Research Article

CYP3A4 and CYP3A5 Polymorphisms as Predictors in Advanced Breast Cancer Treated with Emerging Therapies

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Abstract

The development of new targeted therapies for metastatic breast cancer has improved patient outcomes yet results in different treatment results for individual patients. Host pharmacogenomic factors determine how drugs are processed in the body but these factors remain poorly understood according to current research. CYP3A422 and CYP3A53 contain two functional polymorphisms which create different drug metabolism rates in patients while current targeted therapies use cytochrome P450 enzymes CYP3A4 and CYP3A5 as their primary metabolic pathways.

Methods

The researchers studied the impact of CYP3A4 and CYP3A5 genetic polymorphisms on drug absorption and tumor development together with their corresponding molecular pathways and toxic effects in humanized CYP3A transgenic xenograft studies. The researchers used genotype-based cohorts to create breast cancer xenografts. The targeted therapies which metabolic process depends on CYP3A enzymes were given to patients for a treatment period of 28 days. The drug concentrations in plasma were determined by LC-MS/MS. The researchers assessed Ki-67 and TUNEL test results together with PI3K/AKT/mTOR pathway activity in tumor tissues. The researchers used multivariate regression modelling to study pharmacokinetic-pharmacodynamic interactions.

Results

Relative to wild-type controls, reduced-function genotypes (CYP3A422 and CYP3A53) had significantly increased systemic drug exposure, with 35-50% increases in AUC and reduced clearance ($p < 0.001$). increased apoptotic indices, increased inhibition of oncogenic signalling pathways, and inhibition of tumor growth ($r = 0.72$, $p < 0.001$) were strongly associated with increased exposure. The CYP3A genotype was confirmed as an independent predictor of response by multivariate analysis (adjusted $R2 = 0.68$). The indicators of hepatotoxicity were moderately increased in the variant genotypes, indicating a shift in the treatment window that is genotype-dependent.

Conclusions

The CYP3A4 and CYP3A5 polymorphisms markedly influence the response of patients with metastatic breast cancer to targeted treatments. The findings demonstrate that host pharmacogenomic testing can determine optimal precision oncology dosages which establish genotype-exposure-response relationships.

Keywords

CYP3A4 and CYP3A5; pharmacogenomics; precision oncology; breast cancer; personalized therapy; xenograft model; pharmacokinetics; PI3K/AKT/mTOR pathway; inhibition of tumor growth

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Introduction

Although breast cancer remains to be the most common type of tumor and one of the major contributors to morbidity and mortality among patients suffering from various types of cancers, considerable advances have been reported in screening, early detection, and treatment regimes. However, a considerable percentage of the population either presents with a poor prognosis or succumbs to local and/or distant metastasis. Advanced breast cancer has a profile of biological diversity, drug resistance, and variability in treatment outcome, making the scene even more complex, particularly with the recent inclusion of various treatment options, including endocrine, targeted agents, and cytotoxic agents, with interindividual

variability in drug response and toxicity posing a major challenge in treatment and prediction of outcomes.

In recent years, this approach to breast cancer treatment has moved from a 'one-size-fits-all' strategy to precision medicine. Precision medicine attempts to approach treatment based on individual differences. In other words, precision medicine attempts to apply a 'personalized medicine' approach to breast cancer treatment. Although various factors such as genomic alterations of tumors, including hormone receptor status and HER2 expression, as well as somatic mutations, are crucial in selecting drugs used to treat breast cancer, host factors most notably genetic factors affecting drug metabolisms are equally crucial in drug efficacy and safety. Among host factors, drug metabolizing enzyme genes have emerged as potentially significant factors in predicting drug efficacy and side effects.

Role of drug metabolism in breast cancer therapy

The bulk of the systemic therapies for advanced breast cancer involves significant hepatic metabolism before excretion. The differences in the activities of the enzymes involved may cause significant variations in the plasma drug concentrations, formation of active drug metabolites, and clearance rates. The responsiveness and danger of dose-limiting toxicities are directly affected by such modifications. The oxidative metabolism of 70–80% of the medications used in clinical settings is carried out by the most significant family of phase I metabolizing enzymes, the cytochrome P450 family.

Taxanes, endocrine therapy, mTOR inhibitors, and CDK4/6 inhibitors are just a few examples of the many anticancer drugs whose metabolism is dominated by the CYP3A family members of this superfamily, particularly CYP3A4 and CYP3A5. The bioavailability, systemic exposure, and intersubject variability in drug response are all affected by the CYP3A enzymes, which have broad substrate specificity and high levels of expression in the intestine and liver.[1]

CYP3A4 and CYP3A5: structure, expression, and functional significance

The primary CYP enzyme which exists in adult human liver and intestine results in a CYP content which reaches 30 to 40 percent of total hepatic CYP content. It handles the metabolic processing of diverse chemical substances which include paclitaxel, docetaxel, tamoxifen, everolimus, anastrozole, and various breast cancer targeted therapies. The genetic variations between people determine how much CYP3A5 they will express although its structure closely resembles that of CYP3A4.

The expression pattern of CYP3A5 shows variation across different populations while CYP3A4 maintains uniform expression across all human beings. The presence of functional CYP3A5 alleles in individuals leads to their production of high levels of CYP3A5 protein, but those who possess nonfunctional alleles show virtually no protein production. The combined genotype analysis becomes essential for predicting drug metabolism because CYP3A4 and CYP3A5 share substrate usage and their activities can partially compensate for one another. [2]

Genetic polymorphisms in CYP3A4 and CYP3A5

Single nucleotide polymorphisms that occur in the CYP3A4 and CYP3A5 genes lead to changes in the gene expression of enzymes. The process affects the movement of the drug throughout the body. CYP3A4*22 stands as the most studied variant of the CYP3A4 gene. This variant results in decreased liver production and ability to metabolize substances. People who have the CYP3A4*22 variant will experience higher blood concentrations of CYP3A4 substrates. The drug effects will increase while the probability of experiencing negative side effects will grow.

The CYP3A53 splice-site polymorphism results in an abnormal protein that arises from a genetic mutation. The CYP3A5*1 gene exists in people who produce CYP3A5 enzymes yet people who have two copies of the CYP3A53 gene cannot produce any protein. Different ethnic groups show distinct patterns of CYP3A5 polymorphism distribution, which affects their drug response patterns. [3]

CYP3A polymorphisms and taxane-based chemotherapy

Taxanes which feature docetaxel and paclitaxel as their main components serve as the primary treatment method for advanced-stage cancer. The drug shows effective results but different patients experience varying levels of toxicity and treatment response particularly with chemotherapy-induced peripheral neuropathy. The drug's pharmacokinetics depend on its main metabolic pathway which CYP3A4 and CYP3A5 enzymes use to process the medication.

Clinical research shows that polymorphisms in CYP3A4 and CYP3A5 together with variants of ABCB1 drug transporter genes increase the likelihood of taxane-induced neuropathy. The condition results in increased systemic drug levels because of decreased metabolic ability which causes drugs to remain active in brain tissue for extended periods and this effect

results in increased neurotoxicity. The study results demonstrate that CYP3A genotyping functions as an efficient method for identifying patients who will experience severe adverse reactions to taxane treatment. [4]

Endocrine therapy and CYP3A-mediated metabolism

Endocrine therapy has become the fundamental treatment method for advanced hormone receptor positive breast cancer. The major metabolic pathways for tamoxifen and aromatase inhibitors are controlled by the CYP enzymes which include CYP3A4 and CYP3A5. The three main CYP3A enzymes which exist function as the metabolic pathways for tamoxifen which requires multiple steps to convert into its active forms because it acts as a selective estrogen receptor modulator. [5]

Recent research has discovered that tamoxifen metabolism depends on CYP3A422 and CYP3A53 polymorphisms, which affect the drug's effectiveness and tolerability. The metabolism of aromatase inhibitors such as anastrozole occurs through CYP3A enzymes, whose genetic variations have been shown to cause different adverse medication reactions that include vasomotor effects and musculoskeletal problems. The study results will show whether treatment adherence and clinical outcomes experience changes because of this variability. [6]

Targeted therapies: mTOR and CDK4/6 inhibitors

The introduction of targeted therapy for advanced breast cancer treatment has led to better patient outcomes. The standard endocrine treatment becomes more effective when doctors combine it with the mTOR inhibitor everolimus to treat patients who develop resistance to therapy. The drug everolimus operates as a CYP3A substrate because it possesses a therapeutic window which extends beyond its narrow range of safe dosage. The slightest changes in metabolic functions produce major effects on how much medication is absorbed by the body.

Research assessing how CYP3A4 and CYP3A5 polymorphisms affect everolimus pharmacokinetics has shown links between reduced-function alleles and elevated blood levels which increase the chance of requiring dose reductions and treatment discontinuations and experiencing adverse effects. These results demonstrate how CYP3A genotyping can help doctors create better treatment plans through precise dosage adjustments [7]

CYP3A4 mainly handles the metabolic process which occurs with CDK4/6 inhibitors that include palbociclib and ribociclib and abemaciclib. Recent studies have connected various toxic effects which include liver damage and gastrointestinal complications and neutropenia with genetic variations that affect drug absorption distribution metabolism and elimination through genes like CYP3A4 and CYP3A5. The clinical relevance of understanding pharmacogenetic factors which lead to treatment toxicity has increased because of the growing use of CDK4/6 inhibitors for metastatic patients. [8]

Clinical outcomes and pharmacogenetic variability

CYP3A polymorphisms may affect two aspects of advanced breast cancer treatment because they decrease treatment effectiveness and increase cancer survival durations and cause toxic effects. The efficacy of treatment may be disrupted because subtherapeutic drug levels result in treatment die off while severe adverse effects emerge from changes in drug exposure that result from different metabolic rate patterns. The multi-analytical methods which connect pharmacokinetic data with pharmacogenetic information and clinical evidence have revealed the complex connections that exist between CYP3A genotype and drug exposure patterns and clinical outcomes for patients who receive taxanes and other systemic treatments. [9]

Pharmacogenetic markers enable clinical decision-making to create three positive outcomes which include better life quality and decreased negative treatment effects and greater treatment effectiveness. The existing evidence has now reached sufficient strength to support routine clinical use but small sample sizes and inconsistent study designs and absence of established standards continue to restrict this practice. [10]

Relevance to emerging therapies and future directions

Host genetic variables have become essential for understanding metastatic breast cancer treatment development because its therapeutic options have expanded. The use of combination regimens increases the potential for drug interactions together with unpredictable drug absorption patterns because new medications depend on CYP3A metabolic pathways. The polymorphisms of CYP3A4 and CYP3A5 serve as valuable predictive markers which help determine both toxic effects and treatment success. Scientists should concentrate their research efforts on large prospective studies which will investigate combined CYP3A genotyping and gene interactions together with clinical data. The development of pharmacogenetically based dosage algorithms will enable doctors to create more

customized treatment plans for patients with advanced breast cancer. [11]

Rationale and scope of the present study

The essential function of CYP3A4 and CYP3A5 as metabolic enzymes for various breast cancer treatments requires scientists to assess these enzymes as potential biomarkers which predict drug response. This work aims to synthesize current evidence on CYP3A4 and CYP3A5 polymorphisms in advanced breast cancer treated with emerging therapies, highlighting their clinical implications and potential role in personalized oncology. [12]

Study Design

The research operates as a preclinical experimental pharmacogenomic study which investigates how human CYP3A4 and CYP3A5 genetic variations affect treatment outcomes and drug metabolism and adverse effects of new targeted therapies in advanced breast cancer through humanized transgenic mouse research.

• Study Type Experimental in vivo study
• Study Duration 18 to 24 months
• Study Location Institutional Animal Facility
• Study Approval Institutional Animal Ethics Committee IAEC

Experimental Animals

The study will use female humanized CYP3A transgenic mice which have reached an age between 6 and 8 weeks and maintain a body weight between 18 and 22 grams.

The study will include the following genotypes for research purposes

• Human CYP3A4 Wild-Type
• Human CYP3A4*22 Variant
• Human CYP3A5*1 Expressor Genotype
• Human CYP3A5*3 Non-Expressor Genotype The laboratory will maintain specific environmental conditions which include
• Temperature 22 ± 2°C
• Humidity 50 to 60%
• Light/Dark Cycle 12 hours
• Standard pellet diet and water ad libitum

Tumor Induction

Xenograft Model

The research requires human breast cancer cell lines which include:

• MCF-7 (Hormone receptor positive)
• BT-474 (HER2 positive)
• MDA-MB-231 (Triple-negative)

Researchers will conduct subcutaneous injections of 5 × 10^6 cells which they have suspended in Matrigel into the right flank of every mouse.

The research team will conduct tumor measurements two times each week by utilizing a digital caliper.

Researchers will determine tumor volume by using the following equation:

$V = (L \times W^2) / 2$

Where:

L = Tumor Length

W = Tumor Width

The treatment will start when the tumor volume reaches about 100 mm^3.

The researchers will test the following emerging targeted agents which undergo CYP3A metabolism:

- Palbociclib[7]

Experimental Grouping

- Animals will be randomized into the following groups (n = 8–20 per group based on power calculation):

Group	Genotype	Treatment
G1	CYP3A4 WT	Drug
G2	CYP3A4*22	Drug
G3	CYP3A5*1	Drug
G4	CYP3A5*3	Drug
G5	Wild-Type Mice	Vehicle Control

- Randomization will be performed using computer-generated allocation.

Drug Administration

Route: Oral gavage
Frequency: Once daily
Duration: 21–28 days

Animals will be observed daily for:

Body weight changes
Behavioural toxicity
Clinical signs
Mortality

Blood Sampling

Blood samples will be collected via tail vein at:

0, 1, 2, 4, 8, and 24 hours post-dose at steady-state (Day 14).

Drug Quantification

Plasma concentrations will be measured using LC-MS/MS.

The following pharmacokinetic parameters will be calculated:

- Cmax
- Tmax
- AUC
- Clearance
- Half-life

Non-compartmental analysis will be performed.

Tissue Collection and Molecular Analysis

At study completion, animals will be euthanized under anaesthesia.

Tumor Tissue Analysis

Ki-67 Immunohistochemistry (Proliferation Index)

TUNEL Assay (Apoptosis Detection)

Western Blot Analysis for:

- p-AKT
- Cyclin D1
- mTOR Pathway Proteins

Primary Outcomes

Tumor Growth Inhibition (%TGI)

Pharmacokinetic variability across genotypes

Secondary Outcomes

Survival Analysis

Hepatotoxicity markers (ALT, AST)

Apoptotic Index

PK–PD Correlation [8]

Ethical Considerations

Approval from Institutional Animal Ethics Committee

Compliance with CPCSEA guidelines

Adherence to ARRIVE reporting standards

Humane endpoints strictly followed

Results

Table 1. Baseline Characteristics of Experimental Groups

Parameter	CYP3A4 WT (n=10)	CYP3A4*22 (n=10)	CYP3A5*1 (n=10)	CYP3A5*3 (n=10)	Control (n=10)	p-value
Initial Body Weight (g)	19.8 ± 1.2	20.1 ± 1.1	19.7 ± 1.3	20.0 ± 1.0	19.9 ± 1.2	0.88
Baseline Tumor Volume (mm^3)	102 ± 12	98 ± 14	101 ± 13	99 ± 11	100 ± 10	0.91
ALT (U/L)	38 ± 6	40 ± 7	39 ± 5	41 ± 6	37 ± 5	0.76

All experimental groups showed equal baseline conditions because body weight and initial tumor volume and liver function markers did not show any significant differences between the groups ($p > 0.05$). The statement confirms that all future pharmacokinetic and therapeutic response differences result from CYP3A genotype instead of initial patient condition differences.

Table 2. Pharmacokinetic Parameters (Steady-State, Day 14)

Parameter	CYP3A4 WT	CYP3A4*22	CYP3A5*1	CYP3A5*3	p-value
Cmax (ng/mL)	1245 ± 110	1580 ± 135*	1180 ± 95	1705 ± 142**	<0.001
$AUC_{0–24}$ (ng·h/mL)	13,200 ± 950	18,950 ± 1100**	12,850 ± 890	19,400 ± 1250**	<0.001
Clearance (mL/h/kg)	1.85 ± 0.22	1.10 ± 0.18**	1.92 ± 0.25	0.98 ± 0.14**	<0.001
Half-life (h)	6.2 ± 0.8	8.9 ± 1.1*	5.9 ± 0.7	9.4 ± 1.2**	<0.001

Reduced-function genotypes demonstrated significantly altered drug exposure:

- CYP3A4*22 and CYP3A5*3 showed:
  - 35–45% increase in AUC
  - Prolonged half-life
  - 30–45% reduction in clearance
  - Significantly higher Cmax ($p < 0.001$)

These data support the concept that changes in CYP3A enzyme function could result in an increased systemic exposure of target therapies.

Table 3. Tumor Growth Inhibition (Day 28)

Parameter	CYP3A4 WT	CYP3A4*22	CYP3A5*1	CYP3A5*3	Control	p-value
Final Tumor Volume (mm^3)	520 ± 48	320 ± 35**	545 ± 52	290 ± 30**	890 ± 75	<0.001

% Tumor Growth Inhibition (TGI)	41%	67%**	38%	71%**	—	<0.001
Apoptotic Index (%)	22 ± 4	39 ± 6**	19 ± 3	42 ± 5**	8 ± 2	<0.001

Enhanced drug exposure translated into superior antitumor efficacy:

- Tumor Growth Inhibition (TGI):
  - CYP3A4*22: 67%
  - CYP3A5*3: 71%
  - WT/Expressor groups: ~40%

Table 4. Molecular Marker Expression (Relative Fold Change)

Marker	CYP3A4 WT	CYP3A4*22	CYP3A5*1	CYP3A5*3	p-value
p-AKT	1.00	0.62 ± 0.08**	1.05 ± 0.09	0.58 ± 0.07**	<0.001
Cyclin D1	1.00	0.70 ± 0.10*	1.08 ± 0.11	0.65 ± 0.09**	<0.001
Ki-67 Index (%)	48 ± 5	28 ± 4**	51 ± 6	25 ± 3**	<0.001

Reduced-function genotypes exhibited stronger inhibition of oncogenic pathways:

- p-AKT reduction: 38–42%
- mTOR suppression: 33–40%
- Cyclin D1 reduction: 30–35%
- Ki-67 proliferation index significantly decreased

These mechanistic findings confirm enhanced pathway blockade in variant genotypes.

Table 5. Toxicity Assessment

Parameter	CYP3A4 WT	CYP3A4*22	CYP3A5*1	CYP3A5*3	p-value
ALT (U/L)	45 ± 7	62 ± 8*	44 ± 6	70 ± 9**	<0.01
AST (U/L)	72 ± 9	95 ± 11*	74 ± 8	102 ± 12**	<0.01
Weight Loss (%)	4.5 ± 1.2	8.2 ± 1.8*	4.1 ± 1.0	9.0 ± 2.1**	<0.01

Median survival was significantly prolonged in:

- CYP3A4*22: 58 days
- CYP3A5*3: 61 days

Compared to WT (42 days) and control (30 days). Hazard ratios indicated nearly 50% reduction in mortality risk ($p < 0.01$).

Table 6. PK–PD Correlation Analysis

Correlation	r-value	p-value
AUC vs %TGI	0.72	<0.001
AUC vs Apoptotic Index	0.69	<0.001
Clearance vs Tumor Volume	-0.65	<0.001

mRNA and protein expression analysis confirmed genotype-driven functional differences:

- CYP3A4*22 → ~45% reduction in CYP3A4 expression
- CYP3A5*3 → ~88–90% reduction in CYP3A5 expression

These results validate the mechanistic basis of altered pharmacokinetics.

Table 7. Kaplan–Meier Survival Analysis (Day 60 Follow-Up)

Group	Median Survival (Days)	Hazard Ratio (HR)	95% CI	p-value
CYP3A4 WT	42	Reference	—	—
CYP3A4*22	58	0.52	0.34 – 0.80	0.003
CYP3A5*1	40	1.08	0.71 – 1.64	0.72
CYP3A5*3	61	0.47	0.29 – 0.74	0.001
Control	30	1.88	1.20 – 2.95	<0.001

While enhanced efficacy was observed, increased systemic exposure was associated with:

- Elevated ALT and AST levels
- Increased inflammatory markers (TNF-α, IL-6)

- Increased oxidative stress (↑MDA, ↓GSH)
- Moderate body weight loss

These findings suggest a genotype-driven efficacy–toxicity balance.

Table 8. CYP3A mRNA and Protein Expression Levels

Parameter	CYP3A4 WT	CYP3A4*22	CYP3A5*1	CYP3A5*3	p-value
CYP3A4 mRNA (Fold Change)	1.00	0.55 ± 0.07**	—	—	<0.001
CYP3A5 mRNA (Fold Change)	—	—	1.00	0.12 ± 0.03**	<0.001
CYP3A4 Protein Expression	1.00	0.60 ± 0.09**	—	—	<0.001
CYP3A5 Protein Expression	—	—	1.00	0.10 ± 0.02**	<0.001

Strong correlations were identified:

- AUC vs Tumor Growth Inhibition: r = 0.72
- AUC vs Apoptotic Index: r = 0.69
- Clearance vs Tumor Volume: r = -0.65

These data demonstrate robust pharmacokinetic–pharmacodynamic coupling.

Table 9. Inflammatory and Oxidative Stress Markers

Biomarker	CYP3A4 WT	CYP3A4*22	CYP3A5*1	CYP3A5*3	p-value
TNF-α (pg/mL)	35 ± 6	48 ± 7*	34 ± 5	52 ± 8**	<0.01
IL-6 (pg/mL)	42 ± 7	60 ± 9*	44 ± 6	65 ± 10**	<0.01
MDA (nmol/mg protein)	2.1 ± 0.4	3.0 ± 0.5*	2.0 ± 0.3	3.2 ± 0.6**	<0.01
GSH (µmol/g tissue)	8.5 ± 1.1	6.2 ± 0.9*	8.7 ± 1.0	5.8 ± 0.8**	<0.01

After adjusting for confounders:

- CYP3A5*3 (β = +0.39, p < 0.001)
- CYP3A4*22 (β = +0.34, p = 0.001)
- AUC (β = +0.42, p < 0.001)

remained independent predictors of tumor growth inhibition.

Model $R^2 = 0.68$, indicating strong predictive strength.

Table 10. Dose–Exposure–Toxicity Modeling

Variable	β-Coefficient	Standard Error	p-value
AUC → ALT Elevation	0.61	0.08	<0.001
AUC → Weight Loss	0.55	0.10	0.002
Cmax → Apoptosis Index	0.68	0.07	<0.001

Genotype–phenotype concordance analysis demonstrated:

- 75% poor metabolizer phenotype in variant carriers.

Predicted clinical dose adjustments:

- CYP3A4*22 → 20–25% dose reduction
- CYP3A5*3 → 25–30% dose reduction

This supports precision oncology–guided dosing strategies.

Table 11. Multivariate Regression Model for Tumor Growth Inhibition

Predictor Variable	β	95% CI	p-value
CYP3A4*22	+0.34	0.18–0.50	0.001
CYP3A5*3	+0.39	0.22–0.56	<0.001
AUC	+0.42	0.25–0.60	<0.001
Clearance	-0.31	-0.49 to -0.14	0.002

Multivariate regression modeling demonstrated that both genetic and pharmacokinetic variables independently predicted tumor growth inhibition (TGI).

- CYP3A5*3 ($\beta = +0.39$, $p < 0.001$)
- CYP3A4*22 ($\beta = +0.34$, $p = 0.001$)
- AUC ($\beta = +0.42$, $p < 0.001$)
- Clearance ($\beta = -0.31$, $p = 0.002$)

The model was able to explain 68% of the variation in tumor suppression (Adjusted $R^2 = 0.68$). This demonstrates strong predictive power. The pharmacokinetic changes, which are genotype-dependent, are the primary determinant of how a person responds to this treatment.

Table 12. Pathway Inhibition Quantification (% Reduction vs WT)

Pathway Marker	CYP3A4*22	CYP3A5*3	p-value
p-AKT	38%	42%	<0.001
mTOR	33%	40%	<0.001
Cyclin D1	30%	35%	0.002

Variant genotypes demonstrated significantly greater suppression of key proliferative signaling pathways:

- p-AKT inhibition: 38–42%
- mTOR reduction: 33–40%
- Cyclin D1 suppression: 30–35%

The decreases were significant ($p < 0.001$) and supported the notion that letting metabolism go to a reduced function state can enhance tumor inhibition.

Table 13. Genotype–Phenotype Concordance Analysis

Genotype	Poor Metabolizer Phenotype (%)	Extensive Metabolizer (%)
CYP3A4 WT	8%	92%
CYP3A4*22	76%	24%
CYP3A5*1	5%	95%
CYP3A5*3	81%	19%

Phenotypic classification showed:

- 75% of CYP3A4*22 and CYP3A5*3 carriers exhibited a poor metabolizer phenotype
- 90% of wild-type and CYP3A5*1 animals demonstrated extensive metabolizer characteristics

These points demonstrate that genetic variants lead to differences in metabolism.

Table 14. Translational Prediction Model for Clinical Dosing

Genotype	Predicted Dose Adjustment	Predicted Exposure Change
CYP3A4*22	Reduce by 20–25%	↑ 35–45%
CYP3A5*3	Reduce by 25–30%	↑ 40–50%
CYP3A5*1	Standard Dose	Baseline

Based on pharmacokinetic modelling

- CYP3A4*22 → Predicted 20–25% dose reduction
- CYP3A5*3 → Predicted 25–30% dose reduction

Those individuals who had reduced function genotypes displayed a 35% to 50% increase in systemic exposure, which supports the use of this approach in order to balance tumor inhibition and toxicity.

Discussion

Targeted therapies have revolutionized treatment of advanced-stage breast cancers, and yet, even when cancers share similar molecular profiles, there can be considerable variability in treatment response from person to person. Although tumor genomic profiles play a critical role in guiding treatment, the role of the host in drug metabolism and its impact on treatment response remains poorly integrated into this new era of precision oncology. Here, we demonstrate, using a mechanistic in vivo preclinical model, that genetic differences in CYP3A4 and CYP3A5 expression significantly impact the amount of drug in the system, the degree of oncogenic pathway inhibition, tumor growth rates, survival, and toxicity. Such an effect places pharmacogenomic differences in drug metabolism as more than just a pharmacokinetic fine-tuning of drug dose and schedule, potentially impacting treatment response in a more significant and clinically relevant manner. [13]

Here, we show that reduced function alleles, such as CYP3A4*22 and CYP3A5*3, significantly impact drug exposure, increasing AUC and half-life, and slowing down clearance. Such differences in pharmacokinetics significantly impact tumor growth inhibition and survival, demonstrating a more significant effect than wild-type or expressor genotypes. Importantly, this effect was significant enough to be considered biologically relevant, suggesting that metabolism via CYP3A enzymes plays a significant role in determining the rate-limiting step in drug efficacy. [14]

Mechanistically, this was associated with a more significant effect on PI3K/AKT/mTOR inhibition and

tumor proliferation biomarkers, such as Cyclin D1 and Ki-67. Such an effect links systemic pharmacokinetics and its impact on oncogenic pathways, potentially implicating a role of the host's genetic background in determining the degree of oncogenic pathway inhibition. Such a role of drug metabolism could potentially explain differences in treatment response, even when choosing the correct drug, as rapid metabolism in extensive metabolizers could potentially prevent adequate oncogenic pathway inhibition.[15]

Notably, the data demonstrate a tight relationship between drug handling and efficacy, as evidenced by the relationship between AUC and tumor regression and apoptosis. Multivariate analysis, which considered drug exposure, suggested that CYP3A genotype was an independent predictor of response. That is, an individual's genetic profile, specifically around CYP3A, seems to influence not just their drug exposure, but also their biological response. Presumably, this is mediated by coordinated regulation of drug metabolism enzymes and transporters. [16]

There are a number of big ideas here. One is that this work undermines the traditional notion of tumors as the primary driver of precision oncology, demonstrating instead the importance of the individual's own biology. Another is the concept of a genotype-dependent change in the therapeutic window, wherein genotypes associated with lower function can enhance efficacy and toxicity. Indeed, variant genotypes did show a small increase in liver toxicity and inflammatory biomarkers, consistent with increased drug exposure. However, this was always within non-lethal, reversible bounds, and suggests that genotype-dependent dosing could enhance efficacy and safety. [17]

From a translational perspective, this research supports incorporating CYP3A genotype into dosing regimens of targeted therapies. Many of these agents have significant metabolism by CYP3A enzymes but currently receive uniform dosing regimens in genetically heterogeneous populations. Our model predicts that modest dose adjustments of 20–30% in carriers of reduced-function alleles could normalize drug exposures across genotypes. This type of stratification has the potential to decrease toxicity without compromising pathway inhibition.

This research also minimizes confounding variables such as liver dysfunction, drug compliance issues, and polypharmacy that often plague pharmacogenomic studies. The humanized CYP3A model is particularly relevant to translation since it expresses human functional CYP3A enzymes. However, there are several limitations to this research. The xenograft model does not recapitulate an intact immune microenvironment. Interactions between the immune system and targeted therapies may affect genotype-response relationships in humans. Combination regimens, such as in advanced breast cancer, may induce synergistic or antagonistic metabolism that has not been explored in this model. [18]

Conclusion

The practical application of this research is obvious and ready to be put into use. The research has shown that CYP3A4 and CYP3A5 genotyping has the potential to be used in real-world settings as a biomarker to refine dosing of CYP3A substrates of targeted therapies in advanced breast cancer. Genomic profiling of cancer has shown that it is useful in determining which drug to use in cancer treatment. However, pharmacogenomic profiling of patients offers us an opportunity to fine-tune the dose of drugs to increase the therapeutic window without losing efficacy. Trials that incorporate pharmacogenomic profiling in their protocol may help us avoid toxicities of drug exposures without losing efficacy in cancer treatment. The integration of pharmacogenomic profiling with cancer genomic profiling offers us an opportunity to minimize variability among patients by taking into account two aspects of cancer treatment: the genomic profiling of cancer and pharmacogenomic profiling of patients.

Ethical Approval: 1962/PO/Re/S/17/CPCSEA

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