

**Characterization of Cancer Gene Mutations in
Human Cancer Cell lines for Correlation with
Drug Activity**

Ogechi Nkemdilim Ikediobi

Clare Hall

September 2007

This dissertation is submitted for the degree of Doctor of Philosophy

DECLARATION:

This dissertation is the result of my own work and includes work done in collaboration where specifically indicated in the text. The dissertation does not exceed the word limit set by the Biology Degree Committee.

Sequencing and mutational analysis of 24 cancer genes in the NCI-60:

The sequencing primers were designed to the 24 cancer genes by the bioinformaticians of the Cancer Genome Project. Optimization of the sequencing primers and preparation of the cell lines for sequencing was performed by the laboratory technicians of the Cancer Genome Project. The sequencing of the 24 genes in the NCI-60 cell lines was done through the Wellcome Trust Sanger Institute sequencing center.

I designed and built the custom *nci60* database to store all sequence variants identified. The perl script, *pass.fail*, was written by myself with help from Steffen Durinck, at the time, a fellow Ph.D. student at the European Bioinformatics Institute.

I identified 117 of the total 156 mutations using Mutation Surveyor v. 2.0 sequence analysis software and manual analysis. The Cancer Genome Project sequence analysis team members, consisting of approximately 24 individuals, identified the remaining 39 mutations using their in-house sequence analysis software (OncoCSA). Sudhir Varma performed the clustering analysis of the U133 expression data on the NCI-60.

Statistical analysis of mutations in cancer genes and drug activity in the NCI-60:

Mark Reimers, statistician at the National Cancer Institute, and Steffen Durinck, a bioinformatician at the National Cancer Institute, performed the statistical analysis of cancer gene mutation in the NCI-60 and activity of 7794 compounds. Paul Blower, a medicinal chemist at Ohio State University, performed the cheminformatic search for phenothiazine compounds tested in the National Cancer Institute, Developmental Therapeutics Program 60 cell line anti-cancer drug screen.

Statistical analysis of phenothiazines' activity in the NCI-60 and validation pharmacology experiments:

I performed additional statistical analyses on BRAF mutation in the NCI-60 and drug activity in the 7794 compounds and led the interpretation of the *in silico* results. I performed all of the subsequent pharmacology experiments, confirming the statistical association between BRAF mutation and phenothiazine activity and validating the predicted phenothiazines' antiproliferative activity in a larger set of cancer cell lines.

ACKNOWLEDGEMENTS

I am grateful to the organizers and founders of the NIH/University of Cambridge Health Sciences Program, Mike Lenardo and Ronald Schwartz, for providing me with the opportunity to obtain a unique research training experience. I would like to thank my NIH supervisor John Weinstein for his support and enthusiasm for the project. I would particularly like to thank my supervisor Mike Stratton for his guidance, support, dedication, and enthusiasm; especially in his assuming responsibility as my Cambridge supervisor at a late stage. I am grateful to my past supervisors: David Bentley and Richard Wooster for their guidance during the early stages of the project. I am thankful to Andy Futreal for his support and advice throughout the project. I am also thankful to the Cancer Genome Project team members for their assistance and timely execution of the sequencing component of the project. I would like to thank the members of the Laboratory of Molecular Pharmacology, especially Uma Shankavaram, Mark Reimers, Bill Reinhold, and Phil Lorenzi for their enthusiasm and support during my transition from Cambridge to NIH. I would also like to thank all of my family members and friends, especially Mama, Daddy, Nneka, Uchenna, Nancy, and Thomas, for their unconditional support and love. I am especially grateful to mijn schatje, Steffen, for his unconditional love, support, and patience during this journey: Ik hou zo veel van jou!

“A word is dead when it is said, some say. I say it just begins to live that day”.

---Emily Dickinson

ABSTRACT

The panel of 60 human cancer cell lines (the NCI-60) assembled by the National Cancer Institute for anticancer drug discovery is a widely used resource. The NCI-60 has been characterized pharmacologically and at the molecular level more extensively than any other set of cell lines. There has not, however, been a systematic sequence analysis of the NCI-60 for key genes causally implicated in oncogenesis. We report the sequence analysis of 24 known cancer genes in the NCI-60 and an assessment of four of the 24 genes for homozygous deletions. Using a pharmacogenomic approach, we have identified an association between mutation in BRAF and the anti-proliferative potential of phenothiazine compounds. Phenothiazine compounds have been used as anti-psychotics and as adjunct anti-emetics during cancer chemotherapy, and more recently reported to have anti-cancer properties. However, to date the phenothiazine anti-cancer mechanism of action has not been elucidated. We demonstrate that BRAF mutation (V600E) in melanoma is predictive of an increased sensitivity to phenothiazines. We also show that RAS mutant and RAS/BRAF wild type melanoma cell lines are approximately two-fold less sensitive to inhibition by phenothiazines than are BRAF mutant melanoma cell lines. This pattern of increased sensitivity to phenothiazines based on the presence of V600E BRAF mutation may be unique to melanomas; we do not observe it in a panel of colorectal cancers. The clinical implications for the use of phenothiazines in the treatment of melanoma, in light of the *in vitro* differential sensitivity between V600E BRAF mutant and RAS mutant melanomas are discussed.

TABLE OF CONTENTS

DECLARATION	2
ACKNOWLEDGEMENTS	4
ABSTRACT	5
TABLE OF CONTENTS	6
1 GENERAL INTRODUCTION	11
1.1 CANCER	11
1.1.1 Epidemiology	11
1.1.2 Multi-stage theory of carcinogenesis	11
1.1.3 A genetic model for colorectal tumorigenesis	13
1.1.4 The hallmarks of cancer	14
1.1.5 Cancer genes	16
1.1.5.1 Definition	16
1.1.5.2 Mutations in cancer genes	16
1.1.5.2.1 Dominant cancer genes	16
1.1.5.2.2 Recessive cancer genes	17
1.1.5.3 Cancer gene census	17
1.1.5.4 Twenty-four cancer genes analyzed	19
1.1.5.4.1 <i>APC</i>	20
1.1.5.4.2 <i>CTNNB1</i>	23

1.1.5.4.3	<i>MADH4</i>	26
1.1.5.4.4	<i>HRAS</i>	28
1.1.5.4.5	<i>KRAS</i>	28
1.1.5.4.6	<i>NRAS</i>	28
1.1.5.4.7	<i>BRAF</i>	35
1.1.5.4.8	<i>PIK3CA</i>	39
1.1.5.4.9	<i>PTEN</i>	41
1.1.5.4.10	<i>ERBB2</i>	45
1.1.5.4.11	<i>EGFR</i>	51
1.1.5.4.12	<i>KIT</i>	56
1.1.5.4.13	<i>PDGFRA</i>	61
1.1.5.4.14	<i>RET</i>	65
1.1.5.4.15	<i>MET</i>	69
1.1.5.4.16	<i>FLT3</i>	73
1.1.5.4.17	<i>MAP2K4</i>	77
1.1.5.4.18	<i>STK11</i>	80
1.1.5.4.19	<i>VHL</i>	84
1.1.5.4.20	<i>CDKN2A</i>	87
1.1.5.4.21	<i>BRCA1</i>	90
1.1.5.4.22	<i>BRCA2</i>	92
1.1.5.4.23	<i>RB1</i>	94
1.1.5.4.24	<i>TP53</i>	97
1.2	PHARMACOGENOMICS	101
1.2.1	Germline variants as predictors of drug response	102
1.2.2	Somatic variants as predictors of drug response	104
1.2.2.1	BCR-ABL, KIT, PDGFRA kinase inhibitors	105
1.2.2.2	ERBB2 kinase inhibitors	110
1.2.2.3	EGFR kinase inhibitors	115
1.3	BRAF as a potential drug target in melanoma	120

1.3.1	BRAF inhibitors	122
1.3.2	MEK inhibitors	123
1.3.3	Heat shock protein (HSP) 90 inhibitors	123
1.3.4	Highthroughput screen for oncogenic BRAF inhibitors	124
1.4	NCI-60 cell lines	125
1.4.1	Major scientific outcomes from the NCI-60 drug screen	126
1.4.1.1	MDR-1 inverse compounds	127
1.4.1.2	L-asparaginase	127
1.4.1.3	MEK inhibitors	128
1.5	Introduction to the thesis project	128
2	MATERIALS AND METHODS	130
2.1	Laboratory Methods	130
2.1.1	Cell culture	130
2.1.2	DNA sequencing	130
2.1.2.1	Cell lines	130
2.1.2.2	Genomic DNA extraction	130
2.1.2.3	Reagents	131
2.1.2.4	Primer design	131
2.1.2.5	PCR	131
2.1.2.6	PCR product sequencing	132
2.1.3	Detection of homozygous deletions	133
2.1.4	Pharmacology	133
2.1.4.1	Cell lines	133
2.1.4.2	Cell plating	134
2.1.4.3	Drug dilutions	134
2.1.4.4	Drug addition	135
2.1.4.5	Proliferation assay	135
2.1.4.6	Data analysis	135
2.2	Bioinformatic Methods	136

2.2.1	Processing of sequence traces	136
2.2.2	Sequence analysis/confirmation sequence variants	137
2.2.3	Storage of sequence variants	138
2.3	Statistical analysis	139
2.3.1	Relationship between mutations and drug activity	139
2.4	Cheminformatic screen of phenothiazine compounds	140
3	DETECTION AND ANALYSIS OF SEQUENCE VARIANTS IN THE NCI-60	
	CELL LINE SET	141
3.1	Introduction	141
3.2	Classification of sequence variants	142
3.3	Results of the mutation analysis of 24 cancer genes	143
3.3.1	<i>APC</i>	143
3.3.2	<i>CTNNB1</i>	149
3.3.3	<i>MADH4</i>	149
3.3.4	<i>HRAS</i>	151
3.3.5	<i>KRAS</i>	152
3.3.6	<i>NRAS</i>	155
3.3.7	<i>BRAF</i>	156
3.3.8	<i>PIK3CA</i>	159
3.3.9	<i>PTEN</i>	160
3.3.10	<i>STK11</i>	164
3.3.11	<i>VHL</i>	166
3.3.12	<i>RB1</i>	167
3.3.13	<i>CDKN2A</i>	168
3.3.14	<i>TP53</i>	170
3.3.15	<i>BRCA2</i>	175
3.3.16	<i>EGFR</i>	177
3.3.17	<i>ERBB2</i>	179
3.3.18	<i>FLT3</i>	180

3.3.19	<i>PDGFRA</i>	180
3.4	Discussion	186
4	RELATIONSHIPS BETWEEN MUTATIONS IN CANCER GENES AND DRUG ACTIVITY	196
4.1	Introduction	196
4.2	Results	196
4.2.1	Selection of compounds	196
4.2.2	Drug-gene relationships	197
4.2.3	Analysis of BRAF mutation and activity of 7794 drugs	203
4.3	Discussion	213
5	EXPERIMENTAL CONFIRMATION AND VALIDATION OF ASSOCIATION BETWEEN PHENOTHIAZINE ACTIVITY AND BRAF MUTATION	217
5.1	Introduction	217
5.2	Results	217
5.2.1	Confirmation of anti proliferative activity of phenothiazine compounds in the NCI-60	217
5.2.2	Validation of differential sensitivity of V600E BRAF mutant and RAS mutant melanoma cell lines	223
5.2.3	Validation of differential sensitivity of V600E BRAF mutant and RAS mutant colorectal cancer cell lines	227
5.3	Discussion	231
6	GENERAL DISCUSSION	241
7	REFERENCES	244