from Molecular to Clinical research in PGx

The Pharmacogenomics Research Center (PGRC), which is the first institute specialized in PGx research in Korea, is operated jointly by the Department of Pharmacology, Clinical Pharmacology, and Bio - Marker Research Center for Personalized Therapy (BMRC) in Inje University College of Medicine.

PGRC is recognized worldwide as frontier research institute in the field of PGx and personalized pharmacotherapy.
PGRC researchers are currently involved in various Korean government and pharmaceutical industry funded projects in drug metabolism and transport, personalized medicine, genotyping chip development, drug interactions, and biomarker discovery.

As the premier research unit for PGx in Korea, Inje University Busan Paik PGRC has significant expertise in genome analysis and utilizes up to date genotyping technologies for clinical application.

Organization

- PGRC
  - Business Development
  - **Personalized Therapy**
    - **Genomics**: SNP/Ht/LD Map; Functional Genetics; DNA Biomarker
    - **Proteomics**: Expression Profile; Molecular Signaling; Protein Marker
    - **Metabolomics**: PD/DMPK; Drug/Phenotype; Metab. Marker
  - **Drug Developmental Science**
    - **Preclinical ADMET**: Metabolism; Transport; Preclinical PK
    - **Clinical PK/PD**: Phase 0; Phase Ⅰ Trial; IVIVE
  - **Core Lab**: PGx Core lab; DMPK Core lab; PGx DM Core lab; CRC Team; Biomedical Resoruce Bank; QA

First and unique research center

in the area of Pharmacogenomics researches in Korea

Full Spectrum Service In Personalized Medicine

Biomarker Discovery
(Candidate gene, GWAS)

- Novel gene biomarker exploration
- Candidate gene approach – Response pathway
- SNPs / Expression profile
- CNV, regulatory, epigenetic

Preclinical Validation
(Molecular, Cell, Animal)

- Evaluate functional changes of biomarker
- Molecular based
- Cell based
- Animal based

Clinical Validation
(Healthy subjects)

- Small scale clinical trial in healthy subjects
- Proof of concept trial
- Translational research : PK/ PD study

Clinical Validation
(Patients, Outcome)

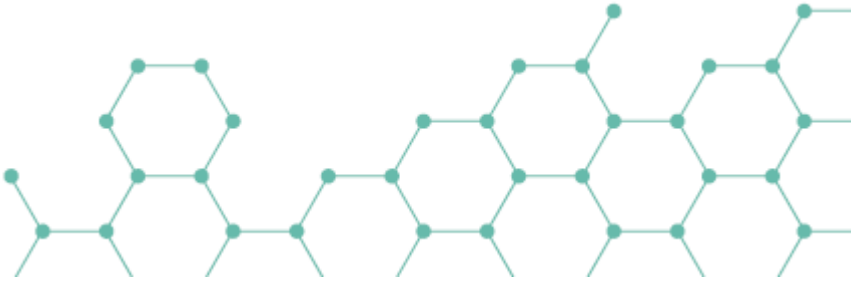
- Large scale, clinical response (outcome) in patients population
- Retrospective, prospective study
- Replication study for the GWAS approach
- Confirmed trials

Clinical Utility Validation
(Algorithm, Cost-effectiveness)

- Development of predictive algorithm
- Large scale, prospective, randomized trial.
- Comparative effectiveness (genotype guided vs. traditional)
- Cost-effectiveness or cost-utility analysis

Clinical Implication

- Regulatory approval of biomarker / diagnostic tool (IVIVD)
- Labeling of PGx information in product label
- Genotype-guided pharmacotherapy
- Ethnic comparison
- Health insurance Education



Local Expertise, Global reach

Research Areas

- Genetic polymorphism of drug metabolism and transport
- Functional genetics of drug metabolism and transport
- High-throughput analysis of drug metabolism
- Genotype to phenotype association in human subjects
  Drug-drug interaction study *in vitro* and *in vivo*

- Discovery of candidate compounds with pharmacological actions
- Pharmacoproteomic approach to discovering markers for drug response
- Construction of pharmacogenomics database for Asian populations
- Development of clinical applications such as drug regimens, practical genotyping methods, pharmacotherapy instructions

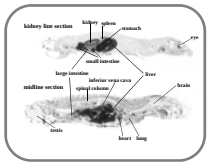
Facilities

Facilities	Equipment
• Genomics Laboratory • Pharmacogenomics Core Lab	ABI 7900HT Real-time PCR, Pyrosequencer, ABI 3130 Genetic Analyzer, Teccan auto prep, Phosphoimager, Ultracentrifuge, Gel Doc. etc.
• Metabolomics Laboratory • DMPK Core Lab	API 3000, 4000 LC/MS/MS, Q-TOF, Qtrap 4000, 5500 LC/MS/MS, Agilent 1100 UV/FLD, Agilent 6410, 6530 TOF/MS, HPLC, UPLC, etc.
• Biomedical Resource Bank	Deep freezer, Nano drop, 2D bar-code reader
• Biomedical Informatics Team	DB Server, UPS

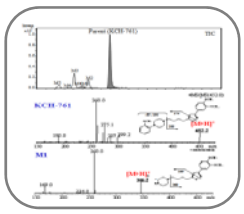
Full DMPK Service

- To increase the success rate of new drug development, many steps from discovery to clinical development were integrated strategically.
- Especially, "Pharmacokinetics, drug metabolism / transport, drug interaction (DM/PK)" is a core technology and the bottle neck in the drug development process.
- Therefore, development of DM/PK Technology Platform is the key to improve the success rate of drug development.
- Here, we introduce the currently established **DM/PK Technology Platform in PGRC, Inje University** which center was established in 2003.

One-Stop DMPK Evaluation Service For Drug Discovery

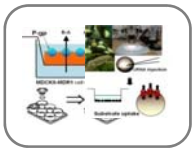


Whole-body Autoradiography

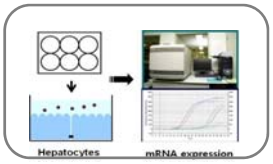


Drug Metaboism
MetID & metabolic pathw

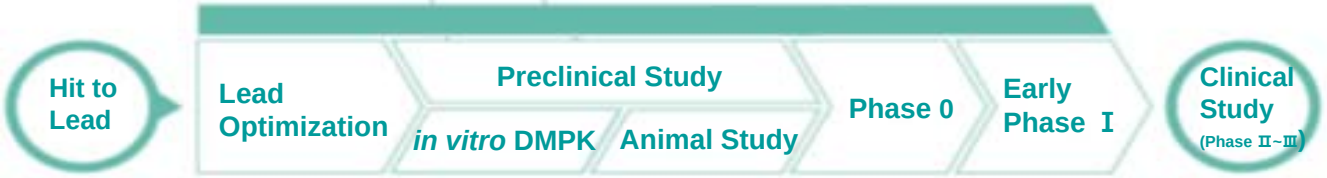
Drug Excretion Study
Metabolic cage



Drug transport characterization



Drug Interaction Enzyme
inhibition/induction/
transporter inhibition



Lead optimization – metabolic stability, permeability

In vitro/ in vivo animal study – in vitro metabolism/ transport study, drug-drug interaction, animal safety and efficacy, rat ADME study

Early Clinical Study – translational research, microdosing, FIH, DDI potential (cocktail), PGx-based PK study, animal PK/PD, PK/PD modeling & simulation

DMPK Core Lab Technology Platform

		Service	Technology	Status
Permeability test	Permeability test	Drug absorption	Caco-2 cell permeability test	○
		Hepatic excretion	Cryopreserved hepatocytes uptake	○
			Sandwich cultured hepatocytes	○
		Renal excretion	LLC-PK1 cell permeability test	○
Metabolism study	Protein binding	Plasma protein binding	Equilibrium dialysis, ultrafiltration	○
		Blood partition	Blood to plasma ratio	P
	in vitro metabolism	Metabolic stability	metabolic stability (hepatocytes, microsomes, S9…), plasma/blood stability	△
		Metabolism	Reaction phenotyping and kinetics : P450s, UGTs, non-CYP Phase I (FMO, MAO…) Enzyme systems: microsome, recombinant enzymes, S9, hepatocytes...)	○
			Species comparison (monkey, dog, mouse, rabbit, rat…)	○
			Pharmacogenetics study (CYP2C19*10, 2D6*10B, 4F2*3…)	△
		Met ID	Metabolites profiling and Met ID	△
Transporter study	*in vitro* transport study	Identification of transport system	Vesicle-based transporter assay	○
			Uptake transport screening in overexpressing cells or oocytes (OCTs, OATs, OATPs, NTCP)	○
			Efflux transport screening in overexpressing cells (MDR1, BCRP, MRP1, MRP2)	○
			Cryopreserved hepatocytes uptake	○
			Kinetic studies (K_m, V_{max}, intrinsic clearance)	○
		Pharmacogenetics study	OCT2A270S, OATP1B1*15, NTCP*2, MDR G2677T/A, BCRP Q141K	○
DDI Study	*in vitro* DDI	Inhibition	Screening of inhibitory potential of P450s(cocktail method), UGTs, Transporters	○
			Estimation of IC_{50} (K_i) value	○
		Time-dependent inhibition	IC_{50} shift assay, Estimation of K_I and K_{inact} value	○
			Reversibility assay	○
		Induction	Reporter assay (PXR)	○
			mRNA expression level in human hepatocytes (P450s, UGTs, Transporters)	○
			Protein expression level in tissue (P450s, UGTs, Transporters)	○
	in vitro to *in vivo* prediction		IVIVE prediction	○
Preclinical study		Linearity / Bioavailability	IV/Oral administration PK	△
		Mass balance	Radio-labeled compound PK	P
		Distribution	Tissue distribution (brain, liver, kidney…)	△
		Metabolic profiling	Metabolites profiling and Met ID	△
		Biliary / Renal excretion	Bile cannulation	△
Early Phase Clinical Development		Preclinical Prediction of Human PKs	Microdosing study of hot compound and cold compound	△
			Allometry	△
			Physiologic Based Pharmacokinetics	△
		Phase I study	Pharmacologically guided dose escalation	○
			Human mass balance (cold/hot compound)	P
			Absolute bioavailability	○
			Drug-drug interaction potential study (cocktail study)	○
			Mechanism based drug-drug interaction study	○
			Genotype based ADME study	○
			Special population study (Renal/Hepatic dysfunction, Elderly, Gender)	○
			Bridging study	○
			Biologics	△
		Pharmacokinetics/ Pharmacodynamics	Non-compartmental/Compartmental Analysis	○
			Population Pharmacokinetics/Pharmacodynamics	○
			Dose-Effect/Concentration Effect analysis	○
			Development of Biomarker	△

(Status: ○ Established, △ Available on request, P: in future)

Leading Pharmaco-genotyping Service

Genotype core lab has explored the genetic variations in these genes and characterized their functions.
Genotype core lab has constructed the multidisciplinary pharmacogenomics database including genotype-to-phenotype correlation data and genotyping tools. We now provide pharmacogenetic tests for most of drug-related genes for clinical application. All genotyping tools that we provide are technically validated by comparative studies.

Our pharmacogenetic test panels are designed to cover most of Asian alleles which are identified from Asian populations and recommended as genetic biomarkers by US FDA. The coverage rate of our genetic tests for Asian populations is generally higher than those of other US- or EC-approved diagnostics. Some of our pharmacogenetic tests can be used for the clinical interpretation, since they are evidenced by previous clinical studies.

Service

One-Stop Genotyping through the website

http://pgrc.inje.ac.kr/corelab

Genotype testing service

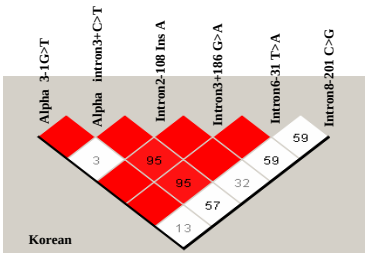
- Genotyped control materials - immortalized cell lines
- SNaPshot, Pyrosequencing, Taqman assay, Sequencing, HRM, Chip, etc.

Research Support Services

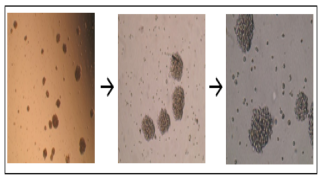
- Cost effective Genotype method development
  : Genotyping analysis based on researcher specifications
- Haplotype and LD analysis
- Functional analysis of SNPs
- Gene expression profiling
- Copy number analysis

Research Resource Management

- 2D barcode system
- Biobank



Linkage disequilibrium (LD) Block of Korean



Genotyped immortalized cell lines

2D barcode system

Pharmacogenetic Core Lab Key Technology Platform

Gene list of Full sequencing and genotyping data

Metabolic Enzymes	CYPs	CYP1A2, CYP2A6, CYP2B6, CYP2C8/9/19, CYP2D6/7, CYP2J2, CYP2S1, CYP3A4/5/7, CYP4F2, CYP7A1, CYP2C19A1
	UGTs	UGT1A1/3/4/5/6/7/8/9/10, UGT2B4, UGT2B7/15
	SULTs	SULT1A1/2, SULT1E1
	Others	ADH2, ALDH2, CES1/2, DPYD, EPHX1, FMO3, MTHFR, NAT2, NQO1, TS, TP, TPMT, POR
Transporters	ABCs	ABCB1, ABCB11(=BSEP), ABCC1/2/3/4/5/6(=MRP1/2/3/4/5/6), ABCC7(=CFTR), ABCG2(=BCRP)
	SLCs	SLCO1A2(=OATP1A2), SLCO1B1(=OATP-C), SLCO1B3(=OATP1B3), SLCO2B1(=OATP2B1), SLC10A1/2(=NTCP, ASBT), SLC15A1/2(=PEPT1/2), SLC22A1/2/3(=OCT1/2/3), SLC22A4/5(=OCTN1/2), SLC22A6/7/9(=OAT1/2/7), SLC28A1/2/3(=CNT1/2/3), SCL29A1/2(=ENT1/2), SLC47A1/2(=MATE1/2)
Nuclear receptor		CAR, FXR, HIF1, HNF1α/3α/4α/6α, LXRα, PXR, SHP(NR0B2)
Pharmacodynamic target Protein of drug response		APOE, CALU, ECGF, GGCX, KLKB1, HLA-DRA, HPCAL1, OPRM1, P2Y1/12, PROC, PROS1, SCN1, SERPINC1, TTN, VKORC1, ADRB2, ALOX5, LTC4S, CysLTR1, DRD2/3/4, 5-HT1A/2A/2C, 5-HTT, COMT, HLAs

*** not include Exome sequencing data**

Genotyping service gene list of US FDA biomarkers

FDA Biomaker	Drugs	Methods
CYP2C19	Clopidogrel, Voriconazole, Prasugrel	Pyrosequening, HRM, SNaPshot
CYP2C9	Celecoxib, Warfarin	Pyrosequening, HRM, SNaPshot
CYP2D6	Atomoxetine, Fluoxetine HCL, Codeine sulfate	SNaPshot
DPD	Capecitabine	Full-sequencing
EGFR	Erlotinib, Cetuximab,	Sequencing
HLA-B*1502	Carbamazepine	SBT
HLA-B*5701	Abacavir	SBT
KRAS	Panitumumab	Sequencing
NAT2	Rifampin, isoniazid, and pyrazinamide	Sequencing
TPMT	Azathioprine	Pyrosequening
UGT1A1	Irinotecan, Nilotinib	Pyrosequening, HRM
VKORC1	Warfarin	Pyrosequening, HRM, SNaPshot

Preemptive genotype kit to be developed.: Example of CYP panel

Preemptive genotype of P450 enzymes					
SNaPshot Multiplex 1					
CYP2D6 dup	CYP2C9*3,*13,*14	CYP2C19*2*3*17	CYP3A4*18	CYP3A5*3	CYP2B6*4*9
SNaPshot Multiplex 2					
CYP2D6*1*2*10,*14*18*21*41*49*52*60 (*4*6*9,*15,17*29)					CYP2D6 del