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***STUDIES TO UNDERSTAND THE EFFECT OF
CANCER ON HEPATIC CYP2C19 ACTIVITY***

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Abstract

Inter-patient variation in effectiveness and toxicity of cancer chemotherapy may be due to differences in pharmacokinetics, influenced by genetic and environmental factors controlling the activity of hepatic drug metabolising enzymes. One such enzyme, CYP2C19, displays genetic variation; homozygous variant individuals have a poor metaboliser (PM) phenotype. Whilst this relationship is valid in healthy populations, genotype-phenotype discordance has been reported in cancer patients. The aim of this thesis was to determine if discordance occurs in a wider range of cancer patients and to elucidate mechanisms responsible for decreased CYP2C19 enzyme activity.

Two independent clinical studies were undertaken. Of 33 patients with terminal cancer, 37% were PM, significantly ($P < 0.0005$) higher than predicted from genotype. For 29 patients with colorectal carcinoma, 27% in stage IV and 14% of resected patients were PM. Although RECIST analysis of stage IV patients did not demonstrate a significant relationship between CYP2C19 activity and tumour burden, the one patient tested both before and after tumour resection, changed from a poor to an extensive metaboliser.

In patients with terminal cancer, no correlation between CYP2C19 status and inflammatory markers was observed. In contrast, PM phenotype in stage IV and resected patients was associated with elevated CRP ($P < 0.05$) and decreased serum TGF- β ($R_s = -0.5331$, $P < 0.005$). Interestingly, six patients changed phenotype categories over three test occasions reflected by changes in TGF- β . There was also an association between BMI and CYP2C19 activity ($R_s = 0.4953$, $P = 0.0063$).

NO-donor compounds reversibly inhibited CYP2C19 activity in human liver microsomes and cells over-expressing CYP2C19. In addition, 24h exposure of cells to NO-donor compounds irreversibly decreased CYP2C19 activity ($P < 0.0005$), which was blocked by MG132, an inhibitor of proteasomal degradation. However, there was no relationship between plasma nitrate/nitrite concentrations and CYP2C19 activity in the patients.

Total plasma protein and unbound drug fraction were determined for individual patients. It was demonstrated that the high drug/metabolite ratio in the PM subjects was not due to altered drug-protein binding and could only be accounted for by decreased enzyme activity (intrinsic clearance, CL_{int}).

In conclusion, some cancer patients have compromised CYP2C19 activity that may be due to factors including inflammation, obesity and nitrosative damage. Non-inherited variation in CYP2C19 activity may account for variable pharmacokinetics of some anticancer drugs, thus identification of phenotypic PM prior to treatment may reduce the wide variation in both toxicity and response to these agents.

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LongCYP: CYP2C19 pharmacogenetics in cancer, the effect of disease progression. **Lo WY**, Laking G, Spells K, Findlay M, Helsby NA. *Cancer Trials New Zealand, CTNZ Annual Scientific Meeting, Auckland New Zealand, 30th July 2009.*

Compromised CYP2C19 function in advanced cancer patients. Helsby NA, **Lo WY**, Sharples, K, Riley, G, Murray, M, Spells, K, Dzhelai, M, Simpson A, Findlay MP. *New Zealand Society for Oncology, Annual meeting, Christchurch New Zealand, 5-6th August 2008.*

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List of Abbreviations and Symbols

°C	Degree Celsius
5'OH OMP	5'hydroxy omeprazole
α-MEM	Alpha-minimal essential media
A	Adenine
AAG	Alpha ₁ -acid glycoprotein
ALP	Alkaline phosphatase
ALT	Alanine transaminase
AST	Aspartate transaminase
AUC	Area under the time-concentration curve
bid	bis in die, twice a day
BMI	Body mass index
bp	Base pair
C	Cytosine
CG	Cycloguanil
CL	Clearance
CL _H	Hepatic clearance
CL _{int}	Intrinsic clearance
CRP	C-reactive protein
CT scan	X-ray computed tomography scan
CYP450	Cytochrome P450
dL	Decilitre
DNA	Deoxyribonucleic acid
E	Hepatic extraction ratio
EDTA	Ethylenediaminetetraacetic acid
EM	Extensive metaboliser
f _u	Fraction unbound
g	Gravitational acceleration
G	Guanine
g	Gram
HDL	High-density lipoprotein
HI	Hydroxylation index
HPLC	High performance liquid chromatography
IC ₅₀	Half maximal inhibitory concentration
IFN-γ	Interferon-gamma
IL-1α	Interleukin-1 alpha
IL-1β	Interleukin-1 beta
IL-6	Interleukin-6

IQR	Interquartile range
IS	Internal standard
iv	Intravenous
kg	Kilogram
K_m	Michaelis constant
L	Litre
LDL	Low-density lipoprotein
M	Molar
m	Metre
mg	Milligram
min	Minutes
mL	Millilitre
mM	Millimolar
mm	Millimetre
mm	Millimetre
mRNA	Micro RNA
n	Number
NADPH	β -Nicotinamide adenine dinucleotide phosphate (reduced form)
NED	No-evaluable disease
ng	Nanogram
nm	Nanometre
NO	Nitric oxide
NOC-18	2,2'-(hydroxynitrosohydrazino)bis-ethanamine
OD	Optical density
OMP	Omeprazole
P	P -value
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
pg	Picogram
PG	Proguanil
PM	Poor metaboliser
po	<i>Per os</i> , oral administration
PVDF	Polyvinylidene difluoride
Q	Liver blood-flow
qPCR	Real-time polymerase chain reaction
R_B	Blood-to-plasma ratio
RECIST	Response evaluation criteria in solid tumours
RFLP	Restriction fragment length polymorphism
R_s	Spearman rank correlation
SD	Standard deviation
SE	Standard error

SNAP	S-nitroso <i>N</i> -acetylpenicillamine
SNP	Single nucleotide polymorphism
SP	Sodium nitroprusside
T	Thymine
$t_{1/2}$	Half-life
TBS	Tris-buffered saline
TGF- β	Transforming growth factor-beta
TNF- α	Tumour necrosis factor-alpha
U	Units
UV	Ultraviolet
v/v	Volume to volume
<i>var</i>	Variant allele
Vd	Volume of distribution
V_{max}	Maximum catalytic rate
w/v	Weight to volume
<i>wt</i>	Wildtype allele
μg	Microgram
μL	Microlitre
μM	Micromolar