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**ANTITUMOUR EFFICACY OF THE
NITROREDUCTASE-ARMED ONCOLYTIC
ADENOVIRUS ONYX-411^{NTR} IN COMBINATION
WITH DINITROBENZAMIDE MUSTARD PRODRUGS
IN PRECLINICAL MODELS**

A thesis submitted in fulfilment of the
requirements for the degree of
Doctor of Philosophy

by

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ABSTRACT

Oncolytic viruses that selectively replicate in and lyse cancer cells are a promising approach for the treatment of tumours that are resistant to conventional therapies. Clinical experience has shown that oncolytic viruses are safe and well tolerated but possess modest single agent activity. One approach to improve the efficacy of oncolytic viruses is to utilise their tumour tropism to deliver genes encoding enzymes able to activate prodrugs. ONYX-411 is an oncolytic adenovirus that replicates in cells that carry dysfunctions in the retinoblastoma (pRb) pathway, a common hallmark of cancer. ONYX-411 was ‘armed’ by inserting the *Escherichia coli* nfsB nitroreductase (NTR) gene into the E3B region of the viral genome under the control of the endogenous E3 viral transcriptional machinery. NTR is an oxygen-insensitive nitroreductase that is capable of activating dinitrobenzamide mustard (DNBM) prodrugs to cytotoxic metabolites. The main objective of this thesis was to determine the extent and mechanism of the therapeutic interaction between ONYX-411^{NTR} and DNBM prodrugs.

A fluorogenic probe was developed to monitor NTR activity non-invasively and revealed robust, replication dependent NTR activity in ONYX-411^{NTR}-infected neoplastic but not primary human cell lines. *In vitro* exposure of ONYX-411^{NTR}-infected cells to therapeutically relevant concentrations of the DNBM prodrugs (SN 27686 or PR-104A) did not inhibit virus replication.

Tumour growth delay studies of systemic ONYX-411^{NTR} followed by prodrug demonstrated different outcomes in three models (H1299, C33A, 22Rv1). To establish predictable viral infection of tumours a pre-infection model was developed using HCT 116 xenografts. This methodology demonstrated that prodrug administration (SN 28343 or PR-104) provided significant inhibition of tumour growth without suppression of ONYX-411^{NTR} replication. Follow-on studies using intravenous virus administration confirmed titre amplification with time (24-fold between day 3 and 13 post administration; $P < 0.001$) and a marked survival gain for the virus/prodrug combinations. Neither the prodrugs nor ONYX-411^{NTR} were active as single agents. The improvement in efficacy for the combination of ONYX-411^{NTR} and prodrug was conditional on NTR-dependent prodrug activation resulting in improved virus distribution within the tumour.

PR-104 is currently in clinical development making the combination of ONYX-411^{NTR} with PR-104 a promising strategy for cancer selective therapy.

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LIST OF ABBREVIATIONS AND DEFINITIONS

α MEM	α -modified minimal essential medium
ATCC	American Type Culture Collection
AUC	area under the curve
CB1954	5-aziridinyl-2,4-dinitrobenzamide
CYPOR	cytochrome P450 reductase
CPA	cyclophosphamide
DMSO	dimethyl sulphoxide
DNBM	dinitrobenzamide mustard
d.p.i.	days post infection
FBS	foetal bovine serum
FSL	Fluorogenic substrate library
FSL 41	1-methyl-6-nitro-4(1 <i>H</i>)-quinolinone
HPLC	high performance liquid chromatography
h.p.i.	hours post infection
IMS	increase in median survival
i.p.	intraperitoneal
i.v.	intravenous
kDa	kiloDalton
LC/MS	liquid chromatography with mass detection
MCL	multicellular layer
MTD	maximum tolerated dose
NTR	nitroreductase (specifically the product of the <i>E. coli</i> nfsB gene)
NQO1	NAD(P)H dehydrogenase, quinone 1
PD	pharmacodynamic
PK	pharmacokinetic
PR-104	2-((2-bromoethyl)-2-{[(2-hydroxyethyl)amino]carbonyl}-4,6-dinitroanilino)ethyl methanesulfonate phosphate ester
PR-104A	2-((2-bromoethyl)-2-{[(2-hydroxyethyl)amino]carbonyl}-4,6-dinitroanilino)ethyl methanesulfonate
P/S	100 U/mL penicillin and 100 μ g/mL streptomycin
SAR	structure-activity relationship
s.c.	subcutaneous
sd	standard deviation
sem	standard error of the mean
SN 27686	2-(bis(2-bromoethyl)amino)-N-(2-hydroxyethyl)-3,5-dinitrobenzamide
SN 28343	2-(bis(2-bromoethyl)amino)-N-(2-hydroxyethyl)-3,5-dinitrobenzamide phosphate ester