# *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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## Publications arising from this thesis

*CYP2C19* pharmacogenetics in advanced cancer: compromised function independent of genotype. Helsby NA, **Lo WY**, Sharples K, Riley G, Murray M, Spells K, Dzhelai M, Simpson A, Findlay M. *British Journal of Cancer*. 2008, 99(8): 1251-1255.

Do 5-fluorouracil therapies alter CYP2C19 metaboliser status? Helsby NA, **Lo WY**, Thompson P, Laking GR. *Cancer Chemotherapy and Pharmacology*. 2010, 66(2): 405-407.

## Conference presentations

Effect of tumour burden on CYP2C19 drug metabolizing activity in patients. **Lo WY**, Laking G, Spells K, Findlay M, Helsby NA. *European Association of Clinical Pharmacology and Therapeutics, 8th EACPT Summer School*, Dresden Germany, 30th September-2nd October 2010.

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Effect of Tumour burden on CYP2C19 drug metabolizing activity in patients. **Lo WY**, Laking G, Spells K, Findlay M, Helsby NA. *New Zealand Society for Oncology, Annual meeting*, Auckland New Zealand, 3-5th May 2010.

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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.

CYP2C19 Pharmacogenetics: Understanding the Relationship Between Genotype and Clinical Phenotype. Helsby NA, **Lo WY**, Murray M, Findlay MPN. *41st Annual Scientific Meeting of the Australasian Society of Clinical and Experimental Pharmacologists*, Adelaide Australia, 2-6th Dec 2007.

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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 | *Per os*, 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 *N*-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 |
| *var* | Variant allele |
| Vd | Volume of distribution |
| $V_{max}$ | Maximum catalytic rate |
| w/v | Weight to volume |
| *wt* | Wildtype allele |
| µg | Microgram |
| µL | Microlitre |
| µM | Micromolar |