Targeted EDV^{TM} Nanocells carrying small interfering RNA (siRNA) molecules to overcome drug resistance in Non-small cell lung cancer

by Eva St. Clair

Thesis submitted in fulfilment of the requirements for
the degree of

Master of Science (Research)

under the supervision of

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June, 2021

CERTIFICATE OF ORIGINAL AUTHORSHIP

I, Eva St. Clair declare that this thesis, is submitted in fulfilment of the requirements for the award of Master of Science (Research), in the Faculty of Life Science at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise referenced or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

This document has not been submitted for qualifications at any other academic institution.

This research is supported by the Australian Government Research Training Program.

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Date: 3/06/2021

Acknowledgments

First and foremost, I would like to thank Dr. Jennifer MacDiarmid and Dr. Himanshu Brahmbhatt for their guidance, encouragement and support to allow me to achieve this master's degree. I feel incredibly grateful to have worked in the innovative company you have built for the past 6 years and cannot thank you enough for your willingness to share your experience and endless knowledge with me. You both inspire me to strive to be the best scientist I can be.

I would like to thank all of the EnGenes for your support over the years. I am incredibly blessed to be working with such lovely people and talented scientists. In particular, thank you to Jocelyn & Natasha for sharing your animal ethics and *in vivo* skills and knowledge. Stacey, for always sharing her extensive tissue culture knowledge and Sharon for being so willing to share her flow cytometry expertise. To Nancy and Steven for their guidance in helping me chose suitable siRNAs, probes and primers and in RT-qPCR analysis. To Lu, Kasia, Estefania and Julia for sharing their knowledge in various *in vitro* experiments, and Ilya, Reema, Vatty and Arash for their guidance in antibody targeting and EDV^{TM} loading and lyophilisation.

To my UTS supervisors, Professor Gyorgy Hutvagner and Dr. Eileen McGowan thank you for your amazing support, encouragement and sharing your extensive knowledge on thesis writing and presentations.

Thank you also to Carmel and David Goggins and the EnGeneIC Cancer Research Foundation that awarded me the Jenna Goggins Scholarship which allowed me to carry out this master's degree. I have heard about what an amazing and courageous woman your daughter Jenna was, and her memory has inspired me throughout my work.

A very big thank you to my own family for their constant support and love and for always being there for me.

Finally, to my husband Sean, thank you for supporting me in everything I wish to achieve, for always making me laugh even during the difficult times and for encouraging me to be the best I can be.

I dedicate this thesis to our beautiful daughter Annabelle, who arrived at the very end of this work and gave me strength and inspiration to complete this master's degree.

Abstract

Background. Over two million people worldwide suffer from lung cancer, the main sub-type (85%) being NSCLC and despite many chemotherapeutics approved for NSCLC, only 2% of patients with NSCLC metastatic disease survive 5 years post diagnosis with *multidrug resistance being the major cause of mortality in NSCLC patients.*

Aim. The overall aim of this project was to evaluate targeted EnGeneIC Dream Vector™ (EDV™) nanocells for loading and delivering small interfering RNA (siRNA) molecules, Polo like kinase-1 (PLK1), Ribonucleoside reductase subunit M1 (RRM1) & Kinesin Spindle Protein (KSP), in order to silence proteins essential to tumour cell survival and proliferation, and to evaluate their therapeutic potential in overcoming the hitherto intractable multiple drug resistance in non- small cell lung cancer (NSCLC).

Methods. The expression of cell cycle genes *PLK*1, *RRM*1 & *KSP* in NSCLC cell lines was measured using RT-qPCR and Western Blot. Efficacy of siRNAs targeting PLK1 (siPLK1), RRM1 (siRRM1) & KSP (siKSP) transfected into NSCLC cell lines was measured by the MTS proliferation assay and Western Blot. Flow cytometric analysis was used to measure apoptosis and cell cycle arrest in NSCLC cell lines transfected with the siRNAs targeting PLK1, RRM1 and KSP. EDV™ nanocells were targeted to the epithelial growth factor receptor (EGFR) and the copy number of siRNAs loaded into the nanocells was measured by staining with an RNA specific dye and measured on a fluorometer compared to known standards. EDV™-siRNAs were used to treat NSCLC cells lines grown as 3D spheroids using the hanging drop plates (Perfecta3D®:HDP1096) and cell proliferation inhibition was assessed using trypan blue cell viability assay. The EDV™s-siRNAs were then tested *in vivo* using the A549-Dox-R a xenograft mouse model, tumours were excised and assessed for gene knockdown by RT-qPCR.

Results and Conclusion. In this study we show that *in vitro* and *in vivo*, EDV™s can effectively deliver targeted-cell cycle-siRNAs to hanging drop 3D spheroids and into a mouse xenograft model to inhibit cell and tumour growth, and that EDV™s can encapsulate and deliver a significant siRNA payload directly inside the tumour cells without affecting non-target tissue. Overall, this study highlights the exciting possibility that siRNAs against mitotic regulators loaded into EDV™s will be safe alone or in combination with drug-loaded EDV™s, and may overcome drug resistance in NSCLC patients. This project has true translational potential for

both delivering hitherto “undeliverable” functional nucleic acids, and for potentially addressing drug-resistance mechanisms in lung cancer.

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Abbreviations

A549-Dox-R	A549-Doxorubicin-Resistant cell line
Ab	Antibody
ACC	Adrenocortical cancer
AF488	Alexa Fluor® 488
AGCA	Australian Government Cancer Australia
AIHW	Australian Institute of Health and Welfare
ALK	Anaplastic lymphoma kinase
ATCC	American Type Culture Collection
BCL2	B-cell lymphoma 2 encoding gene
BRAF	B-Raf Proto-Oncogene
BSA	Bovine Serum Albumin
BsAb	Bispecific Antibody
c-myc	Myc Proto-Oncogene Protein
CA-19-9	Carbohydrate Antigen 19-9
CAR	Chimeric Antigen Receptor
CTLA-4	Cytotoxic T-Lymphocyte-associated Antigen-4
DDT	Dithiothreitol
DNA	Deoxyribonucleic Acid
DOPC	1,2-Dioleoyl-sn-glycero-3-phosphocholine
dsDNA	Double Stranded DNA
dsRNA	Double Stranded RNA
EBSS	Earles Balanced Salt Solution
EMEM	Eagles Minimum Essential Medium
ECACC	European Collection of Animal Cell Cultures
EDTA	Ethylenediaminetetraacetic acid
EDV™	EnGeneIC Dream Vector™
EGFR	Epidermal Growth factor receptor
EML4-ALK	Endocrine Microtubules associated protein-like protein
EMT	Epithelial to Mesenchymal Transition
EphA2	Ephrin type-A Receptor 2
EPR	Enhance Permeation and Retention

ERK	Extracellular Signal-Regulated Kinase
FAK	Focal Adhesion Kinase
FBS	Foetal Bovine Serum
FTI	Farnesyltransferase inhibitors
HDM2	Human Double Minute-2 protein
HER2	Human Epidermal growth factor Receptor 2
Hh	Hedgehog
IC50	Half maximal inhibitory concentration
IFN	Interferon
IL	Interleukin
iNOP	Interfering Nanoparticle
KRAS	Kirsten Rat Sarcoma viral proto-oncogene
KSP	Kinesin Spindle Protein
LCP	Lipid Calcium Phosphate
LODER™	Local Drug Eluter
mAb	Monoclonal Antibody
MAGE-3	Melanoma-associated antigen 3
MAPK1	Mitogen-Activated Protein Kinase 1
MAP	Mitogen-Activated Protein
MEK-1	MAP Kinase/ERK Kinase 1
MET	MET proto-oncogene, receptor tyrosine kinase
MDR	Multi Drug Resistance
MDR 1	Multi Drug Resistant Protein
miRNA	Micro RNA
MESF	Molecules of Soluble Fluorochrome
MNP	Micellar Nanoparticles
mRNA	Messenger RNA
MRP3	Multi Drug Resistance-associated Protein 3
MST	Median Survival time
NEAA	Non-Essential Amino Acid
nm	Nanometer
nM	Nanomolar
NSCLC	Non-Small Cell Lung Cancer

NS-NSCLC	Non-Squamous Non-Small Cell Lung Cancer
NY-ESO-1	Human tumour antigen of the cancer/testis family
ORR	Objective Response Rate
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PD-1	Programmed cell death protein
PDK4	Pyruvate dehydrogenase lipoamide kinase isozyme 4
PFS	Progression-free survival
PLGA	Poly (DL-lactide-co-glycolide acid)
PLK1	Polo-like kinase 1
PTGS	Post-Transcriptional Gene Silencing
PDR	Progressive Disease Rate
QC	Quality Control
RET	Rearranged during Transfection Proto-oncogene
RNA	Ribonucleic Acid
ROS1	ROS proto-oncogene 1, receptor tyrosine kinase
RISC	RNA Induced Silencing Complex
RNAi	RNA interference
RPMI	Roswell Park Memorial Institute medium
RRM1	Ribonucleotide Reductase Subunit M1
RT	Room Temperature
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
S. Typhimurium	*Salmonella typhimurium*
SCLC	Small-cell lung cancer
siLuc	Luciferase siRNA
shRNA	Short hairpin RNA
siRNA	Small interfering RNA
siPLK1	Polo-Like Kinase 1 targeted siRNA
sLDH	Small layered Double Hydroxide
TKI	Tyrosine Kinase Inhibitor
Tp53	Tumour Protein 53 gene
TRAE	Treatment Related Adverse Effects
VEGF	Vascular endothelial growth factor

WHO	World Health Organisation

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