
**REGROWTH RESISTANCE IN
PLATINUM-DRUG RESISTANT
SMALL CELL LUNG CANCER
CELLS**

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PhD 2006

Certificate of Authorship/Originality

I hereby certify that the work in this thesis has not been previously submitted for a degree. I also certify that I wrote this thesis and any help received in my research work or in the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

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Abstract

The H69CIS200 cisplatin-resistant and H69OX400 oxaliplatin-resistant cell lines developed as part of this study, are novel models of low-level platinum resistance. These resistant cell lines do not have common mechanisms of platinum resistance such as increased expression of glutathione or decreased platinum accumulation. Rather, these cell lines have alterations in their cell cycle allowing them to proliferate rapidly post drug treatment in a process known as 'regrowth resistance'. This alteration in cell cycle control has come at the expense of DNA repair capacity. The resistant cell lines show a decrease in nucleotide excision repair and homologous recombination repair, the reverse of what is normally associated with platinum resistance. The alterations in these DNA repair pathways help signal the G₁/S checkpoint to allow the cell cycle to progress despite the presence of DNA damage. The decrease in DNA repair capacity has also contributed to the development of chromosomal alterations in the resistant cell lines. Similarities in chromosomal change between the two platinum resistant cell lines have been attributed to inherent vulnerabilities in the parental H69 cells rather than part of the mechanism of resistance.

The H69CIS200 and H69OX400 resistant cells are cross-resistant to both cisplatin and oxaliplatin. This demonstrates that oxaliplatin does not have increased activity in low-level cisplatin-resistant cancer. Oxaliplatin resistance also developed more rapidly than cisplatin resistance suggesting that oxaliplatin may be less effective than cisplatin in the treatment of SCLC. The resistant cell lines have also become hypersensitive to taxol but show no alterations in the expression, polymerisation or morphology of tubulin. Rather, the PI3K/Akt/mTOR pathway is involved in both platinum resistance and taxol sensitivity as both are reversed with rapamycin treatment. mTOR is also phosphorylated in the resistant cell lines indicating that platinum resistance is associated with an increase in activity of this pathway. The mechanism of regrowth resistance in the platinum-resistant H69CIS200 and H69OX400 cells is a combination of activation of PI3K/Akt/mTOR signalling and alterations in control of the G₁/S cell cycle checkpoint. However, more work remains to determine which factors in these pathways are governing this novel mechanism of platinum resistance.

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Table of Contents

Certificate of authorship/originality	ii
Abstract	iii
Acknowledgements	iv
List of Figures	xiii
List of Tables	xviii
Publications	xx
List of Abbreviations	xxii
Chapter 1.0 Introduction	1
1.1 Cisplatin	2
1.2 Oxaliplatin	4
1.3 Small cell lung cancer (SCLC)	9
1.4 Previous platinum-resistant SCLC sublines	11
1.5 Mechanism of action of platinum chemotherapeutics	13
1.6 Mechanisms of resistance to platinum chemotherapeutics	15
1.6.1 Redox detoxification of platinum chemotherapeutics	17
1.6.1.1 Glutathione	17
1.6.1.2 Thioredoxin	20
1.6.2 Reduced intracellular accumulation of platinum chemotherapeutics	20
1.6.3 Inhibition of the apoptotic response	22
1.6.3.1 PI3K/Akt signalling pathway and platinum resistance	24
1.6.4 Increased bypass of platinum adducts	27
1.6.5 DNA repair	29
1.6.5.1 Nucleotide excision repair	31
1.6.5.2 Cross links and double strand breaks	34
1.6.5.3 Base excision repair	36
1.6.5.4 Mismatch repair	36
1.6.5.5 Chromosomal aberrations and genomic instability	41
1.7 Conclusions and Aims of PhD Project	42
Chapter 2.0 Materials and methods	43

2.1 Materials	44
2.1.1 Cytotoxic drugs	44
2.1.2 General solutions	44
2.2 Cell lines and tissue culture conditions	45
2.3 MTT cytotoxicity assay	46
2.3.1 Radiation resistance	46
2.4 Flow cytometry cell cycle analysis	47
2.5 Glutathione assay	47
2.6 Platinum accumulation	49
2.6.1 Analysis of electrolytes which could interfere with the platinum assay	51
2.7 Genomic DNA extraction	51
2.7.1 Assessing yield and purity of genomic DNA	52
2.8 Affymetrix genechip mapping 10K array	53
2.9 Cytogenetics	56
2.9.1 Preparation of metaphase chromosome spreads	56
2.9.2 Fluorescent in-situ hybridisation (FISH)	56
2.9.2.1 Slide preparation	56
2.9.2.2 Probes used for FISH	57
2.9.2.3 FISH probe hybridisation and visualisation	58
2.9.3 G-banding of metaphase slides for karyotyping	58
2.10 Total RNA purification	59
2.10.1 DNase treatment of total RNA	60
2.10.2 Assessing yield and purity of total RNA	60
2.11 Atlas nylon array analysis	61
2.11.1 Poly A ⁺ RNA enrichment and probe synthesis	61
2.11.2 Column chromatography purification of cDNA probe	63
2.11.3 Nylon array hybridisation	63
2.11.4 Analysis of Atlas arrays	65
2.12 Real-time PCR	65
2.12.1 Conversion of RNA to cDNA	65
2.12.2 Designing of primers for real-time PCR	67
2.12.3 PCR reaction and cycling conditions for real-time PCR	67

2.12.4 Testing of primers for real-time PCR	69
2.12.5 Analysis of real-time PCR data	70
2.13 Protein analyses	70
2.13.1 Preparation and quantitation of total protein extracts	70
2.13.2 Preparation of cell fractions for soluble/polymerised tubulin analysis	73
2.13.3 Sodium dodecyl sulphate - polyacrylamide gel electrophoresis (SDS- PAGE)	73
2.13.4 Western blotting	74
2.13.4.1 Development of Western blots	75
2.14 Immunocytochemistry	75
2.15 Statistics	78
Chapter 3.0 Development of platinum-resistant small cell lung cancer cell lines	79
3.1 Introduction	80
3.2 Development of platinum resistance	81
3.3 Cell cycle changes associated with platinum resistance	87
3.4 Cross resistance to other chemotherapeutics	87
3.5 Cellular glutathione and resistance	93
3.6 Platinum accumulation	93
3.7 Discussion	98
3.8 Conclusion	103
Chapter 4.0 Chromosomal change associated with platinum resistance	104
4.1 Introduction	105
4.2 Affymetrix 10K SNP array	109
4.3 H69 parental cell line	109
4.4 H69CIS200 and H69OX400 resistant cell lines	119
4.5 H69CIS200-S and H69OX400-S sensitive revertant cell lines	124
4.6 Discussion	129
4.6.1 Inherent chromosomal changes in the H69 cells	129
4.6.2 Changes induced by cisplatin and oxaliplatin treatment	131

4.6.3 Changes associated with reversion of resistance	133
4.7 Conclusion	139
Chapter 5.0 Identification of genes associated with platinum resistance and platinum cytotoxicity	141
5.1 Introduction	142
5.2 Atlas toxicology array results	142
5.3 Functional classification of candidate genes	147
5.4 Chromosomal location of candidate genes	149
5.5 Real-time PCR results for candidate genes	151
5.6. Discussion	153
5.6.1 H69 parental cells response to cisplatin and oxaliplatin	162
5.6.2 H69CIS200 and H69OX400 control cells	164
5.6.3 H69CIS200 response to cisplatin and oxaliplatin	166
5.6.4 H69OX400 response to cisplatin and oxaliplatin	168
5.6.5 Consistency between Atlas nylon array and real-time PCR data	169
5.7 Conclusion	169
Chapter 6.0 The inverse relationship between platinum and taxane resistance	171
6.1 Introduction	172
6.2 Analysis of α -tubulin	180
6.2.1 α -tubulin mRNA and protein expression	180
6.2.2 α -tubulin protein soluble to polymerised ratio	183
6.2.3 α -tubulin immunocytochemistry	183
6.3 Analysis of β -actin	187
6.3.1 β -actin mRNA and protein expression	193
6.3.2 β -actin immunocytochemistry	193
6.4 MAP4	193
6.5 RANBP1	198
6.6 Identification of genes associated with PI3K/Akt/mTOR signalling	198
6.6.1 PTPL1	199

6.6.2 IGFBP2	199
6.6.3 FAP48	202
6.7 Response of cell lines to co-treatment with rapamycin	202
6.8 Protein expression of mTOR	204
6.9 Discussion	204
6.9.1 α -tubulin	207
6.9.2 β -actin	208
6.9.3 <i>Vinca</i> alkaloid resistance and taxane sensitivity	209
6.9.4 PI3K/Akt/mTOR signalling	210
6.9.5 PI3K/Akt/mTOR signalling and platinum	212
6.9.6 PI3K/Akt/mTOR signalling and taxanes	214
6.10 Conclusion	215
Chapter 7.0 Glutathione metabolism and platinum resistance	216
7.1 Introduction	217
7.2 mRNA analysis of glutathione related genes	217
7.2.1 Glutathione-S-transferases	217
7.2.1.1 Glutathione-S-transferase theta 1 (GSTT1)	217
7.2.1.2 Glutathione-S-transferase pi 1 (GSTP1)	219
7.2.1.3 Glutathione-S-transferase omega 1 (GSTO1)	219
7.2.2 Glutathione reductase (GSR)	219
7.2.3 Gamma glutamyl cysteine synthetase regulatory and catalytic subunits (γ GCS-Reg and γ GCS-Cat)	220
7.3 γ GCS catalytic subunit protein expression	220
7.4 Thioredoxin analysis	222
7.4.1 Thioredoxin protein expression	224
7.4.2 Thioredoxin immunocytochemistry	224
7.5 Influence of sodium selenite on platinum cytotoxicity	229
7.6 Discussion	229
7.6.1 BSO Resistance	229
7.6.2 Platinum resistance and the glutathione pathway	233
7.6.3 Platinum resistance and the thioredoxin pathway	234

7.6.4 Redox related signalling and platinum resistance	237
7.7 Conclusion	238
Chapter 8.0 DNA repair pathways, cell cycle checkpoints and platinum drug resistance	241
8.1 Introduction	242
8.2 DNA repair	242
8.2.1 MutY homolog (MutY)	242
8.2.2 RAD51 Homolog 2 (RAD51B)	242
8.2.3 Xeroderma pigmentosum group A complementing protein (XPA)	250
8.2.4 DNA polymerase delta catalytic subunit (POLD1)	250
8.2.5 Excision repair deficiency complementation group 1 (ERCC1)	251
8.2.6 MSH2	251
8.2.7 RPS3	253
8.2.8 NM23-H1 (NDK1)	253
8.3 Determination of radiation resistance	255
8.4 Analysis of genes associated with the cell cycle	255
8.5 Discussion	255
8.5.1 Nucleotide excision repair	255
8.5.2 Homologous recombination repair	258
8.5.3 Mismatch repair	260
8.5.4 Base excision repair	261
8.5.5 NM23-H1 (NDK1)	262
8.5.6 Cell cycle checkpoints	263
8.5.7 DNA repair pathways and the G ₁ /S checkpoint	265
8.5.8 Cell cycle genes and the G ₁ /S checkpoint	269
8.5.9 Cross resistance, DNA repair and the G ₁ /S checkpoint	270
8.5.9.1 Topoisomerase inhibitors	270
8.5.9.2 Radiation	272
8.5.9.3 G ₁ /S checkpoint and cross resistance	274
8.5.10 DNA repair pathways and chromosomal stability	274
8.6 Conclusion	276

Chapter 9.0 Conclusions and Future Directions	277
9.1 Conclusions	278
9.2 Future Directions	281
Chapter 10.0 References	285
Appendix 1.0 Journal Publications	323

List of Figures

Chapter 1.0 Introduction	1
Figure 1.1 Cisplatin and oxaliplatin structure and adduct-DNA structure	3
Figure 1.2 Cross resistance between cisplatin and oxaliplatin literature review	6
Figure 1.3 Mechanisms of action of platinum chemotherapeutics	14
Figure 1.4. Mechanisms of resistance to platinum chemotherapeutics	16
Figure 1.5. Glutathione metabolism	19
Figure 1.6. Basic apoptotic pathways	23
Figure 1.7 PI3K/Akt signalling	26
Figure 1.8 Types of platinum adducts	30
Figure 1.9 Nucleotide excision repair	32
Figure 1.10 Double-strand break repair	35
Figure 1.11 Base excision repair	37
Figure 1.12 Mismatch repair and apoptosis	38
Figure 1.13 Mismatch repair	40
Chapter 2.0 Materials and methods	43
Figure 2.1 Glutathione assay standard curve	48
Figure 2.2 Platinum accumulation standard curve	50
Figure 2.3 Genome coverage of the Affymetrix 10K SNP array	54
Figure 2.4 Methodology of the Affymetrix 10K SNP array	55
Figure 2.5 Atlas toxicology 1.2 nylon array	66
Figure 2.6 β -actin standard curve for real-time PCR analysis	71
Figure 2.7 Pierce protein assay standard curve	72
Chapter 3.0 Development of platinum-resistant small cell lung cancer cell lines ...	79
Figure 3.1. Drug treatment regimens and the development of resistance	82
Figure 3.2. The recovery time following each treatment	84
Figure 3.3. Size, morphology and growth rate of resistant cell lines	85
Figure 3.4. Resistance to cisplatin and oxaliplatin following each treatment	86

Figure 3.5. Effect of acute platinum drug treatment on cell growth	88
Figure 3.6. Effect of acute platinum drug treatment on cell cycle	89
Figure 3.7. Effect of dose escalation on cell cycle	90
Figure 3.8. Effect of acute drug treatment on cell cycle at treatment 4	91
Figure 3.9. Resistance and cross resistance of the H69 sublines	92
Figure 3.10. Cellular glutathione	94
Figure 3.11. Effect of glutathione depletion on cell growth and drug resistance	95
Figure 3.12. Platinum accumulation	97
Chapter 4.0 Chromosomal change associated with platinum resistance	104
Figure 4.1 Literature review of chromosomal changes in cisplatin-resistant cell lines ..	107
Figure 4.2 Chromosomal copy number of chromosomes 1, 2, 3 and 4	110
Figure 4.3 Chromosomal copy number of chromosomes 5, 6, 7 and 8	111
Figure 4.4 Chromosomal copy number of chromosomes 9, 10, 11 and 12	112
Figure 4.5 Chromosomal copy number of chromosomes 13, 14, 15 and 16	113
Figure 4.6 Chromosomal copy number of chromosomes 17, 18, 19 and 20	114
Figure 4.7 Chromosomal copy number of chromosomes 21, 22, and X	115
Figure 4.8 Summary of chromosomal copy number changes in the resistant cell lines .	116
Figure 4.9 Karyotype of the H69 SCLC cell line	117
Figure 4.10 H69 metaphase stained with c-myc FISH probe	118
Figure 4.11 H69, H69CIS200 and H69OX400 metaphases stained with 6q15 probe ...	120
Figure 4.12 H69, H69CIS200 and H69OX400 metaphases stained with chromosome 6 paint	122
Figure 4.13 Number of copies of 6p probes in interphase nuclei	123
Figure 4.14 Loss of the platinum-resistant phenotype in the H69CIS200 and H69OX400 cells	125
Figure 4.15 Comparison of chromosomal copy number of chromosomes 1, 6, 10 and 13 before and after the loss of resistance	126
Figure 4.16 Comparison of chromosomal copy number of chromosomes 16, 19, 21 and X before and after the loss of resistance	127
Figure 4.17 Summary of chromosomal copy number changes associated and not associated with resistance	128

Figure 4.18 Additional chromosomal copy number changes associated with the loss of resistance on chromosome 4 and 12	130
Figure 4.19 %GC content maps of chromosomes 1 and 6	134
Figure 4.20 %GC content maps of chromosomes 10 and 13	135
Figure 4.21 %GC content maps of chromosomes 16 and 19	136
Figure 4.22 %GC content maps of chromosomes 21 and X	137
Chapter 5.0 Identification of genes associated with platinum resistance and platinum cytotoxicity	141
Figure 5.1 Atlas toxicology 1.2 nylon arrays	143
Figure 5.2 Flow chart of the selection of candidate genes from Atlas toxicology nylon array data	145
Figure 5.3 Analysis of chromosomal location of candidate genes	150
Figure 5.4 Real-time PCR data	152
Figure 5.5 Biological association network of the H69 parental cell line treated with cisplatin and oxaliplatin	156
Figure 5.6 Biological association network of the H69CIS200 and H69OX400 control cells	157
Figure 5.7 Biological association network of the H69CIS200 cell line treated with cisplatin and oxaliplatin	158
Figure 5.8 Biological association network of the H69OX400 cell line treated with cisplatin and oxaliplatin	159
Chapter 6.0 The inverse relationship between platinum and taxane resistance	171
Figure 6.1 A summary of reported relationships between cisplatin and taxol resistance in cell lines	173
Figure 6.2 mRNA expression data for cytoskeletal genes	181
Figure 6.3 α -tubulin total protein expression determined by Western blot	182
Figure 6.4 α -tubulin soluble and polymerised fractions determined by Western blot ...	184
Figure 6.5 α -tubulin polymerised fractions in response to high dose taxol determined by Western blot	185

Figure 6.6 α -tubulin immunocytochemistry control cells	186
Figure 6.7 α -tubulin immunocytochemistry cisplatin treated cells	188
Figure 6.8 α -tubulin immunocytochemistry oxaliplatin treated cells	189
Figure 6.9 α -tubulin immunocytochemistry low dose taxol treated cells	190
Figure 6.10 Percentage of cells with microtubule bundles in response to 12.5 nM taxol for 24 hours	191
Figure 6.11 α -tubulin immunocytochemistry high dose taxol treated cells	192
Figure 6.12 β -actin protein expression determined by Western blot	194
Figure 6.13 β -actin immunocytochemistry control cells	195
Figure 6.14 β -actin immunocytochemistry cisplatin treated cells	196
Figure 6.15 β -actin immunocytochemistry oxaliplatin treated cells	197
Figure 6.16 Interaction of cisplatin and taxol with mTOR signalling	200
Figure 6.17 mRNA expression data for PI3K/Akt/mTOR signalling related genes	201
Figure 6.18 Cell growth in response to cisplatin, oxaliplatin and taxol in the presence or absence of rapamycin	203
Figure 6.19 mTOR protein expression determined by Western blot	205
Figure 6.20 mTOR signalling in cell growth, proliferation and survival	206
Chapter 7.0 Glutathione metabolism and platinum resistance	216
Figure 7.1 mRNA expression data for glutathione related genes	218
Figure 7.2 γ GCS-catalytic subunit protein expression determined by Western blot	221
Figure 7.3 Homology between γ GCS-Cat and γ GCS-Reg proteins	223
Figure 7.4 Thioredoxin protein expression determined by Western blot	225
Figure 7.5 Thioredoxin immunocytochemistry control cells	226
Figure 7.6 Thioredoxin immunocytochemistry cisplatin treated cells	227
Figure 7.7 Thioredoxin immunocytochemistry oxaliplatin treated cells	228
Figure 7.8 Influence of sodium selenite on platinum cytotoxicity	230
Figure 7.9 Cisplatin metabolism and inhibition of the thiol redox systems	236
Figure 7.10 Regulation of ASK1 kinase activity by cisplatin	239
Chapter 8.0 DNA repair pathways, cell cycle checkpoints and platinum drug resistance	241

Figure 8.1 mRNA expression data for DNA repair genes	243
Figure 8.2 RAD51B protein expression determined by Western blot	245
Figure 8.3 RAD51B immunocytochemistry control cells	246
Figure 8.4 RAD51B immunocytochemistry cisplatin treated cells	247
Figure 8.5 RAD51B immunocytochemistry oxaliplatin treated cells	248
Figure 8.6 Percentage of cells staining positive for RAD51B foci	249
Figure 8.7 ERCC1 protein expression determined by Western blot	252
Figure 8.8 MSH2 total protein expression determined by Western blot	254
Figure 8.9 Radiation resistance in the H69, H69CIS200 and H69OX400 cells	256
Figure 8.10 mRNA expression of p107	257
Figure 8.11 The G1/S cell cycle checkpoint	264
Figure 8.12 Differential expression of genes and proteins reflecting the phase of the cell cycle	267
Figure 8.13 Model of G1/S maintenance phase versus progression to S phase	268
Figure 8.14 DNA repair pathways involved in the repair of the topoisomerase I cleavage complex	271
 Chapter 9.0 Conclusions and Future Directions	 277
 Figure 9.1 Summary of the mechanism of resistance	 282

List of Tables

Chapter 1.0 Introduction	1
Table 1.1 Cell lines with cross resistance to cisplatin and oxaliplatin	7
Table 1.2 Cell lines with acquired resistance to either cisplatin or oxaliplatin and hypersensitivity to the other compound	8
Table 1.3 Cell lines with acquired resistance to either cisplatin or oxaliplatin and are non-cross-resistant to the other compound	8
Table 1.4 Examples of cisplatin-resistant SCLC sublines developed with cisplatin	12
Table 1.5 Changes in apoptotic proteins associated with acquired platinum resistance ..	25
Table 1.6 Properties of DNA polymerases in respect to platinum adduct bypass	28
Chapter 2.0 Materials and methods	43
Table 2.1 Cytotoxic drugs	44
Table 2.2 Atomic absorption furnace operating conditions for platinum	49
Table 2.3 Atomic absorption instrument parameters for platinum	51
Table 2.4 Probes used for FISH	58
Table 2.5 Primers designed for real-time PCR	68
Table 2.6 Antibodies used for Western blotting	76
Table 2.7 Antibodies used for immunocytochemistry	77
Chapter 4.0 Chromosomal change associated with platinum resistance	104
Table 4.1 Cisplatin-resistant cell lines previously analysed for chromosomal changes..	106
Chapter 5.0 Identification of genes associated with platinum resistance and platinum cytotoxicity	141
Table 5.1 Top 40 candidate genes selected from Atlas array data	146
Table 5.2 Analysis of functional classification of candidate genes	148
Table 5.3A Candidate genes function and association with platinum resistance	154

Table 5.3B Candidate genes function and association with platinum resistance	155
Table 5.4A References for biological association network diagrams – Part 1	160
Table 5.4B References for biological association network diagrams – Part 2	161
Chapter 6.0 The inverse relationship between platinum and taxane resistance	171
Table 6.1A Hypersensitive cell lines with resistance to either cisplatin or taxol and hypersensitivity to the other drug – Developed with either cisplatin or taxol	174
Table 6.1B Hypersensitive cell lines with resistance to either cisplatin or taxol and hypersensitivity to the other drug – Developed with other compounds	175
Table 6.2A Non-cross-resistant cell lines showing an inverse relationship between cisplatin and taxol resistance – Developed with cisplatin or taxol	176
Table 6.2B Non-cross-resistant cell lines showing an inverse relationship between cisplatin and taxol resistance – Developed with other compounds	177
Table 6.3 Cross-resistant cell lines resistant to both cisplatin and taxol	178
Chapter 7.0 Glutathione metabolism and platinum resistance	216
Table 7.1 Mechanisms of acquired BSO resistance	231

Journal Publications

- 1) Stordal, B.K., Davey, M.W. and Davey, R.A. (2006). Oxaliplatin induces drug resistance more rapidly than cisplatin in H69 small cell lung cancer cells. *Cancer Chemotherapy and Pharmacology*. 58(2): 256-265.
- 2) Stordal, B., Peters, G. and Davey, R. (2006). Similar chromosomal changes in cisplatin-resistant and oxaliplatin-resistant sublines of the H69 SCLC cell line are not associated with platinum resistance. *Genes, Chromosomes and Cancer*. 45(12):1094-1105.
- 3) Stordal, B., Pavlakis N. and Davey, R. Oxaliplatin for the treatment of cisplatin-resistant cancer: a systematic review. Accepted for publication in *Cancer Treatment Reviews* 23.1.2007

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- 1) Stordal, B.K., Davey, M.W. and Davey, R.A. (2005). Oxaliplatin does not have activity against low-level cisplatin resistance in a lung cancer cell model. American Association for Cancer Research Annual Meeting, Anaheim California, USA. Poster – 1488.
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- 1) Stordal, B.K., Davey, M.W. and Davey, R.A. (2003). Development of platinum drug resistant lung cancer cell lines. UTS/RNSH 20th Scientific Research Meeting. Sydney, Australia. Poster – P36.

2) Stordal, B.K., Davey, M.W. and Davey, R.A. (2004). Comparative study on the development of cisplatin and oxaliplatin resistance in a lung cancer cell model. UTS/RNSH 21st Scientific Research Meeting. Sydney, Australia. Oral – O32.

3) Stordal, B.K, Peters, G., St. Heaps L., Davey M.W. and Davey R.A. (2005). Chromosome rearrangements and platinum drug resistance: Part of the resistance phenotype or “collateral damage”? UTS/RNSH 22nd Scientific Research Meeting. Sydney, Australia. Oral – O17.

4) Stordal B.K. and Davey R.A. (2006). Decreased rather than increased DNA repair proteins in platinum-resistant small cell lung cancer cell lines. UTS/RNSH 23rd Scientific Research Meeting. Sydney, Australia. Oral – O12.

List of Abbreviations

Abbreviation	Full Name
4E-BP	eIF-4E Binding Protein
Acc	Accumulation
ASK1	Apoptosis Signal-regulating Kinase 1
AMPS	Ammonium Persulfate
ATP	Adenosine Triphosphate
AP	Alkaline Phosphatase
bp	base pair
BAN	Biological Association Network
BER	Base Excision Repair
BCIP	5-bromo-4-chloro-3-indolyphosphate
BCNU	1,3-Bis(2-chloroethyl)-1-nitrosourea
BSA	Bovine Serum Albumin
BSO	Buthionine sulfoximine
Car	Carboplatin
cDNA	Complementary DNA
Cis	Cisplatin (<i>cis</i> -diamminedichloroplatinum (II))
DAPI	4',6-diamidino-2-phenylindole, dihydrochloride
dCK	deoxycytidine kinase
DMSO	Dimethylsulphoxide
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
Dox	Doxorubicin
D-PBS	Dulbecco's Phosphate Buffered Saline
DTNB	5,5'-dithio-bis(2-Nitrobenzoic acid)
EDTA	Ethylene diamine tetra acetic acid
EGFR	Epidermal Growth Factor Receptor
EGTA	Ethyleneglycol-bis(β -amino-ethyl ether)N,N'-tetra acetic acid
EtBr	Ethidium bromide
ERCC1	Excision Repair Deficiency Complementing Group 1
FCS	Foetal Calf Serum

FISH	Fluorescent In Situ Hybridisation
FITC	Fluorescein isothiocyanate
Flav	Flavopiridol
FKBP	FK506-binding protein
γ GCS	gamma-Glutamyl Cysteine Synthetase
γ GCS-Cat	γ GCS Catalytic subunit
γ GCS-Reg	γ GCS Regulatory subunit
GPX	Glutathione Peroxidase
GRX	Glutaredoxin
GSH	Glutathione
GSK3	Glycogen synthase kinase 3
GSR	Glutathione Reductase
GST	Glutathione-S-Transferase
γ GT	gamma-Glutamyl Transpeptidase
Gy	Grey – Unit of measurement of radiation
H69	H69 Small Cell Lung Cancer Cell Line
H69CIS200	H69 Cisplatin Resistant Small Cell Lung Cancer Subline
H69OX400	H69 Oxaliplatin Resistant Small Cell Lung Cancer Subline
H69CIS200-S	Sensitive Revertant of H69CIS200
H69OX400-S	Sensitive Revertant of H69OX400
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HRR	Homologous Recombination Repair
IC ₅₀	50% Inhibitory Concentration
IKK	I κ B kinase
JNK	c-Jun N-terminal Kinase
kb	Kilobase
kDa	Kilodaltons
LOH	Loss of Heterozygosity
LRP	Lung Resistance-related Protein (MVP)
NADP	Nicotinamide Adenine Dinucleotide Phosphate
NPC	Nuclear Pore Complex
NSCLC	Non-Small Cell Lung Cancer
MAP kinase	Mitogen Activated Protein kinase

MAP4	Microtubule Associated Protein 4
MMR	Mismatch Repair
mRNA	messenger Ribonucleic Acid
MRP1	Multidrug Resistance-associated Protein 1 (ABCC1)
MRP2	Multidrug Resistance-associated Protein 2 (ABCC2)
MRP4	Multidrug Resistance-associated Protein 4 (ABCC4)
MOPS	3-[N-morpholino]propane-sulphonic acid (free acid)
MSH2	DNA mismatch repair protein 2
mTOR	mammalian Target Of Rapamycin
MTT	3-4,5-dimethylthiazol-2,5-diphenol tetrazolium bromide
MW	Molecular Weight
nd	not determined
NER	Nucleotide Excision Repair
NBT	Nitroblue tetrazolium
Ox	Oxaliplatin
p21	p21 ^{WAF-1/Cip1}
PAGE	Polyacrylamide Gel Electrophoresis
PBS	Phosphate Buffered Saline
PCR	Polymerise Chain Reaction
P-gp	P-glycoprotein (ABCB1)
PI3K	Phosphatidylinositol 3-kinase
PIP3	Phosphatidylinositol-3,4,5-trisphosphate
Pol	Polymerase
POLD1	DNA Polymerase Delta Catalytic Subunit
pRB	Retinoblastoma tumor suppressor gene
Pt	Platinum
RAD51B	DNA Repair Protein Rad51 Homolog 2
RAR	Retinoic Acid Receptor
RNA	Ribonucleic Acid
rRNA	Ribosomal Ribonucleic Acid
RPMI	Culture Media (Roswell Park Memorial Institute)
RNase	Ribonuclease
S6K	ribosomal protein S6 Kinase
SSA	Sulphosalicylic acid

SCLC	Small Cell Lung Cancer
SDS	Sodium Dodecyl Sulphate
SNP	Single Nucleotide Polymorphism
SSC	Sodium Chloride and Sodium Citrate Buffer
Tax	Taxol
TBE	Tris Borate Buffer with EDTA
TBS	Tris Buffered Saline
TE	Tris EDTA Buffer
TEMED	Tetramethylethylenediamine
T _m	Melting Temperature
TNF	Tumour Necrosis Factor
TopoI	Topoisomerase I
TopoII	Topoisomerase II
TRX	Thioredoxin
TrxR	Thioredoxin Reductase
Vin	Vindesine
XPA	Xeroderma Pigmentosum Complementing Protein