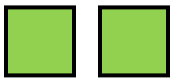
R.G.C.C.- RESEARCH GENETIC CANCER CENTRE LTD

Florina, __ / __ /2015

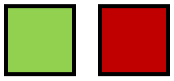
Dear Colleague,

We report the allelic discrimination results for patient **Mr/ Ms** _____ whose sample receipt on __/__/2015. 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.

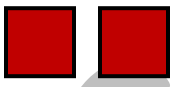
The "green" square represents the normal allele, while the "red" represents the defect allele. The person can either be equipped with two normal alleles, or two defective, or be heterozygous, namely to one normal and one defective allele.



Homozygote for the wild type (normal) allele



Heterozygote



Homozygote for the mutant (defect) allele

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BASIC

Gene	Polymorphism	Result		Outcome
CYP2D6	CYP2D6*2-1			Normal Metabolizer
	CYP2D6*2-2			Normal Metabolizer
	CYP2D6*3-1			Normal Metabolizer
	CYP2D6*3-2			None Activity
	CYP2D6*6			Poor Metabolizer/None Activity
	CYP2D6*9			Normal Metabolizer
	CYP2D6*10			Decrease or None Activity
CYP2C19	CYP2C19*2			Normal Metabolizer
	CYP2C19*3			Normal Metabolizer
	CYP2C19*17			Ultra Fast Metabolizer
CYP1A2	CYP1A2*1F			Normal Metabolizer
	CYP1A2*1K			Normal Metabolizer
CYP1A1	CYP1A1*2C			Normal Metabolizer
CYP2C9	CYP2C9*2			Normal Metabolizer
	CYP2C9*3			Poor Metabolizer
CYP1B1	Leu432Val			Possible Slow Metabolizer
CYP3A4	CYP3A4*1B			Poor Metabolizer
	CYP3A4*20			Normal Metabolizer
GSTP1	Ala114Val			Normal Metabolizer
	Ile105Val			Normal Metabolizer
EPHX1	His139Arg			Poor Metabolizer
	Tyr113His			Normal Metabolizer
NAT2	NAT2*5D			Possible Slow Metabolizer
	NAT2*6B			Normal Metabolizer
	NAT2*7A			Normal Metabolizer
	NAT2*11A			Normal Metabolizer
	NAT2*12A			Possible Slow Metabolizer
	NAT2*13			Normal Metabolizer
	NAT2*14A			Normal Metabolizer
TPMT	TPMT*3C			Normal Metabolizer
	TPMT*4A			Normal Metabolizer
	TPMT*3A			Normal Metabolizer
	TPMT*2			Normal Metabolizer
ABCB1	Ile1145Ile			Possible Slow Metabolizer
	Ser893Ala			Possible Slow Metabolizer
ABCG2	Gln141Lys			Normal Metabolizer

Mr/Ms _____

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ALKYLATING AGENTS

Gene	Polymorphism	Result	Outcome
TP53	Pro33Arg		Possible increased risk for toxicity/ decreased survival
ABCB1	Ile1145Ile		Possible decreased risk of lymph node metastasis/ increased survival
TPMT	TPMT*3C		Possible decreased risk for hearing loss (Cisplatin)
ERCC1	Asn118Asn		Possible increased risk for toxicity/ decreased survival
ERCC2	Lys751Gln		Possible increased survival
GSTP1	Ile105Val		Possible increased risk for toxicity
GSTM3	110280254delC		Possible increased risk of side effects
ERCC2	Asp288Asn		Possible increased overall survival (Cisplatin)
TPMT	TPMT*3A		Possible decreased risk for hearing loss (Cisplatin)
NQO1	Pro149Ser		Possible better outcome (overall survival and progression-free survival)
MTHFR	Glu429Ala		Associated with overall survival and progression free survival
MTHFR	Ala222Val		Possible increased likelihood of response to chemotherapy and drug toxicity
MTR	Asp919Gly		Possible decreased likelihood of drug toxicity
LRP2	Lys4094Glu		Possible decreased risk for hearing loss (Cisplatin)
XPC	Gln902Lys		Possible increased risk for toxicity
XRCC1	Gln399Arg		Possible decreased survival and risk of neutropenia
SLC22A2	Ser270Ala		Possible increased risk of nephrotoxicity
CD3EAP	Gln504Lys		Possible increased risk of nephrotoxicity
COMT	19955692C>T		Possible decreased risk for hearing loss (Cisplatin)
UBE2I	157C>G		Possible decreased response to cisplatin and irinotecan
GALNT14	10069401G>T		Possible decreased response and survival
EGFR	Leu858Arg		Possible decreased overall survival time/progression-free survival time (carboplatin-erlotinib-paclitaxel)
DSCAM	27076915T>C		Possible increased survival (carboplatin-paclitaxel)
PTGS2	427T>C		Possible increased progression-free survival/ overall survival (capecitabine-oxaliplatin)
NOS3	Asp298Glu		Possible longer disease-free survival (cyclophosphamide-doxorubicin-5FU-MTX)
ABCB1	Ser893Ala		Possible decreased survival in (cyclophosphamide-doxorubicin, Breast)

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ALDH3A1	Pro329Ala			Possible increased likelihood of cystitis (carboplatin-cyclophosphamide-thiotepa)
CYP2B6	CYP2B6*4			Possible decreased risk for oral mucositis (cyclophosphamide)
CYP3A4	CYP3A4*1B			Possible shorter period of time before chemotherapy-induced ovarian failure (cyclophosphamide)
CYP2B6	CYP2B6*6			Possible no requirement for dose reduction (cyclophosphamide)
SOD2	Val16Ala			Possible decreased survival (cyclophosphamide)
CYP2B6	CYP2B6*2			Possible decreased risk for hemorrhagic cystitis (cyclophosphamide)
ABCC4	8803391G>T			Possible decreased risk of adverse drug reaction (cyclophosphamide-doxorubicin-5FU)
PPP1R13L	18171984T>C			Possible increased overall survival (melphalan-multiple myeloma)
CD3EAP	18178152G>A			Possible increased overall survival (melphalan-multiple myeloma)
EPM2AIP1	36974946G>A			Possible decreased likelihood for breast neoplasms/t-ML (dacarbazine-procarbazine)
BLMH	Ile443Val			Possible increased survival (bleomycin-testicular neoplasms)

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TOPO I Inhibitors

Gene	Polymorphism	Result		Outcome
ENOSF1	145-370delT			Possible decreased risk of disease progression (5FU-irinotecan-leucovorin)
UGT1A	172270T>G			Possible increased risk of Neutropenia (irinotecan)
UGT1A1	Gly71Arg			Possible decreased risk of Neutropenia (irinotecan)
UBE2I	157C>G			Possible decreased response to cisplatin-irinotecan

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TOPO II Inhibitors

Gene	Polymorphism	Result	Outcome
ABCB1	Ile1145Ile		Possible decreased response to anthracycline regimens and doxorubicin metabolites
RAC2	12536A>T		Possible increased risk for cardiotoxicity (doxorubicin-non Hodgkin lymphoma)
ABCC2	Val1188Glu		Possible decreased risk for cardiotoxicity (doxorubicin-non Hodgkin lymphoma)
NOS3	Asp298Glu		Possible longer disease-free survival (cyclophosphamide-doxorubicin-5FU-MTX)
NCF4	-368G>A		Possible decreased risk for cardiotoxicity (doxorubicin-non Hodgkin lymphoma)
ABCB1	Ser893Ala		Possible decreased metabolism of doxorubicin (breast)
CBR1	Ala209Ala		Possible increased clearance of doxorubicin
CYB2B6	CYP2B6*6		Possible no requirement for dose reduction (cyclophosphamide-doxorubicin)
ABCC1	Gly671Val		Possible decreased risk for cardiotoxicity (doxorubicin-non Hodgkin lymphoma)
CYBA	Tyr72His		Possible increased risk for cardiotoxicity (doxorubicin-non Hodgkin lymphoma)
SLC22A16	His49Arg		Possible decreased likelihood of dose delay when treated with cyclophosphamide and doxorubicin
ABCC2	Cys1515Tyr		Possible increased risk for cardiotoxicity (doxorubicin-non Hodgkin lymphoma)
CBR1	133G>A		Possible increased clearance of doxorubicin
ABCC4	8803391G>T		Possible decreased risk of adverse drug reaction (cyclophosphamide-doxorubicin-5FU)
GSTP1	Ile105Val		Possible increased drug response/decreased severity of toxicity (cyclophosphamide-epirubicin)
NRP2	110077C>G		Possible increased response to daunorubicin
CBR3	Val244Met		Possible increased risk of cardiac damage after anthracycline exposure
Chromosome 12	47386987A>G		Possible lower risk of toxicity (etoposide)
MTHFR	Ala222Val		Possible decreased likelihood of drug toxicity (cisplatin-cyclophosphamide-dactinomycin-doxorubicin-MTX-vincristine) in Osteosarcoma
MTR	Asp919Gly		Possible decreased likelihood of drug toxicity (cisplatin-cyclophosphamide-dactinomycin-doxorubicin-MTX-vincristine) in Osteosarcoma

ANTIMETABOLITES

Gene	Polymorphism	Result	Outcome
TP53	Pro33Arg		Possible increased risk for toxicity/ decreased survival
ABCB1	Ile1145Ile		Possible increased risk of Neutropenia and Neurotoxicity syndromes
ERCC2	Lys751Gln		Possible increased risk of drug toxicity (5FU-leucovorin-oxaliplatin)
GSTP1	Ile105Val		Possible poorer treatment outcome (5FU-oxaliplatin in colorectal)
NOS3	Asp298Glu		Possible longer disease-free survival (cyclophosphamide-doxorubicin-5FU-MTX)
MTHFR	Glu429Ala		Possible decreased risk of drug toxicity (capecitabine)
MTHFR	Ala222Val		Possible decreased risk and severity of mucositis (MTX- leukemia or lumphoma)/ Possible increased risk of drug toxicity (5FU-capecitabine)
DPYD	Ile543Val		Possible decreased likelihood of middle-severe nausea/ vomiting/white blood cell decreases (5FU)
DPYD	Cys29Arg		Possible increased likelihood or middle-severe nauseas/vomiting (5FU)
DPYD	Met166Val		Possible decreased risk of drug toxicity (fluoropyrimidines)
XRCC1	Gln399Arg		Possible decreased response to fluorouracil-containing chemotherapy regimens
ENOSF1	145-370delT		Possible decreased risk of disease progression (5FU-irinotecan-leucovorin)
DPYD	1905+1G>A		Possible increased risk of drug toxicity (fluoropyrimidines based chemotherapy)
DPYD	Asp949Val		Possible increased clearance and decreased risk of drug toxicity (fluoropyrimidines)
ABCC4	8803391G>T		Possible decreased risk of adverse drug reaction (cyclophosphamide-doxorubicin-5FU)
GALNT14	10069401G>T		Possible decreased response (cisplatin-5-FU-mitoxantrone)
CDA	20915590delC		Possible increased risk of drug toxicity (cytarabine)
SLC19A1	His27Arg		Possible decreased risk of hepatotoxicity (MTX)
SLCO1B1	103492T>C		Possible increased clearance of methotrexate/Increased risk for GI toxicity
MTRR	Ile22Met		Possible increased likelihood of methotrexate induced toxicity

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MTR	Asp919Gly			Possible decreased likelihood of drug toxicity/ increased response to MTX
SHMT1	Leu435Phe			Possible decreased catalytic activity of TYMS/ Decreased likelihood of toxic liver disease
MTHFD1	Arg653Gln			Possible decreased event free survival
SLCO1B1	Val174Ala			Possible increased clearance of methotrexate
SLCO1B1	14138145G>A			Possible increased clearance of methotrexate/ Increased risk of GI toxicity
CCND1	Pro241Pro			Possible increased time-to-tumor recurrence when treated with 5FU
ABCC3	-260T>A			Possible decreased event-free survival/ Decreased risk of thrombocytopenia when treated with MTX
CDA	Lys27Gln			Possible decreased risk of toxicity when treated with cytarabine/ Possible lower frequency of GI toxicity/ Decreased risk of developing Neutropenia when treated with gemcitabine
CDA	-451C>T			Possible reduced toxicity / Increased survival time when treated with cytarabine
CDA	-92A>G			Possible decreased risk of diarrhea or dehydration when treated with capecitabine
CDA	Ala70Thr			Possible increased clearance of gemcitabine/ Decreased severity of neutropenia
RRM1	Thr741Thr			Possible decreased risk of toxicity (Neutropenia) when treated with gemcitabine

SPINDLE POISONS

Gene	Polymorphism	Result		Outcome
ABCB1	Ser893Ala			Possible decreased risk for resistance to taxanes
TP53	Pro33Arg			Possible increased risk for toxicity/ decreased survival
ABCB1	Ile1145Ile			Possible increased risk of Neutropenia and Neurotoxicity syndromes (paclitaxel)
CYP1B1	Leu432Val			Possible shorter disease-free progression (docetaxel-paclitaxel-taxanes)
CYP2C8	23210C>G			Possible increased risk of neurotoxicity (paclitaxel)
CYP2C8	Arg69Lys			Possible decreased risk of neurotoxicity (paclitaxel)
CYP3A5	12083G>A			Possible increased risk of neurotoxicity (paclitaxel)
DSCAM	27076915T>C			Possible increased survival (carboplatin-paclitaxel)
NAT2	NAT2*7A			Possible decreased risk of toxicity (docetaxel-thalidomide)
CYP3A4	CYP3A4*1B			Possible decreased clearance of docetaxel

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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.

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- 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.

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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

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