STRATEGIC PRE-CLINICAL DEVELOPMENT OF RIMINOPHENAZINES AS RESISTANCE CIRCUMVENTING ANTICANCER AGENTS

by

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This thesis is dedicated to my parents and grandparents.

DECLARATION BY CANDIDATE

I (D.J. Koot) declare that this thesis, which I hereby submit for the degree PhD: Pharmacology at the University of Pretoria is my own work and has not previously been submitted for a degree at this or any other tertiary institution.

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Dwayne Jonathan Koot Date

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SUMMARY

Cancer is responsible for upward of 13% of human deaths. Contemporary chemotherapy of disseminated cancer is often thwarted by dose limiting systemic toxicity and by multi-drug resistance (MDR). Riminophenazines are a novel class of potential anticancer agents that possess a potent multi-mechanistic antineoplastic action. Apart from their broad action against intrinsic, non-classical resistance, Riminophenazines inhibit the action of Pgp and hypothetically all ABC transporters demonstrating their great utility against classical MDR.

Considering that combination chemotherapy is the norm, the vision directing R&D efforts was that Riminophenazines could be used with benefit within many standard chemotherapeutic regimes.

The strategic intent of this project was to attain improved therapeutic benefit for patients through gains in both pharmacodynamic and pharmacokinetic specificity for cancer cells over what is currently available. Tactically, this was driven through the use of synergistic Fixed-Ratio Drug Combinations (FRDC) encapsulated within tumour-targeting Nanoparticulate Drug Delivery Systems (NDDS).

Long-term aims of this R&D project were to:

1) Screen FRDC of clofazimine (B663) and the lead derivative (B4125) with etoposide, paclitaxel and vinblastine for synergistic drug interactions *in vitro*.
2) Design, assemble and characterize a novel nanoparticulate, synergistic, anticancer co-formulation.
3) Evaluate the *in vivo* safety and efficacy of the developed product/s in accordance with international regulatory guidelines.

Using the median effect and combination index equations, impressive *in vitro* synergistic drug interactions (CI<1) were shown for various FRDC of the three standard chemotherapeutics tested (etoposide, paclitaxel and vinblastine) in combination with either B663 or B4125 against MDR neoplastic cell cultures.

Considering *in vitro* results and with the view to advance quickly to clinical studies, the already approved clofazimine (B663) was elected as the combination partner for paclitaxel (PTX). Considering the potency and wide action of PTX, a novel co-formulation (designed to circumvent drug resistance) has the potential to greatly impact upon virtually all cancer types, particularly if selectively delivered through innovative delivery systems and loco-regional administration.

A passively tumour targeting, micellular NDDS system called Riminocelles™ that encapsulates a synergistic FRDC of B663 and PTX has been designed, assembled using thin film hydration methods and characterized in terms of drug loading, particle size, zeta potential, CMC and drug retention under sink conditions. An acute toxicity and a GLP repeat dose toxicity study confirmed Riminocelles to be well tolerated and safe at clinically relevant dosages whilst Taxol® (QDx7) produced statistically significant ($P<0.05$) weight loss within 14 days. The same study demonstrated statistically significant ($P<0.05$) tumour growth delays superior to that of Taxol at an equivalent PTX dosage of 10 mg/kg. Importantly, all components (amphiphiles and drugs) used in assembly of Riminocelles are already individually approved for medicinal use - this promotes accelerated development towards advanced clinical trials and successful registration. Although these results are very promising (outperforming Taxol), this system was however found in a pharmacokinetic study to suffer from *in vivo* thermodynamic instability due to the high concentration (abundance) of albumin present in plasma. For this reason, *in vivo* longevity within circulation, permitting passive tumour accumulation was not fully realized.

A second NDDS called the RiminoPLUS™ imaging system was additionally developed. This lipopolymeric nanoemulsion system has successfully entrapped Lipiodol® Ultra fluid (an oil based contrast agent) within the hydrophobic core of a monodisperse particle population with a size of roughly 100 nm and a stability of one week. This formulation is therefore thought capable of CT imaging of tumour tissue and drug targeting after either intravenous or loco-regional injection. *In vivo* proof of the imaging concept is warranted

The results of this study serve to highlight the great potential of *in vitro* optimized synergistic FRDC against drug resistant cancers. Lipopolymeric micelles are an effective way to formulate multiple hydrophobic drugs for intravenous administration and present a means by which cancer can be readily targeted; provided that the delivery system possess the prerequisite *in vivo* stability and surface attributes. Further experiments exploring synergistic drug and biological combinations as well as "intelligent" NDDS actively guided through specific molecular recognition are called for.

Key terms:

Cancer; multi-drug resistance; fixed-ratio drug combination; Riminophenazine; clofazimine; paclitaxel; nanoparticulate drug delivery systems; thin film hydration method; lipopolymeric micelle; ternary phase diagram; aqueous titration method; Lipiodol; nanoemulsion; pre-clinical; *in vivo* models; toxicity; pharmacokinetics; LC-MS/MS; efficacy

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GLOSSARY OF ABBREVIATIONS

ABC	ATP-binding cassette
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
API	Active pharmaceutical ingredient
AST	Aspartate aminotransferase
ATCC	American tissue culture collection
ATP	Adenosine triphosphate
AUCC	Animal use and care committee
B663	Clofazimine
BCRP	Breast cancer resistant protein
BSA	Bovine serum albumin
$CDCl_3$	Deuterated chloroform
CE	Collision energy
$CHCl_3$	Chloroform
CI	Combination index
CMC	Critical micelle concentration
cP	Centipoise
CRL	Charles River Laboratory
CSIR	Council for Scientific and Industrial Research
CT	Computed tomography
CV	Coefficient of Variation
CXP	Collision-cell exit potential
D_2O	Deuterium oxide
DLS	Dynamic light scattering
DMEM	Dulbecco's Modified Eagle's Medium
DMSO	Dimethyl Sulphoxide
DP	Declustering potential
DTX	Docetaxel
EDTA	Ethylenediaminetetraacetic acid
EMEA	European agency for the evaluation of medicinal products
EO	Ethylene oxide

EP	Entrance potential
EPR	Enhanced permeability and retention
ESI	Electrospray ionization
ETOP	Etoposide
fa	Fraction affect level
FCS	Fetal calf serum
FDA	Food and drug administration (USA)
FRDC	Fixed-ratio drug combination
GGT	Gamma-glutamyl transpeptidase
GLP	Good laboratory practise
GMP	Good manufacturing practise
HBSS	Hank's Balanced Salt Solution
HEPA	High efficiency particulate air
HCC	Hepatocellular carcinoma
HCO_2H	Formic acid
HLB	Hydrophilic-Lipophilic Balance
HNSTD	Highest non-severely toxic dose
HTS	High throughput screening
IC	Inhibitory Concentration
IS	Internal Standard
IV	Intravenous
JCRB	Japanese Collection of Research Bioresources
LC-MS	Liquid Chromatography tandem Mass Spectrometry
LD	Lethal Dose
LOQ	Limit of quantification
MAb	Monoclonal Antibody
MCC	Medicines Control Council (South Africa)
MDR	Multi-drug resistance
ME	Matrix effect
MeCN	Acetonitrile
MeOH	Methanol
MHLW	Ministry of Health, Labour and Welfare (Japan)
MPS	Mononuclear phagocyte system
MRC	Medical Research Council

MRM	Multiple reaction monitoring
MRP	Multidrug Resistance Associated Protein
MtBE	Methyl *tert* butyl ether
MTD	Maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NCI	National Cancer Institute
NDDS	Nanoparticulate Drug Delivery System
nm	Nanometre
NMR	Nuclear Magnetic Resonance spectroscopy
PC	Phosphatidylcholine
PdI	Polydispersity index
PEG	Polyethylene glycol
Pgp	P-glycoprotein
PLA_2	Phospholipase A_2
PTFE	Polytetrafluoroethylene
PTX	Paclitaxel
PVP	Polyvinylpyrrolidone
QC	Quality control
QSAR	Quantitative structure activity relationships
R&D	Research and Development
R*f*	Retention / retardation factor
RPMI	Roswell Park Memorial Institute Medium
SC	Standard chemotherapeutic
S_{mix}	Surfactant mixture
SPE	Solid phase extraction
STD	Severely toxic dose
T_d	Tumour-volume doubling time
TEM	Transmission Electron Microscopy
TMP	Tetramethylpiperidine
UPBRC	University of Pretoria Biomedical Research Centre
UV	Ultraviolet
VIN	Vinblastine
WHO	World Health Organisation