R.G.C.C.- RESEARCH GENETIC CANCER CENTRE S.A.

Florina __/__/_____

Dear Colleague,

We report the allelic discrimination results for patient **Mr./Ms._____** whose sample receipt on __/__/_____. DNA was extracted from blood sample and was used as template in PCR reactions. Molecular-based assays and spectrophometer analysis were used to verify the DNA. In all reactions genomic DNA was used as a positive control. The reactions were performed in triplicates.

The graduated bars indicate any potential positive or negative outcome. An arrow on the green or the red part of the bar demonstrates the outcome.

Mr/Ms_____
Industrial Area of Florina, GR 53100 – Florina, Greece
Tel.: +30 23850 41950, 41951, 41960, 41961, Fax.: +30 23850 41931
Website: www.rgcc-genlab.com E-mail:papasotiriou.ioannis@rgcc-genlab.com

BASIC

Polymorphism	Outcome
CYP2D6*2	Normal Metabolizer
CYP2D6*3A	Poor Metabolizer
CYP2D6*3B	Normal Metabolizer
CYP2D6*6	Poor Metabolizer
CYP2D6*9	Normal Metabolizer
CYP2D6*10	Poor Metabolizer
CYP2C19*2	Normal Metabolizer
CYP2C19*3	Normal Metabolizer
CYP2C19*17	Possible Ultra-Fast Metabolizer
CYP1A2*1F	Possible Normal Metabolizer
CYP1A2*1K	Normal Metabolizer
CYP1A1*2C	Normal Metabolizer
CYP2C9*2	Normal Metabolizer
CYP2C9*3	Normal Metabolizer
CYP3A4*1B	Poor Metabolizer
CYP3A4*20	Possible Poor Metabolizer
CYP1B1	Possible Normal Metabolizer

Mr/Ms_____
Industrial Area of Florina, GR 53100 – Florina, Greece
Tel.: +30 23850 41950, 41951, 41960, 41961, Fax.: +30 23850 41931
Website: www.rgcc-genlab.com E-mail:papasotiriou.ioannis@rgcc-genlab.com

Polymorphism	Outcome
GSTP1*Ala114Val	Normal Metabolizer
GSTP1*Ile105Val	Possible Normal Metabolizer
EPHX1* His139Arg	Normal Metabolizer
EPHX1*Tyr113His	Normal Metabolizer
NAT2*5	Normal Metabolizer
NAT2*6	Possible Normal Metabolizer
NAT2*7	Normal Metabolizer
NAT2*14	Normal Metabolizer
NAT2*11A	Normal Metabolizer
NAT2*12	Normal Metabolizer
NAT*13	Normal Metabolizer
TPMT*4A	Normal Metabolizer
TPMT*2	Normal Metabolizer
ABCB1*Ile1145Ile	Slower Metabolizer
ABCB1*Ser893Ala	Slower Metabolizer
ABCG2*Gln141Lys	Normal Metabolizer

ALKYLATING AGENTS

Drug	Polymorphism	Outcome
Cisplatin	ERCC1*Asn118Asn	Decreased likelihood of nephrotoxicity
	LRP2*Lys4094Glu	Increased risk of Ototoxicity
	ERCC1*Gln504Lys	Increased likelihood of nephrotoxicity
	COMT*19955692C>T	Decreased risk of Deafness
	XPC*Gln902Lys	Increased risk of toxicity
	GSTP1*Ile105Val	Increased risk of toxicity
	NQO1*Pro149Ser	Increased overall, progression-free survival (platinum compounds, anthracyclines, nucleodise inhibitors)
Cyclopshosphamide	ALDH3A1*Pro329Ala	Decreased likelihood of Cystitis (carboplatin, cyclophosphamide,thiotepa)

TOPO I Inhibitors

Drug	Polymorphism	Outcome
Irinotecan	UGT1A1*172270T>G	Decreased risk of Neutropenia
	UGT1A1*Gly71Arg	Decreased risk of Neutropenia

TOPO II Inhibitors

Drug	Polymorphism	Outcome
Anthracyclines	CBR3*Val244Met	Increased risk of Heart Failure
	CBR3*Val244Met	Decreased risk of cardiomyopathies (low to moderate dose)
	CBR1*133G>A	Decreased risk of cardiomyopathies (low to moderate dose)
Daunorubicin	NRP2*110077C>G	Decreased IC50

ANTIMETABOLITES

Drug	Polymorphism	Outcome
5-Fluorouracil	DPYD*Cys29Arg	Increased metabolism
	DPYD*Cys29Arg	Increased likelihood of overall gastrointestinal toxicity
	DPYD*Cys29Arg	Decreased likelihood of Nausea and Vomiting
	DPYD*Met166Val	Increased likelihood of Neutropenia
	DPYD*Met166Va	Increased metabolism
	DPYD*1905+1G>A	Increased likelihood of drug toxicity, Mucositis, Leukopenia, Thrombocytopenia
	DPYD*1905+1G>A	Increased likelihood of drug toxicity (5-fluorouracil, capecitabine)
	DPYD*1905+1G>A	Decreased metabolism
	DPYD*Asp949Val	Decreased severity of drug toxicity
Cytarabine	CDA*Lys27Gln	Decreased drug toxicity
	CDA*20915590delC	Decreased drug toxicity
	CDA*-92A>G	Decreased drug toxicity
Gemcitabine	CDA*Ala70Thr	Decreased severity of Neutropenia

Mr/Ms_____
Industrial Area of Florina, GR 53100 – Florina, Greece
Tel.: +30 23850 41950, 41951, 41960, 41961, Fax.: +30 23850 41931
Website: www.rgcc-genlab.com E-mail:papasotiriou.ioannis@rgcc-genlab.com

SPINDLE POISONS

Drug	Polymorphism	Outcome
Paclitaxel	CYP2C8*23210C>G	Decreased risk of Neurotoxicity
	CYP2C8*Arg69Lys	Decreased risk of neurotoxicity
	CYP2C8*Arg69Lys	Increased metabolism
	CYP3A5*12083G>A	Increased risk of Neurotoxicity
Docetaxel	CYP3A4*1B	Decreased clearance

Appendix:

Drug Metabolism:

Phase I:

Phase I enzymes are responsible reactions that convert parent compound into a more polar metabolite by adding or unmasking functional groups. Usually these metabolites are inactive. Phase I reactions include, oxidation, reduction, hydrolytic cleavage, alkylation, methylation, ring cyclization etc. These reactions prepare chemicals for phase II metabolisms and subsequent excretion.

The Cytochrome P450 (CYP) enzyme superfamily is the most important system in the biotransformation of many endogenous and exogenous substances, such as drugs, toxins and carcinogens. For drug metabolism the most important polymorphisms are those of the genes coding for CYP2C9, CYP2C19, CYP2D6 and CYP3A4. CYP1A1 and CYP1A2 are among the most responsible for biotransformation of chemicals, especially for the metabolic activation of pre-carcinogens. Genetic polymorphism is an important reason for variations in drug response of the human body. There are four distinct phenotypes: poor metaboliser (PM), intermediate metaboliser (IM), extensive metaboliser (EM) and ultrarapid metaboliser (UM). A poor metaboliser lacks active allele and may present adverse effects at usual doses, due to reduced metabolism and increased drug concentration. Individuals with intermediate metabolic phenotype are homozygous for two reduced activity alleles or are heterozygous for an inactive allele. Extensive metabolisers have two fully active

allele and show the expected response to a standard dose. Ultra extensive metabolisers are individuals with more than two copies of active gene.

- Cytochrome P450 2D6 is one of the most important enzymes, involved in the metabolisms of xenobiotics in the body, but also in activation of many substances in their active compounds.

- Cytochrome P450 2C19 is responsible for metabolisation or activation of many hormones and drugs (anti-epileptics, anti-depressants, anti-platelet clopidogrel, esomeprazole).
- Cytochrome P450 1A2 is involved in metabolism of xenobiotics substrates such caffeine, aflatoxin B1 and acetaminophen.
- Cytochrome P450 3A4 is one of the most important enzymes involved in xenobiotics metabolism in human body. It metabolizes some steroids and carcinogens. Approximately half of the drugs that are used are metabolized by this protein, such acetaminophen, codeine, cyclosporine, diazepam and erythromycin.
- Cytochrome P450 2C9 is an enzyme with a major role in the oxidation of both xenobiotics and endogenous compounds. Warfarin, phenytoin, acenocoumarol, tolbutamide, losartan glipizide and a few nonsteroidal anti-inflammatory drugs (aspirin, ibuprofen, naproxen) are metabolized by CYP2C9.

Phase II:

The Phase II reactions are conjugations with endogenous substrate to further increase aqueous solubility and conjugations with glucoronide, sulfate, acetate, amino acid etc. N-acetyltransferase 2 (NAT2), Epoxide hydrolase 1 (EPHX1), Glutathione S-transferase P (GSTP1) and Thiopurine methyltransferase (TPMT) are the major enzymes involved in phase II drug metabolism.

- N-acetyltransferase 2 (NAT2), is an enzyme that activates and deactivates arylamine and hydrazine drugs and carcinogens. Human populations segregated into rapid, intermediate and slow acetylator phenotypes, according to different polymorphisms combinations.
- Glutathione S-transferases are responsible for the detoxification of a range of drugs and potential carcinogens, through glutathione conjucation. The GSTP1 is associated with xenobiotics metabolism and susceptibility to cancer and other diseases.
- Thiopurine S-methyltransferase (TPMT) is an enzyme that metabolises thiopurine drugs such as azathioprine, 6-mercaptopurine and 6-thioguanine. Individual homozygous for two non-

functional TPMT variants are at high risk for toxic side effects, due to decreased methylation and decreased inactivation of 6MP.

Pharmacodynamics:

- P-glycoprotein 1, or multidrug resistance protein 1, or ATP-binding cassette sub-family B member 1 (ABCB1), or CD243, is an ATP-dependent drug efflux pump for xenobiotics compounds with broad substrate specificity. ABCB1 regulates the distribution and bioavailability if drugs, removes toxic metabolites and xenobiotics from cells, transports compounds out of brain and protects hematopoietic stem cells from toxins.
- ATP-binding cassette sub-family G member 2 (ABCG2), is a xenobiotic transporter with important role in the multidrug resistance phenotype of several cancer cell lines.

Sincerely,

Panagiotis Apostolou
Molecular Biologist

Ioannis Papasotiriou MD., PhD
Head of molecular medicine dpt. of
R.G.C.C.-RESEARCH GENETIC CANCER CENTRE SA