC metabolism was observed between groups (**Fig. 4G**).

In AML mice subjected to intensive induction chemotherapy, liver tissues from each group were analyzed to assess inflammatory gene expression at varying drug concentrations. The results revealed a dose-dependent upregulation of *Il10* gene expression in liver tissue with increasing 1-MNA concentrations (**Fig. 4H**). Additionally findings suggest that 1-MNA plays a crucial role in IL-10 secretion, rather than being influenced by CXCL1 (**Figure S3**). Furthermore, liver macrophages exhibited a marked increase in anti-inflammatory factor IL-10 expression in Kupffer cells following 1-MNA administration (**Fig. 4I**).

To investigate whether 1-MNA specifically induces M2 polarization, we quantified M2 polarization levels in mouse liver tissues post-1-MNA treatment (**Fig. 4J-L and Figure S7C-D**). The data demonstrated a significant elevation of M2 polarization, evidenced by increased expression of M2 markers (F4/80⁺iNOS⁻CD206⁺ cells) (**Fig. 4K and Figure S7D**). Notably, the 1-MNA-treated group showed a significantly reduced M1/M2 ratio compared to controls (**Fig. 4L**), indicating 1-MNA's specific induction of M2-type macrophage

polarization. Immunohistochemical analysis further confirmed these findings, revealing a substantial increase in IL-10 expression in 1-MNA-treated livers (**Fig. 4M**).

3.7. 1-MNA promotes VRC metabolism via Kupffer cell polarization rather than direct hepatocyte action

In our *in vitro* experiments, we initially isolated hepatocytes and Kupffer cells from AML mice post-chemotherapy and established a co-culture system to elucidate the indirect regulatory mechanism of 1-MNA on VRC metabolism in hepatocytes. **Fig. 5A** schematically presents the cellular co-culture model, while **Fig. 5B** demonstrates the evaluation of inflammatory factor expression in hepatocyte culture medium following 1-MNA treatment of Kupffer cells, along with quantification of IL-10-secreting cells in the Kupffer cell population.

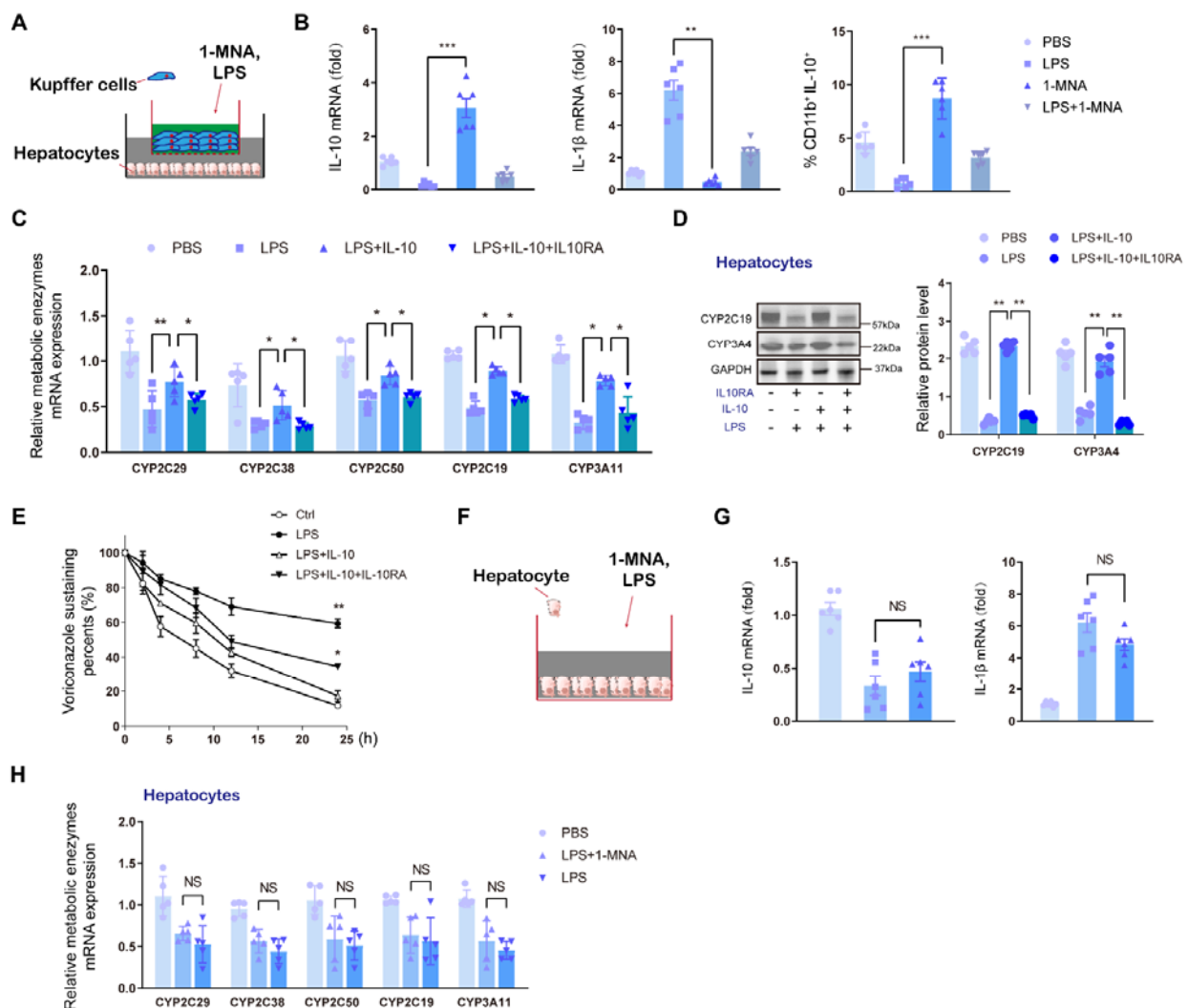


Fig. 5. 1-MNA indirectly promotes the expression and activity of drug metabolizing enzymes in hepatocytes through IL-10 secretion in Kupffer cells.

A, Diagram of co-culture and drug administration of hepatocytes and Kupffer cells. B, Analysis of differences in secretion of inflammatory factors and M2 polarization of Kupffer cells after administration of 1-MNA, $n = 6$. C, Isolated hepatocytes were stimulated by IL-10 and the expression of VRC-related drug metabolizing enzyme genes, $n = 6$. D, Analysis of protein expression of drug metabolizing enzymes induced by IL-10 stimulation in hepatocytes, assays were conducted in triple. E, Effects of IL-10 knockdown in hepatocytes on VRC metabolism. F, Experimental model diagram of the direct effect of 1-MNA on hepatocytes. G, Analysis of the differences in inflammatory factors between the groups after 1-MNA acts directly on hepatocytes, $n = 6$. H, Analysis of gene expression of drug metabolizing enzymes after the direct effect of 1-MNA on hepatocytes, $n = 6$. NS, no significance, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, t-test.

Given the observed capacity of 1-MNA to augment IL-10 secretion in Kupffer cells, we conducted *in vitro* stimulation experiments involving either LPS or IL-10 administration to hepatocytes. As illustrated in **Fig. 5C**, contrasting effects were observed between LPS and IL-10 treatments, with IL-10 administration resulting in a significant upregulation of mRNA expression for VRC-metabolizing enzymes in hepatocytes. The regulatory effect of IL-10 on hepatocyte metabolic enzymes was further corroborated by immunoblot analysis (**Fig. 5D**). Additionally, metabolic profiling following inhibition of hepatocyte IL-10 receptors and subsequent VRC administration revealed a compromised metabolic capacity for VRC (**Fig. 5E**), providing further evidence for the critical role of Kupffer cell-derived signaling in this regulatory pathway.

In this investigation examining the direct effects of 1-MNA on hepatocytes, the experimental model is depicted in **Fig. 5F**. Following 1-MNA administration, no significant differences were observed in the mRNA expression levels of both IL-10 and IL-1 β (**Fig. 5G**). Furthermore, analysis of drug-metabolizing enzyme gene expression in hepatocytes indicated that 1-MNA exerted no influence on the expression profiles of these metabolic enzymes (**Fig. 5H**). Collectively, these experimental findings demonstrate that 1-MNA modulates VRC metabolism in hepatocytes indirectly through Kupffer cell-mediated secretion of inflammatory factors, rather than through direct action on hepatocytes.

3.8. IL-10 induces VRC-related metabolizing enzyme overexpression through the Hsp90 β -PXR pathway

We then investigated the mechanism by which IL-10 modulates the activity of VRC metabolic enzymes. The experimental setup is shown in **Fig. 6A**. Following IL-10 administration, we performed a thorough examination of the transcription factors associated with the metabolic enzymes, focusing particularly on those that are primarily regulated by IL-10. The results of these experiments are shown in **Fig. 6B**. Remarkably, no detectable changes in the expression of the transcriptional regulator *CAR* were observed after IL-10 administration. In contrast, a significant increase in the expression of *PXR* was observed. Regarding gene expression related to metabolism-associated enzymes, a notable increase in the expression of *CYP2C19* and *CYP3A4* metabolic enzyme genes was observed, while no significant differences were observed in the expression of *P-gp*.

In order to investigate the regulatory mechanism of IL-10 on PXR transcription factors in hepatocytes, we performed *in vitro* experiments in which we stimulated or inhibited key factors involved in the downstream signaling pathway of IL-10 in isolated hepatocytes. After knockdown of Hsp90 β , there was a notable decrease in both cytoplasmic and nuclear expression of PXR compared to the normal control group (**Fig. 6C**). In addition, the ability of hepatocytes to metabolize VRC *in vitro* was significantly decreased (**Fig. 6D**). Notably, IL-10 stimulation was able to reverse the *in vitro* metabolism of VRC by mouse hepatocytes after chemotherapy. Immunofluorescence analysis after addition of geldanamycin (an Hsp90 β inhibitor) to hepatocytes also confirmed that IL-10 can upregulate the expression of Hsp90 β (**Fig. 6E**).

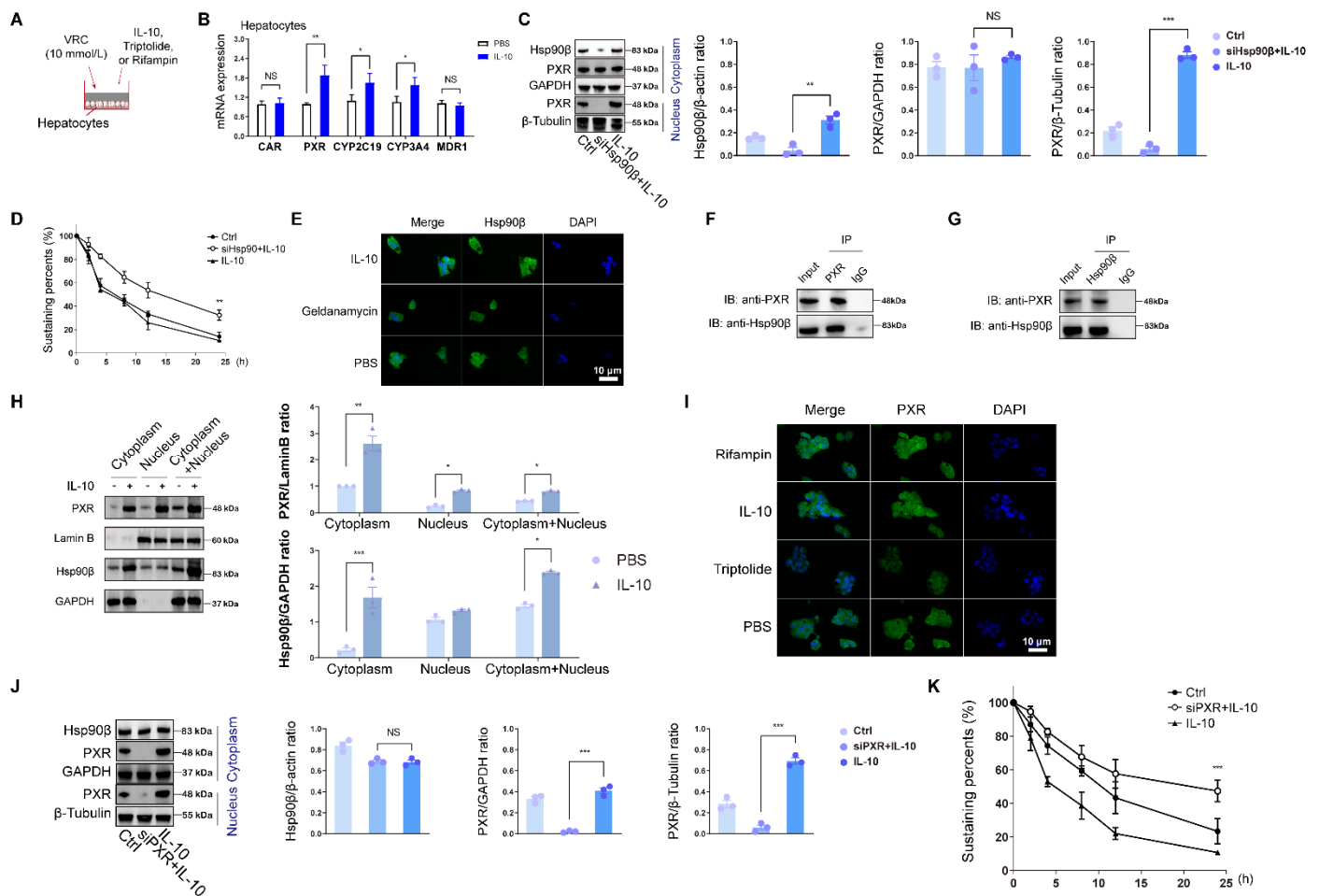


Fig. 6. PXR interacts with Hsp90β in the cytoplasm and is translocated to the nucleus, where it alone induces the activity of VRC-related metabolizing enzymes.

A, IL-10 stimulates hepatocytes to metabolize VRC in the experimental model. B, Expression of VRC-related metabolic enzyme genes and transcription factors after IL-10 stimulation. C, Western blotting analysis of protein expression that may be involved in PXR transcriptional regulation after IL-10 stimulation, assays were conducted in triple. D, Effects of Hsp90β gene knockdown on VRC metabolism in hepatocytes. E, Intracellular distribution of DAPI (blue) and Hsp90β (green) was confirmed with or without geldanamycin treatment by immunofluorescence analysis in hepatocytes, Bar = 10 μm. F, Interaction between PXR and Hsp90β. Cell lysates from PXR-hepatocyte cells were immunoprecipitated with anti-Hsp90β antibody, IgG as negative control, assays were conducted in triple. G, Cell lysates from Hsp90β-hepatocyte cells were immunoprecipitated with anti-PXR antibody, assays were conducted in triple. H, Hepatocytes were treated with IL-10 for 24 hours and then cells were fractionated into cytosol and nuclear extract. The indicated proteins were detected by Western blot analysis. The columns (cytoplasm + nucleus) were the sum of 50% cytosolic and 50% nuclear extracts. Lamin B and GAPDH served as nuclear and cytosolic markers, respectively. Assays were conducted in triple. I, Effects of IL-10 on VRC metabolism in hepatocytes. Intracellular distribution of DAPI (blue) and PXR (green) was confirmed with or without rifampin/triptolide treatment by immunofluorescence analysis in hepatocytes, Bar = 10 μm. J, Validation of PXR knockdown: IL-10 administration effects on Hsp90β and PXR intracellular protein bands in hepatocytes, with statistical analysis column charts, assays were conducted in triple. K, Effects of PXR gene knockdown on VRC metabolism in hepatocytes, n = 6. ****P* < 0.001, t-test.

PXR, a crucial transcription factor for the expression of metabolic enzymes, has been detected in both the cytoplasm and the nucleus in previous studies^[30]. To investigate the possible interaction between Hsp90β and PXR specifically in the cytoplasm, co-immunoprecipitation was used to detect the association between these two proteins using isolated hepatocytes from AML mice. The results of protein detection by Western blot analysis are shown in **Fig. 6F-G**. However, when immunoprecipitation was performed with IgG antibodies, no signal was detected on the blot.

Following the administration of IL-10 to the isolated hepatocytes of AML mice, the presence of PXR in both the cytoplasm and the nucleus was examined. The localization of PXR in the cytoplasm and nucleus was confirmed. In contrast, Hsp90 β was not translocated to the nucleus. To distinguish between the cytoplasmic and nucleic acid compartments, GAPDH and lamin B were used as markers, respectively. These results indicate that PXR interacts with Hsp90 β in the cytoplasm before translocating independently to the nucleus (**Fig. 6H**). Immunofluorescence analysis was performed on hepatocytes from AML mice treated both with and without rifampicin, IL-10 or triptolide to confirm the intracellular localization of PXR in hepatocytes (**Fig. 6I**), as well as its nuclear distribution, which was confirmed by DAPI staining. PXR was found to be distributed throughout the cell, encompassing both the nucleus and the cytoplasm. Notably, there were no detectable differences in PXR protein expression between hepatocytes treated with IL-10 and those stimulated with rifampicin. In contrast, the IL-10 group showed a significant increase in PXR expression compared to the triptolide group, providing evidence for the ability of IL-10 to stimulate PXR expression.

To further elucidate the signaling pathways of key proteins within the IL-10/Hsp90 β –PXR axis, we conducted functional validation through PXR gene knockout. The results demonstrated that PXR knockdown did not affect IL-10's stimulatory effect on Hsp90 β expression, suggesting that PXR acts as an effector downstream of Hsp90 β (**Fig. 6J**). Additionally, following gene knockout, the drug-metabolizing capacity of voriconazole was significantly reduced (**Fig. 6K**). Collectively, these findings indicate that IL-10 promotes the transcriptional regulatory activity of PXR, thereby upregulating the activity of drug-metabolizing enzymes and enhancing drug metabolism capacity. This mechanism ultimately reduces the accumulation of voriconazole in liver.

PXR, a pivotal transcriptional regulator, is well-established for its capacity to upregulate CYP enzyme gene expression. In this study, we utilized siRNA-mediated knockout of the CYP enzymes CYP2C19 and CYP3A4 (**Fig. 7A - B**) to investigate their role in counteracting IL-10-induced effects. Our findings revealed that following these enzyme gene knockouts, IL-10 administration significantly induced PXR transcriptional expression and enhanced the expression of *CYP2C19* and *CYP3A4* genes (**Fig. 7C - F**), demonstrating that IL-10 activates the transcription of PXR and its downstream effectors, CYP2C19 and CYP3A4.

The transcriptional activity of CYP2C19 in hepatic tissue is enhanced through pregnane X receptor (PXR) activation in acute myeloid leukemia (AML) mouse models. PXR demonstrates significant transcriptional regulatory plasticity, functioning as both an activator and repressor of target genes—including *CYP2C19* and *CYP3A4* [31]. To investigate the regulatory role of PXR in CYP2C19 transcription, luciferase reporter assays were systematically performed. Results demonstrated that PXR overexpression induced dose-dependent activation of luciferase expression under the control of a 2.1-kb CYP2C19 promoter region (-2.0/+0.1 kb) in hepatocytes (**Fig. 7G–H**).

Bioinformatic analysis further identified a putative PXR binding site (-456/-487) within the *CYP2C19* promoter. The binding potential was quantified using established databases including JASPAR and TRANSFAC. Each predictive entry was associated with clearly defined scoring metrics, with annotations

specifying that scores ≥ 1.5 indicate high binding probability. Detailed promoter fragment prediction scores are documented in **Supplementary Table 5**.

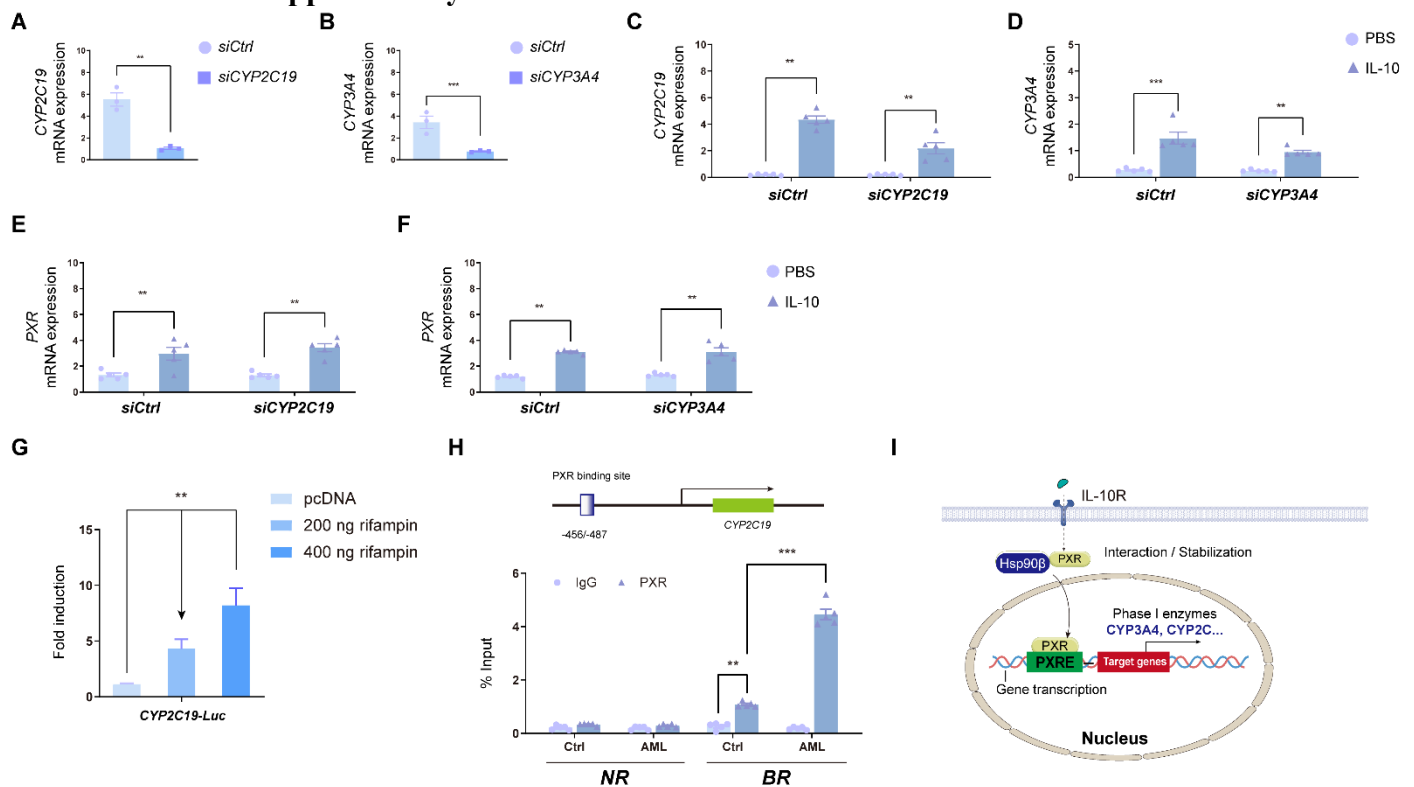


Fig. 7. PXR activated by IL-10 promotes the expression of VRC drug-metabolizing enzymes CYP2C19 and CYP3A4.

A & B, Bar charts depicting the differential expression of *CYP2C19* and *CYP3A4* genes following gene knockdown, respectively. C & D: Investigation into whether IL-10-driven transcriptional activation promotes the expression of *CYP2C19* and *CYP3A4* genes, both of which are involved in VRC drug metabolism. E & F, Bar charts depicting the differential expression of *PXR* genes following *CYP2C19* or *CYP3A4* gene knockdown, respectively. G, Effect of PXR overexpression on *CYP2C19*-Luc reporter activity in Hepa1–6 cells. H, ChIP assays show enrichment of PXR to binding site of *CYP2C19* promoter in the liver. BR, binding region. NR, non-binding region. I, Schematic representation of the regulatory mechanism of VRC-related metabolizing enzymes induced by IL-10. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, t-test.

The transcription of *CYP2C19* in the liver is upregulated by PXR activation in AML mouse liver models. PXR exhibits transcriptional regulatory versatility, capable of either activating or repressing target genes (including *CYP2C19* and *CYP3A4*) [31]. To determine whether PXR regulates *CYP2C19* transcription in the liver, we conducted luciferase reporter assays. These experiments showed that *PXR* overexpression led to dose-dependent activation of luciferase activity driven by a 2.1-kb *CYP2C19* promoter (-2.0/+0.1 kb) in hepatocytes (**Fig. 7G - H**). In silico analysis further predicted a PXR binding site (-456/-487) within the *CYP2C19* promoter region. Detailed scoring data from established databases such as JASPAR and TRANSFAC. Each entry will be accompanied by clearly defined scoring criteria, with annotations specifying that a score ≥ 1.5 indicates high binding probability. Promoter fragment prediction scoring report can be refer to the **Supplementary Table 5**.

To explore the in vivo regulatory effect of PXR on *CYP2C19*, we performed chromatin immunoprecipitation (ChIP) analysis. The results demonstrated hepatic PXR protein recruitment to *CYP2C19* binding sites in both AML and control mice (**Fig. 7H**). However, PXR recruitment to the binding region (BR)

was significantly more pronounced in AML mice compared to controls. No significant difference in PXR enrichment was observed in the distal non-binding region (NR), which served as a negative control (**Fig. 7H**).

Collectively, these findings suggest that PXR activation in mouse liver specifically upregulates CYP2C19 transcription, with this effect being particularly evident in AML mice. We hypothesize that IL-10 facilitates the cytoplasmic interaction between Hsp90 β and PXR, thereby promoting PXR translocation to the nucleus where it regulates transcription factor activity (**Fig. 7I**).

4. Discussion

Voriconazole, a broad-spectrum antifungal agent, demonstrates potent inhibitory effects on fungal proliferation within the intestinal microbiota. Our previous investigation into fungal diversity in the gut microbiome of AML patients revealed no substantial differences in intestinal fungal composition across distinct VRC metabolic subgroups, as illustrated in **Figure S1**. Consequently, this study was designed to elucidate the critical involvement of intestinal bacteria and their metabolites in regulating VRC hepatic metabolism. The current findings indicate that alterations in intestinal bacterial populations, particularly *Bacteroides fragilis*, and their metabolic processing of nicotinic acid and nicotinamide may contribute to the observed intergroup variability in VRC metabolic capacity. Both in vitro and in vivo experimental models involving 1-MNA administration following intensive induction chemotherapy revealed its capacity to induce M2 polarization of Kupffer cells, stimulate interleukin-10 (IL-10) secretion, and subsequently elevate the expression of VRC-metabolizing enzymes in hepatocytes.

Biological specimens were systematically collected from patients who had successfully undergone VRC prophylaxis or treatment for invasive fungal infections. Comparative analysis between hepatotoxicity cases and control subjects demonstrated a significant correlation between VRC-induced liver injury and supratherapeutic drug concentrations in peripheral circulation. These empirical observations are consistent with established clinical pharmacodynamic relationships^[10]. Importantly, the therapeutic trough concentration range associated with minimal hepatotoxicity risk corresponds with the established therapeutic window of 1.5–5.5 mg/L. The present investigation discloses markedly enhanced VRC metabolic clearance in the non-hepatotoxicity cohort relative to the hepatotoxicity group. The elucidation of precise molecular mechanisms governing these metabolic disparities constitutes a primary research objective of our ongoing investigation.

Concerning potential drug-drug interactions within the AML treatment framework, current investigations have not identified substantial effects of cytarabine or daunorubicin on voriconazole metabolism or the functional activity of CYP2C19 and CYP3A4 metabolic enzymes. This study further substantiates these observations by demonstrating that mice subjected to intensive induction chemotherapy consistently display differentiated hepatotoxicity phenotypes, thereby reinforcing the conception that AML chemotherapeutic regimens produce negligible interference with voriconazole-related metabolic pathways. Our research uncovers a significant aspect of the gut-liver axis in AML pathophysiology, specifically its regulatory influence on VRC metabolism. Previous research has firmly established the anti-inflammatory properties of *Bacteroides fragilis* and its polysaccharide A in modulating inflammatory responses^[6,32], while the current

investigation additionally reveals that AML-specific intestinal dysbiosis dynamically modulates hepatic drug-metabolizing capacity^[10]. Contemporary clinical and preclinical investigations uniformly document substantial modifications in gut microbial ecology in AML patients, typically manifesting diminished alpha-diversity indices and reduced prevalence of short-chain fatty acid-producing bacterial taxa, such as *Faecalibacterium* and its metabolic product butyrate^[33,34]. Furthermore, intensive chemotherapeutic protocols exacerbate intestinal epithelial barrier integrity impairment, consequently facilitating bacterial translocation and promoting immune homeostasis dysregulation^[35].

The gut microbiota plays a fundamental role in modulating anticancer immune surveillance and determining clinical outcomes^[36,37]. Dietary intervention with grain proteins effectively ameliorates glucose metabolic disorders through gut microbiota remodeling that enriches indole-derived metabolites. These microbial metabolites demonstrate dual regulatory functions by enhancing intestinal barrier integrity through AhR-dependent signaling pathways, while simultaneously suppressing hepatic gluconeogenesis via regulation of the NLK/FOXO1 signaling cascade. These mechanistic insights highlight the therapeutic potential of grain protein supplementation for type 2 diabetes prevention and management^[38]. In the AML treatment paradigm, intensive induction chemotherapy combined with broad-spectrum antibiotics induces profound intestinal dysbiosis that accelerates disease progression. This pathological transition is characterized by elevated inflammatory mediators including C-reactive protein and interleukin-6, accompanied by substantial reduction in short-chain fatty acid production^[39]. Such alterations in microbial composition and metabolic output contribute to disease pathogenesis by compromising epithelial barrier function and promoting systemic inflammation^[5,40]. These microbiota-driven changes establish a pro-inflammatory milieu conducive to leukemogenesis while indirectly impairing hepatic metabolic capacity through persistent inflammatory signaling^[15].

The current investigation identified the intestinally derived metabolite 1-MNA as a key regulatory component within this molecular network. Through Kupffer cell-hepatocyte co-culture systems, we demonstrated that 1-MNA stimulates IL-10 secretion, which subsequently augments voriconazole metabolism via the Hsp90 β -PXR regulatory axis. This discovery provides new understanding of how gut microbial metabolites regulate hepatic drug metabolism in AML, emphasizing the therapeutic relevance of targeting gut microbiota composition in disease management. The research further revealed that dysregulated cytokine expression contributes to both immunodeficiency and immunopathology. Among critical regulatory elements, heat shock protein 90 β functions as a central coordinator of immunomodulation, apoptosis regulation, and immune response modulation^[41]. A primary research aim was to delineate the regulatory mechanisms governing drug-metabolizing enzymes in hepatocytes. Significant knowledge gaps persist concerning how IL-10 regulates the transcription factor PXR, despite its essential role in controlling these metabolic enzymes. To address this deficiency, we utilized rifampicin as a pharmacological control for PXR pathway activation^[42]. Comprehensive investigations have established that rifampicin induces Hsp90 β phosphorylation^[43]. The cytoplasmic interaction between Hsp90 β and PXR facilitates PXR nuclear translocation, thereby enabling

transcriptional regulation of drug-metabolizing enzymes^[44]. Our immunoprecipitation analyses verified the physical interaction between Hsp90 β and PXR following IL-10 administration, thereby validating their functional association in the cytoplasmic compartment. Interestingly, experimental outcomes revealed an unexpected divergence from rifampicin's classical mechanism. Specifically, Hsp90 β suppression attenuated IL-10's regulatory effects on PXR, indicating that IL-10 acts through Hsp90 β expression and activation, thus establishing Hsp90 β as an essential mediator in IL-10's regulation of VRC drug-metabolizing enzymes.

The nuclear receptors pregnane X receptor (PXR) and constitutive androstane receptor (CAR) are recognized as pivotal sensors that detect both endogenous metabolic byproducts and exogenous chemical compounds, orchestrating their metabolic processing and elimination^[45]. PXR exhibits species-specific regulatory control over multiple cytochrome P450 enzyme genes, including *CYP3A4*, *CYP2B6*, *CYP2B9*, *CYP2C8*, *CYP2C9*, and *CYP2C19*^[46]. In our current investigation, AML mice were initially stratified according to the presence or absence of hepatotoxicity following VRC administration. Subsequent comparative analysis of VRC metabolic patterns revealed that PXR serves as the principal transcriptional regulator governing the activity of key enzymes involved in VRC metabolism within this experimental model, whereas CAR demonstrated substantially less regulatory impact. Following PXR gene knockdown, when hepatocytes were exposed to IL-10, cytoplasmic Hsp90 β expression remained unaffected, while the cells' capacity to metabolize VRC significantly declined (**Fig.6J-K**). This confirms PXR as the effector of the Hsp90 β -PXR signaling axis and underscores its central role in regulating VRC-metabolizing enzyme activity. The enhanced VRC metabolic clearance primarily stems from PXR's dependent regulation of these cytochrome P450 enzymes. However, recent studies suggest that PXR overexpression may increase the activity of P-glycoprotein efflux transporters, potentially leading to doxorubicin/adriamycin resistance^[47]. Notably, our study observed no statistically significant changes in P-gp expression levels in the livers of AML mouse models (**Fig.2G**). Furthermore, 1-MNA had minimal effect on hepatic P-gp levels and did not induce doxorubicin resistance, thereby establishing its unique therapeutic potential for preventing and managing VRC-induced hepatotoxicity.

Collectively, these findings advance our understanding of 1-MNA's functional role and its potential to facilitate hepatic metabolism and elimination of pharmaceutical compounds. The peripheral blood concentration of 1-MNA following chemotherapy demonstrates considerable clinical prognostic value for predicting VRC-induced hepatotoxicity without undermining voriconazole's clinical effectiveness (**Figure S4**). Experimental evidence from in vitro systems confirms that hepatic 1-MNA stimulates IL-10 secretion from Kupffer cells while upregulating both expression and catalytic activity of CYP2C19 and CYP3A4 metabolic enzymes in hepatocytes. Therefore, integrating 1-MNA supplementation into the nutritional protocol for VRC-treated patients may constitute an effective approach for minimizing the incidence of VRC-related hepatotoxic events.

Ethics approval and consent to participate

This study was approved by the Ethical Committee of Shenzhen Hospital of Southern Medical University (No. NYSZYEC20180017) as well as the Ethical Committee of Shenzhen Longhua District Central Hospital (No. 2022-040-02), and all individuals provided informed consent for inclusion of their tissue in this study. The study protocol was approved by the ethics committee of Animal Experiments of Southern Medical University and in conformity with the Guide for the Care and Use of Laboratory Animals.

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Conflict of interest

All authors confirm the absence of any competing interests in relation to this research. The financial supporters of this investigation were not involved in formulating the research framework, conducting data acquisition and analytical procedures, interpreting research findings, preparing the manuscript, or determining publication outcomes.

References

- [1] Peterlin P, Garnier A, Le Bourgeois A, Guillaume T, Le Bris Y, Theisen O, et al. Tocilizumab in combination with a standard induction chemotherapy in acute myeloid leukaemia patients (TOCILAM study): a single-centre, single-arm, phase 1 trial. *EClinicalMedicine* 2023;64:102254. doi: 10.1016/j.eclinm.2023.102254.
- [2] Rosenblatt J, Stone RM, Uhl L, Neuberg D, Joyce R, Levine JD, et al. Individualized vaccination of AML patients in remission is associated with induction of antileukemia immunity and prolonged remissions. *Sci Transl Med* 2016;8(368):368ra171..
- [3] Secreto C, Chean D, van de Louw A, Kouatchet A, Bauer P, Cerrano M, et al. Characteristics and outcomes of patients with acute myeloid leukemia admitted to intensive care unit with acute respiratory failure: a post-hoc analysis of a prospective multicenter study. *Ann Intensive Care* 2023;13(1):79. doi: 10.1186/s13613-023-01172-3.
- [4] Wang X, Zhao J, Wen T, Liao X, Luo B. Predictive Value of FMO3 Variants on Plasma Disposition and Adverse Reactions of Oral Voriconazole in Febrile Neutropenia. *Pharmacology* 2021;106(3-4):202-210. doi: 10.1159/000510327.
- [5] Wang X, Hu X, Ye C, Zhao J, Tan SC, Zhou L, et al. Astragalus Polysaccharide Enhances Voriconazole Metabolism under Inflammatory Conditions through the Gut Microbiota. *Journal of Clinical and Translational Hepatology* 2024;12(5):481-495. doi: 10.14218/JCTH.2024.00024.
- [6] Wang X, Ye C, Xun T, Mo L, Tong Y, Ni W, et al. Bacteroides Fragilis Polysaccharide A Ameliorates Abnormal Voriconazole Metabolism Accompanied With the Inhibition of TLR4/NF- κ B Pathway. *Frontiers In Pharmacology* 2021;12:663325. doi: 10.3389/fphar.2021.663325.

- [7] Rausch CR, DiPippo AJ, Jiang Y, DiNardo CD, Kadia T, Maiti A, et al. Comparison of Mold Active Triazoles as Primary Antifungal Prophylaxis in Patients With Newly Diagnosed Acute Myeloid Leukemia in the Era of Molecularly Targeted Therapies. *Clin Infect Dis* 2022;75(9):1503-1510. doi: 10.1093/cid/ciac230.
- [8] Li J-J, Li Y-L, Chu W, Li G-Q, Zhang M, Dong J-J, et al. Astragaloside IV alleviates cytarabine-induced intestinal mucositis by remodeling macrophage polarization through AKT signaling. *Phytomedicine* 2023;109:154605. doi: 10.1016/j.phymed.2022.154605.
- [9] Ahmad R, Kumar B, Thapa I, Talmon GA, Salomon J, Ramer-Tait AE, et al. Loss of claudin-3 expression increases colitis risk by promoting Gut Dysbiosis. *Gut Microbes* 2023;15(2):2282789. doi: 10.1080/19490976.2023.2282789.
- [10] Mafuru M, Wu S, He S, Lu X, Huang J, Jiang H. The Influence of Proinflammatory Cytokines on Voriconazole Trough Concentration in Patients With Different Forms of Hematologic Disorders. *J Clin Pharmacol* 2019;59(10):1340-1350. doi: 10.1002/jcph.1422.
- [11] Gong S, Yan Z, Liu Z, Niu M, Fang H, Li N, et al. Intestinal Microbiota Mediates the Susceptibility to Polymicrobial Sepsis-Induced Liver Injury by Granisetron Generation in Mice. *Hepatology* 2019;69(4):1751-1767. doi: 10.1002/hep.30361.
- [12] Li R, Xie L, Li L, Chen X, Yao T, Tian Y, et al. The gut microbial metabolite, 3,4-dihydroxyphenylpropionic acid, alleviates hepatic ischemia/reperfusion injury via mitigation of macrophage pro-inflammatory activity in mice. *Acta Pharm Sin B* 2022;12(1):182-196. doi: 10.1016/j.apsb.2021.05.029.
- [13] Zeng Y, Wu R, Wang F, Li S, Li L, Li Y, et al. Liberation of daidzein by gut microbial β -galactosidase suppresses acetaminophen-induced hepatotoxicity in mice. *Cell Host & Microbe* 2023;31(5) doi: 10.1016/j.chom.2023.04.002.
- [14] Deng X, Li Y, Jiang L, Xie X, Wang X. 1-methylnicotinamide modulates IL-10 secretion and voriconazole metabolism. *Front Immunol* 2025;16:1529660. doi: 10.3389/fimmu.2025.1529660.
- [15] Taniki N, Nakamoto N, Chu P-S, Mikami Y, Amiya T, Teratani T, et al. Intestinal barrier regulates immune responses in the liver via IL-10-producing macrophages. *JCI Insight* 2018;3(12) doi: 10.1172/jci.insight.91980.
- [16] Tallman M, Wang E, Altman J, Appelbaum F, Bhatt V, Bixby D, et al. Acute Myeloid Leukemia, Version 3.2019, NCCN Clinical Practice Guidelines in Oncology. *Journal of the National Comprehensive Cancer Network : JNCCN* 2019;17(6):721-749. doi: 10.6004/jnccn.2019.0028.
- [17] Zhang X, Xiong Z, Zhang S, Li K, Bu Y, Zheng N, et al. Enrichment of milk antioxidant activity by dietary supplementation of red clover isoflavone in cows and its improvement on mice intestinal health. *Food Chem* 2024;446:138764. doi: 10.1016/j.foodchem.2024.138764.
- [18] Xu X, Wang J, Tong T, Zhang W, Wang J, Ma W, et al. A self-assembled leucine polymer sensitizes leukemic stem cells to chemotherapy by inhibiting autophagy in acute myeloid leukemia. *Haematologica* 2022;107(10):2344-2355. doi: 10.3324/haematol.2021.280290.
- [19] Wunderlich M, Mizukawa B, Chou F-S, Sexton C, Shrestha M, Saunthararajah Y, et al. AML cells are differentially sensitive to chemotherapy treatment in a human xenograft model. *Blood* 2013;121(12):e90-e97. doi: 10.1182/blood-2012-10-464677.
- [20] Luo L, Kay J, Zhang J, Holliman CL, Rodrigues AD, Dowty M, et al. LC-MS/MS assay for N1-methylnicotinamide in humans, an endogenous probe for renal transporters. *Bioanalysis* 2018;10(9):673-689. doi: 10.4155/bio-2017-0272.
- [21] Ghanem CI, Rudraiah S, Bataille AM, Vigo MB, Goedken MJ, Manautou JE. Role of nuclear factor-erythroid 2-related factor 2 (Nrf2) in the transcriptional regulation of brain ABC transporters during acute acetaminophen (APAP) intoxication in mice. *Biochem Pharmacol* 2015;94(3):203-211. doi: 10.1016/j.bcp.2015.01.013.
- [22] Xiao K, Li H, Li Y, Zhan B, Fang X, Zhao B, et al. Protective effects and mechanism of Sangyu granule on acetaminophen-induced liver injury in mice. *J Ethnopharmacol* 2024;331:118282. doi: 10.1016/j.jep.2024.118282.
- [23] Xu X-S, Feng Z-H, Cao D, Wu H, Wang M-H, Li J-Z, et al. SCARF1 promotes M2 polarization of Kupffer cells via calcium-dependent PI3K-AKT-STAT3 signalling to improve liver transplantation. *Cell Prolif* 2021;54(4):e13022. doi: 10.1111/cpr.13022.
- [24] Wang Z, Gao L, Ren S, Sun G, Lin Y, Wang S, et al. E4BP4 Regulates Hepatic Solute Carrier Family 2 Member 9 and Uric Acid Disposition in Mice. *Drug Metabolism and Disposition: the Biological Fate of Chemicals* 2022;50(5):591-599. doi: 10.1124/dmd.121.000790.

-
- [25] Wang S, Lin Y, Yuan X, Li F, Guo L, Wu B. REV-ERB α integrates colon clock with experimental colitis through regulation of NF- κ B/NLRP3 axis. *Nat Commun* 2018;9(1):4246. doi: 10.1038/s41467-018-06568-5.
- [26] Horst AK, Kumashie KG, Neumann K, Diehl L, Tiegs G. Antigen presentation, autoantibody production, and therapeutic targets in autoimmune liver disease. *Cell Mol Immunol* 2021;18(1) doi: 10.1038/s41423-020-00568-6.
- [27] Balmer ML, Slack E, de Gottardi A, Lawson MAE, Hapfelmeier S, Miele L, et al. The liver may act as a firewall mediating mutualism between the host and its gut commensal microbiota. *Sci Transl Med* 2014;6(237):237ra266. doi: 10.1126/scitranslmed.3008618.
- [28] Wang X, Rao J, Tan Z, Xun T, Zhao J, Yang X. Inflammatory signaling on cytochrome P450-mediated drug metabolism in hepatocytes. *Frontiers In Pharmacology* 2022;13:1043836. doi: 10.3389/fphar.2022.1043836.
- [29] Xu H, Fang F, Wu K, Song J, Li Y, Lu X, et al. Gut microbiota-bile acid crosstalk regulates murine lipid metabolism via the intestinal FXR-FGF19 axis in diet-induced humanized dyslipidemia. *Microbiome* 2023;11(1):262. doi: 10.1186/s40168-023-01709-5.
- [30] Florke Gee RR, Huber AD, Wu J, Bajpai R, Loughran AJ, Pruett-Miller SM, et al. The F-box-only protein 44 regulates pregnane X receptor protein level by ubiquitination and degradation. *Acta Pharm Sin B* 2023;13(11):4523-4534. doi: 10.1016/j.apsb.2023.07.014.
- [31] Zhang Y, Lan H, Yu X, Zhuo L, Zhao B, Liu F, et al. Schisandrin B Targets PXR to Enhance Bile Acid Metabolism and Alleviate ANIT-Induced Cholestatic Liver Injury via Dual Pathways. *Int J Biol Sci* 2026;22(1):387-409. doi: 10.7150/ijbs.121475.
- [32] Lin Y, He L, Cai Y, Wang X, Wang S, Li F. The role of circadian clock in regulating cell functions: implications for diseases. *MedComm (2020)* 2024;5(3):e504. doi: 10.1002/mco2.504.
- [33] Renga G, Nunzi E, Stincardini C, Pariano M, Puccetti M, Pieraccini G, et al. CPX-351 exploits the gut microbiota to promote mucosal barrier function, colonization resistance, and immune homeostasis. *Blood* 2024;143(16):1628-1645. doi: 10.1182/blood.2023021380.
- [34] Wang R, Yang X, Liu J, Zhong F, Zhang C, Chen Y, et al. Gut microbiota regulates acute myeloid leukaemia via alteration of intestinal barrier function mediated by butyrate. *Nat Commun* 2022;13(1):2522. doi: 10.1038/s41467-022-30240-8.
- [35] Hueso T, Ekpe K, Mayeur C, Gatse A, Joncquel-Chevallier Curt M, Gricourt G, et al. Impact and consequences of intensive chemotherapy on intestinal barrier and microbiota in acute myeloid leukemia: the role of mucosal strengthening. *Gut Microbes* 2020;12(1):1800897. doi: 10.1080/19490976.2020.1800897.
- [36] Lin Y-Y, Ding JL, Shen H-T, Lin Y-M, Adhidjaja EC, Chang S-C. PD-1/PD-L1 Cancer Immunotherapeutics Reshape Tumor Microenvironment - Clinical Evidence and Molecular Mechanisms for AI-based Precision Medicine. *Clin Rev Allergy Immunol* 2025;68(1):91. doi: 10.1007/s12016-025-09105-7.
- [37] Li A, Jin L, Xia H, Feng M, Zhou X, Chu Y, et al. Effects of oat β -glucan-based prebiotic blended formulas on defecation and gut microbiota in Chinese adults: a randomized clinical trial. *Food Science and Human Wellness* 2025;14(6):9250278. doi: 10.26599/FSHW.2024.9250278.
- [38] Dai Z, Bao X, Jiang H, Zhang Y, Shen Q, Xue Y. Grain proteins ameliorate glucose metabolism disorders by activating intestinal AhR and the hepatic NLK/FOXO1 pathway via gut microbiota-derived indole metabolites. *J Adv Res* 2025 doi: 10.1016/j.jare.2025.12.010.
- [39] Malard F, Thepot S, Cluzeau T, Carré M, Lebon D, Bories P, et al. Gut microbiota restoration with oral pooled fecal microbiotherapy after intensive chemotherapy: the phase 1b CIMON trial. *Blood Adv* 2025;9(15):3739-3749. doi: 10.1182/bloodadvances.2024015571.
- [40] Masetti R, Muratore E, Leardini D, Zama D, Turrone S, Brigidi P, et al. Gut microbiome in pediatric acute leukemia: from predisposition to cure. *Blood Adv* 2021;5(22):4619-4629. doi: 10.1182/bloodadvances.2021005129.
- [41] Saha B, Momen-Heravi F, Furi I, Kodys K, Catalano D, Gangopadhyay A, et al. Extracellular vesicles from mice with alcoholic liver disease carry a distinct protein cargo and induce macrophage activation through heat shock protein 90. *Hepatology* 2018;67(5):1986-2000. doi: 10.1002/hep.29732.
- [42] Li F, Lu J, Cheng J, Wang L, Matsubara T, Csanaky IL, et al. Human PXR modulates hepatotoxicity associated with rifampicin and isoniazid co-therapy. *Nat Med* 2013;19(4):418-420. doi: 10.1038/nm.3104.

- [43] Beuers U, Wolters F, Oude Elferink RPJ. Mechanisms of pruritus in cholestasis: understanding and treating the itch. *Nat Rev Gastroenterol Hepatol* 2023;20(1):26-36. doi: 10.1038/s41575-022-00687-7.
- [44] Lv C, Huang L. Xenobiotic receptors in mediating the effect of sepsis on drug metabolism. *Acta Pharm Sin B* 2020;10(1):33-41. doi: 10.1016/j.apsb.2019.12.003.
- [45] Thibaut MM, Bindels LB. Crosstalk between bile acid-activated receptors and microbiome in entero-hepatic inflammation. *Trends Mol Med* 2022;28(3):223-236. doi: 10.1016/j.molmed.2021.12.006.
- [46] Zhang L, Yan J, Liu J, Meng C, Liu F, Xia C. Panaxytriol upregulates CYP3A4 expression based on the interaction of PXR, CAR, HSP90 α , and RXR α . *Phytomedicine* 2022;101:154097. doi: 10.1016/j.phymed.2022.154097.
- [47] Niu X, Wu T, Li G, Gu X, Tian Y, Cui H. Insights into the critical role of the PXR in preventing carcinogenesis and chemotherapeutic drug resistance. *Int J Biol Sci* 2022;18(2):742-759. doi: 10.7150/ijbs.68724.