4 RELATIONSHIPS BETWEEN MUTATIONS IN CANCER GENES AND DRUG ACTIVITY IN THE NCI-60 CELL LINES

4.1 Introduction

In the previous chapter I discussed mutations of twenty-four known cancer genes in the NCI-60 cell line set. The major aim of the sequencing study was to define mutation profiles of cancer genes in the NCI-60 and ultimately provide potential genetic markers of differential drug sensitivity between cell lines of the NCI-60. Approximately 100,000 compounds have been screened for anti-cancer activity in the NCI-60 cell lines. The 50-percent growth-inhibitory concentration (GI_{50}) for 42,000 of those compounds has been stored in a public database. In this chapter I discuss the statistical analysis of relationships between mutations in cancer genes and drug activity in the NCI-60 cell lines.

4.2 Results

4.2.1 Selection of compounds

In the first instance I interrogated the relationship between mutations in cancer genes and 7,794 of the 42,000 compounds screened in the NCI-60 and made publicly available. The sub selection of 7,794 compounds was made with the following criteria:

i) The compounds had to be tested at least twice in the NCI-60 cell lines.

ii) All of the compounds had to have GI_{50} values within the range of the dose-response curve for at least 50% of the NCI-60 cell line set.

iii) The standard deviation of -logGI_{50} values of the NCI-60 cell lines had to be equal to or greater than one log unit.

Selecting on the basis of those criteria provides us with a more robust set of data with which to perform my analyses than if I had used the full dataset.

4.2.2 Drug-gene relationships

First, I used a Wilcoxon rank sum test to interrogate the relationship between mutations of the eleven cancer genes (*APC, RB1, KRAS, NRAS, BRAF, PIK3CA, PTEN, STK11, MADH4, TP53, CDKN2A*) with LOMs mutated in three or more cell lines of the NCI-60 and the activity of 7,794 compounds. I also included cell lines with TOVs in those eleven genes. Secondly, I employed a pathway approach to the analysis to enrich for compounds whose activity is associated with mutations of genes in the same pathway. That was done by combining cell lines with mutations of genes involved in the same pathway. For example, to identify active compounds that may act on the WNT pathway, cell lines mutant for *APC* or *CTNNB1* were placed in the same group. I assessed the contributions to differential drug sensitivity of the following mutation combinations: *APC* or *CTNNB1*, *RAS* and/or *BRAF*, *RB1* and/or *CDKN2A*, *RAS* and/or *PIK3CA*, *PIK3CA* and/or *PTEN, RAS and/or PIK3CA and/or PTEN*. In total, therefore, I interrogated the relationship between mutations in eleven individual genes and mutations in six gene combinations and activity of 7794 compounds tested in the NCI-60. The data are presented as the distribution of nominal p-values associated with the activity of 7794 compounds in each gene mutation category (Figure 4-1).

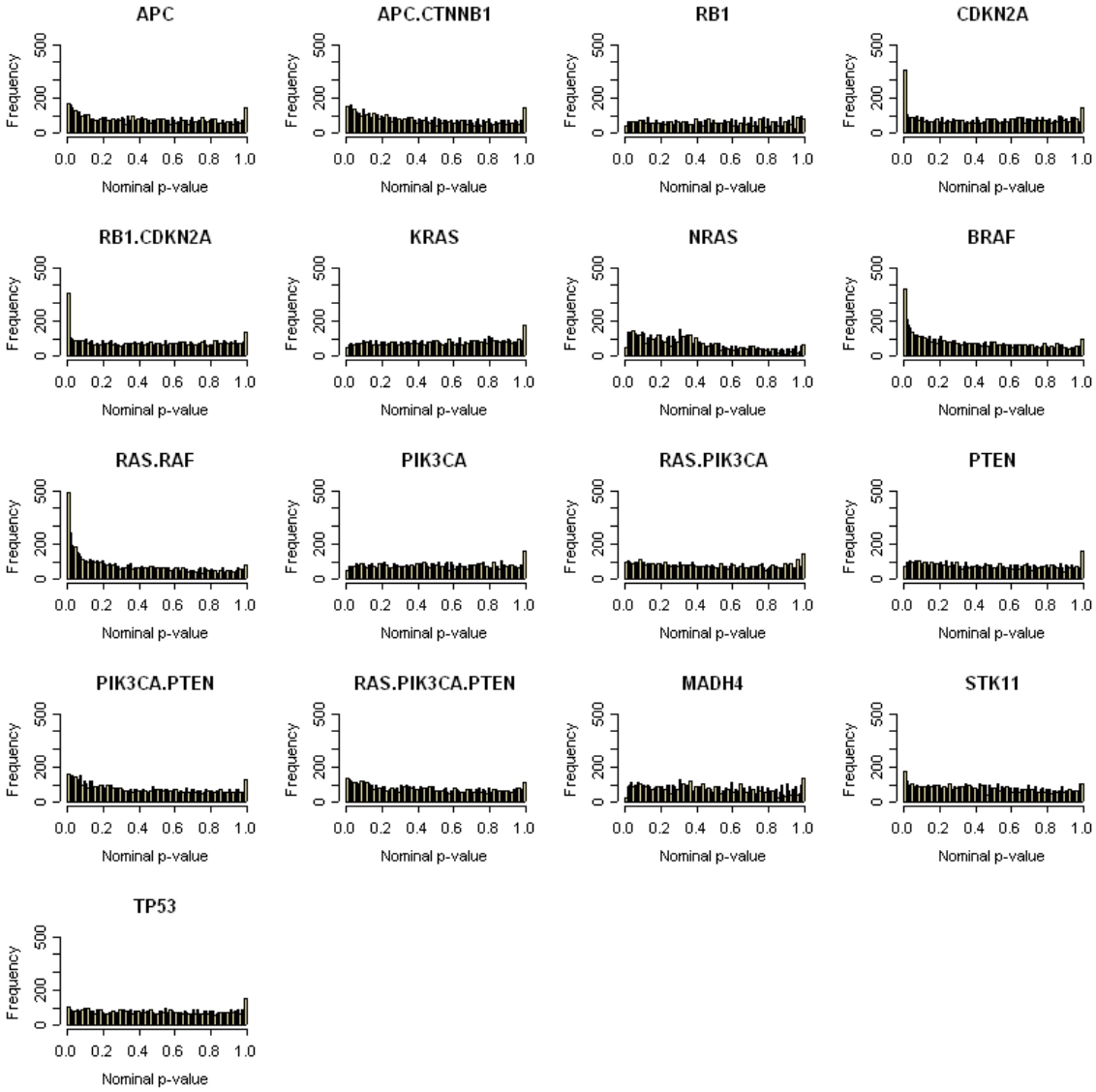

Figure 4-1. Histograms showing the relation between mutations of seventeen gene categories and patterns of growth inhibition in the NCI-60 cell line screen. The parameter calculated for each drug has the form of a Wilcoxon rank sum p-value. P < 0.05 indicates a compound that tends to be active in either wild type or mutant cells for the gene or combination of genes.

After correcting the nominal p-values for testing multiple hypotheses, with a False Discovery Rate (FDR) = 0.25, only four of the seventeen gene /

gene combination categories retained candidate compounds significantly associated (FDR adjusted p-values < 0.05) with mutations in those genes. Controlling the FDR at 0.25 allowed for one out of four apparent drug-gene associations to be false positives. The two most significant gene-drug relationships were found for *CDKN2A* and *BRAF.* No significant associations were detected for *APC, RB1, KRAS, NRAS, TP53, PIK3CA, PTEN, STK11 or MADH4*. Two gene combination pathways, RB1/CDKN2A and RAS/BRAF, showed compounds that were significantly associated with mutation in those genes. Therefore the pathway approach did not yield substantially more information since both pathways were indicated by single genes within them (*CDKN2A* and *BRAF*). Mutations of *APC* independently and mutations of (*APC* or *CTNNB1*) together did not yield compounds that are statistically significantly associated with mutations in those genes.

Similarly, mutations of *PIK3CA* and *PTEN* did not independently yield statistically significant compounds associated with those mutations, nor did combining cell lines with mutations of (*PIK3CA and/or PTEN)* result in the identification of compounds statistically significantly associated with those mutations. In the same vein, combining mutations of (*RAS* or *PIK3CA)* did not yield statistically significant compounds associated with inhibition of the RAS-

PIK3CA pathway. Neither did combining mutations of (*RAS* and/or *PIK3CA* and/or *PTEN)*.

Top ten significant compounds associated with CDKN2A mutation				
NSCID	Chemical Name	Nominal p-value	Adjusted p-value	Mean difference
95382	Camptothecin acetate	2.2E-06	1.2E-02	6.0
651850	No name	6.6E-06	1.2E-02	6.7
645737	No name	8.8E-06	1.2E-02	6.8
653860	No name	1.0E-05	1.2E-02	6.8
99445	Aracytidine 5'-phosphate	1.2E-05	1.2E-02	5.4
644947	No name	1.2E-05	1.2E-02	6.3
63878	Cytarabine hydrochloride	1.2E-05	1.2E-02	5.5
628672	Furo[3',4':6,7]naphtha[2,3-d]-1,3-dioxol-6(5aH)-one, 5,8,8a,9-tetrahydro-5-(4-hydroxy-3,5-dimethoxyphenyl)-9-[(4-nitrophenyl)amino]-	1.3E-05	1.2E-02	6.2
644961	No name	1.8E-05	1.4E-02	4.8
668281	(-)-beta-L-1-[(2-Hydroxymethyl)-1,3-dioxolan-4yl]cytosine	2.0E-05	1.4E-02	5.6

Table 4-1. NSCID, chemical name, Wilcoxon nominal p-values, and FDR adjusted p-values of compounds associated with CDKN2A mutation. Mean difference is calculated by taking the difference between the means of mutant and wild type cells. The same was done for all other tables presented below.

Top ten compounds associated with RB1 and/or CDKN2A mutation				
NSCID	Chemical Name	Nominal p-value	Adjusted p-value	Mean difference
95382	Camptothecin acetate	9.7E-07	5.6E-03	6.0
651850	No name	1.4E-06	5.6E-03	6.7
645737	No name	3.4E-06	9.0E-03	6.8
644961	No name	6.0E-06	1.2E-02	4.8
628670	No name	9.7E-06	1.5E-02	5.8
644947	No name	1.2E-05	1.6E-02	6.4
357885	CI-941	1.8E-05	1.7E-02	7.4
642329	Naphtho[2,3-c]furan-1(9a)-one,3,3a,4,9-tetrahydro-4-(4-fluorophenyl)-6,7-dimethoxy-9-(4-hydroxy-3,5-dimethoxyphenyl)-	1.6E-05	1.7E-02	6.3
295500	1H-Pyrano[3',4':6,7]indolizino[1,2-b]quinoline-4-acetic acid, 3,4,12,14-tetrahydro-4-hydroxy-11-methoxy-3,14-dioxo-,methyl ester	2.1E-05	1.8E-02	6.7
63878	Cytarabine hydrochloride	6.7E-05	1.9E-02	5.4

Table 4-2. NSCID, chemical name, Wilcoxon nominal p-values, and FDR adjusted p-values of compounds associated with RB1 and/or CDKN2A mutation.

I found that the compounds significantly associated with *CDKN2A* mutation became slightly more statistically significant with the addition of cell lines harboring an *RB1* mutation (Table 4-2). Interestingly, the most significant compound associated with mutation of *CDKN2A* independently and mutation of *RB1* and/or *CDKN2A* was camptothecin (NSC 95382), an FDA approved anti-cancer agent whose mechanism of action is inhibition of DNA topoisomerase I (Table 4-1). Cell lines mutant for *CDKN2A* demonstrated increased sensitivity to camptothecin compared to wild type cells.

Top ten compounds associated with BRAF mutation				
NSCID	Chemical Name	Nominal p-value	Adjusted p-value	Mean difference
676879	Phenothiazine, 2-azido-10-[4-(4-methyl-1-piperazinyl)butyl]-difumarate	2.3E-07	1.8E-03	5.6
46061	Phenothiazine, 10-[3-(4-methyl-1-piperazinyl)propyl]-2-(trifluoromethyl)-, disuccinate	5.7E-07	2.2E-03	5.5
658874	No name	4.8E-06	1.2E-02	6.0
644902	Benzo[b]naphtha[2,3-d]furna-6,11-diome, 4-chloro-3-hydroxy	3.3E-05	2.1E-02	5.4
658443	2-Chloro-3-amino-5,8-dihydroxy-1,4-naphthoquinone	1.9E-05	2.1E-02	6.0
661193	Propanamide, 2-[4-[[4-chlorophenyl]carbonyl]-2-chlorophenoxy]-2-methyl-N-[2-(dimethylamino)ethyl]-	1.2E-05	2.1E-02	5.5
664565	No name	2.8E-05	2.1E-02	4.6
678932	1H-Benzimidazole-4-carboxamide, N-[2-(dimethylamino)ethyl]-2-(4-pyridinyl)-, hydrochloride	2.8E-05	2.1E-02	5.7

689620	No name	2.2E-05	2.1E-02	5.6
708550	No name	3.1E-05	2.1E-02	5.5

Table 4-3. NSCID, chemical name, Wilcoxon nominal p-values, and FDR adjusted p-values of compounds associated with BRAF mutation.

Top ten compounds associated with RAS and/or BRAF mutation				
NSCID	Chemical Name	Nominal p-value	Adjusted p-value	Mean difference
717827	No name	6.2E-06	2.2E-02	4.6
46061	Phenothiazine, 10-[3-(4-methyl-1-piperazinyl)propyl]-2-(trifluoromethyl)-, disuccinate	1.2E-05	2.2E-02	5.3
717841	No name	1.3E-05	2.2E-02	4.5
644902	Benzo[b]naphtha[2,3-d]furna-6,11-diome, 4-chloro-3-hydroxy	1.3E-05	2.2E-02	5.4
616511	No name	2.3E-05	2.2E-02	4.3
686411	No name	2.4E-05	2.2E-02	4.2
691207	No name	2.4E-05	2.2E-02	4.2
117274	No name	2.4E-05	2.2E-02	4.7
90829	No name	2.6E-05	2.2E-02	4.6
680094	17-(O-Diethylaminoethyl)oxamino-3-methoxy-1,3,5(10)-estratriene hydrochloride	2.9E-05	2.2E-02	5.8

Table 4-4. NSCID, chemical name, Wilcoxon nominal p-values, and FDR adjusted p-values of compounds associated with RAS and/or BRAF mutation.

BRAF yielded the most statistically significant associations between mutations and drug activity (Table 4-3). In contrast, *KRAS* and *NRAS* did not yield any statistically significant associations between mutations and drug activity. Combining mutations of *BRAF* or *RAS* yielded statistically significant associations between mutation and drug activity. One of the most significant compounds, NSC 46061, identified in RAS-BRAF pathway analysis, was also most associated with *BRAF* mutation (Table 4-4). However, compounds

significantly associated with mutation in *BRAF* became less statistically significant with the addition of cell lines harboring *RAS* mutations.

Although the observation of increased sensitivity of *CDKN2A* and/or *RB1* mutants to camptothecin is interesting, I made a pragmatic decision to follow up on the compounds I identified to be significantly associated with BRAF mutation for the following reasons:

i) The p-values associated with the magnitude of effect of compounds in BRAF mutants are more significant than those of CDKN2A.

ii) The smaller numbers of BRAF mutants and statistically significant p-values indicate greater consistency of the drug-mutation effect.

iii) The appearance of multiple compounds from one chemical or biological class, (phenothiazines and MEK inhibitors), below my threshold of statistical significance (FDR adjusted p-value < 0.05) provides additional circumstantial evidence that the associations may be genuine and therefore merit follow-up.

4.2.3 Analysis of BRAF mutation and activity of 7794 compounds screened in the NCI-60 cell lines

I identified two BRAF mutation types in the NCI-60 cell lines. The predominant one is the V600E BRAF mutation, for which 10 cell lines of the NCI-60 are mutant. One cell line of the NCI-60 harbored a G464V BRAF mutation. For the purposes of the statistical analyses of BRAF mutation and drug activity, I decided it was preferable to include the G464V BRAF mutant cell line in the grouping of BRAF mutants. One could argue that V600E BRAF

mutants are different from the G464V BRAF mutant, as the former increases the kinase activity of the BRAF protein to a greater extent (Davies et al. 2002). However, I found that exclusion of the G464V BRAF mutant from analysis did not make a difference in the ranking of the top two compounds associated with presence of BRAF mutation.

Following statistical analysis of BRAF mutation status and activity of 7794 compounds tested in the NCI-60 cell lines, I identified classes of compounds statistically significantly associated with mutation (Table 4-5). The first class consisted of the phenothiazine compounds exemplified by NSC 676879, NSC 46061, NSC 17474, NSC 676963, NSC 677395, and NSC 674092. The second class consisted of MEK inhibitors exemplified by NSC 706829 and NSC 354462. The third class consisted of the naphthazarins exemplified by NSC 661416 and NSC 661941.

Top 50 statistically significant compounds associated with BRAF mutation				
NSCID	Chemical Name	Nominal p-value	Adjusted p-value	Mean difference
676879	Phenothiazine, 2-azido-10-[4-(4-methyl-1-piperazinyl)butyl]-difumarate	2.3E-07	1.8E-03	5.6
46061	Phenothiazine, 10-[3-(4-methyl-1-piperazinyl)propyl]-2-(trifluoromethyl)-, disuccinate	5.7E-07	2.2E-03	5.5
658874	No name	4.8E-06	1.2E-02	6.0
644902	Benzo[b]naphtha[2,3-d]furna-6,11-diome, 4-chloro-3-hydroxy	3.3E-05	2.1E-02	5.7
708550	No name	3.1E-05	2.1E-02	5.5
661193	Propanamide, 2-[4-[[4-chlorophenyl]carbonyl]-2-chlorophenoxy]-2-methyl-N-[2-(dimethylamino)ethyl]-	1.2E-05	2.1E-02	5.5
678932	1H-Benzimidazole-4-carboxamide, N-[2-(dimethylamino)ethyl]-2-(4-pyridinyl)-, hydrochloride	2.8E-05	2.1E-02	5.7

658443	2-Chloro-3-amino-5,8-dihydroxy-1,4-naphthoquinone	1.9E-05	2.1E-02	5.8
717507	No name	1.4E-05	2.1E-02	4.7
715767	No name	1.9E-05	2.1E-02	5.2
689620	No name	2.2E-05	2.1E-02	5.6
664565	No name	2.8E-05	2.1E-02	4.6
626482	1,5,10-Trihydroxy-7-methoxy-3-methyl-1H-naphtho[2,3-c]pyran-6,9-dione	5.8E-05	2.3E-02	6.3
686324	1-Methyl-3-(4-[2-dimethylaminoethoxy]phenyl)-2-phenylindolizine	6.0E-05	2.3E-02	5.6
17474	Phenothiazine, 10-[3-(4-methyl-1-piperazinyl)propyl]-2-(trifluoromethyl)-dihydrochloride	4.9E-05	2.3E-02	5.6
708551	No name	5.3E-05	2.3E-02	5.2
303612	Mequitazine	6.3E-05	2.3E-02	5.6
718579	No name	5.8E-05	2.3E-02	5.6
676963	3-Azido-10-[4-(4-(4-benzoylphenyl)methyl)-1-piperazinyl)butyl]phenothiazine, bismaleate salt	4.3E-05	2.3E-02	5.6
706829	1,6-Bis[4-(4-aminophenoxy)phenyl]diamantine	5.0E-05	2.3E-02	6.1
715580	No name	5.6E-05	2.3E-02	4.8
661941	2-(3-chloropropyloxy) naphthazarin	7.2E-05	2.5E-02	6.5
661416	2-(2-(2-Methoxyethoxy)ethoxy)naphthazarin	7.5E-05	2.5E-02	6.3
354462	Hypothemycin	9.5E-05	3.0E-02	6.6
635366	No name	1.1E-04	3.3E-02	5.6
707847	No name	1.0E-04	3.3E-02	4.6
676931	1-Amino-2-hydroxy-3-naphthoic acid hydrochloride	1.1E-04	3.3E-02	4.7
708073	No name	1.2E-04	3.5E-02	4.7
677395	2-Azido-10-[(4-dimethylamino)butyl]phenothiazine, oxalate salt	1.4E-04	3.7E-02	5.7
656204	Discorhabdin G	1.5E-04	3.7E-02	5.6
721393	No name	1.5E04	3.7E-02	5.6
699452	No name	1.5E-04	3.7E-02	5.6
627991	Benzo[g]pteridine-2,4-dione,8-chloro-10-(4-chlorophenyl)-3-methyl-	1.6E-05	3.8E-02	5.6
79563	No name	1.6E-05	3.8E-02	4.6
617131	No name	1.7E-05	3.8E-02	5.7
674092	Quinoline-4-carboxamide,N,N'-[(1,4-piperizinediyl)bis(3,1-propanediyl)]bis(2-phenyl-,dihydrochloride	1.7E-05	3.8E-02	5.4
682223	2H-Pyrano[3,2-g]quinoline-5,10-	1.9E-04	4.0E-02	5.7

	dione,4-hydroxy-2,2,6-trimethyl-			
708864	No name	1.9E-04	4.0E-02	4.7
717827	No name	2.0E-04	4.1E-02	4.3
669995	No name	2.1E-04	4.2E-02	5.4
658450	2-Acetamido-6-methyl-8-hydroxy-1,4-naphthaquinone	2.6E-04	4.8E-02	5.6
649750	No name	2.5E-04	4.8E-02	4.7
90829	No name	2.9E-04	5.1E-02	4.8
681603	No name	2.9E-04	5.1E-02	4.7
713546	No name	2.8E-04	5.1E-02	5.5
707452	No name	3.0E-04	5.1E-02	4.7
708075	No name	3.1E-04	5.2E-02	5.6
13028	No name	3.3E-04	5.4E-02	5.6
689078	No name	3.5E-04	5.7E-02	5.5
656211		4.0E-04	6.1E-02	5.6

Table 4-5. NSCID, chemical name, Wilcoxon nominal p-values, and FDR adjusted p-values of the top 50 compounds after statistical analysis of BRAF mutation and activity of 7794 compounds.

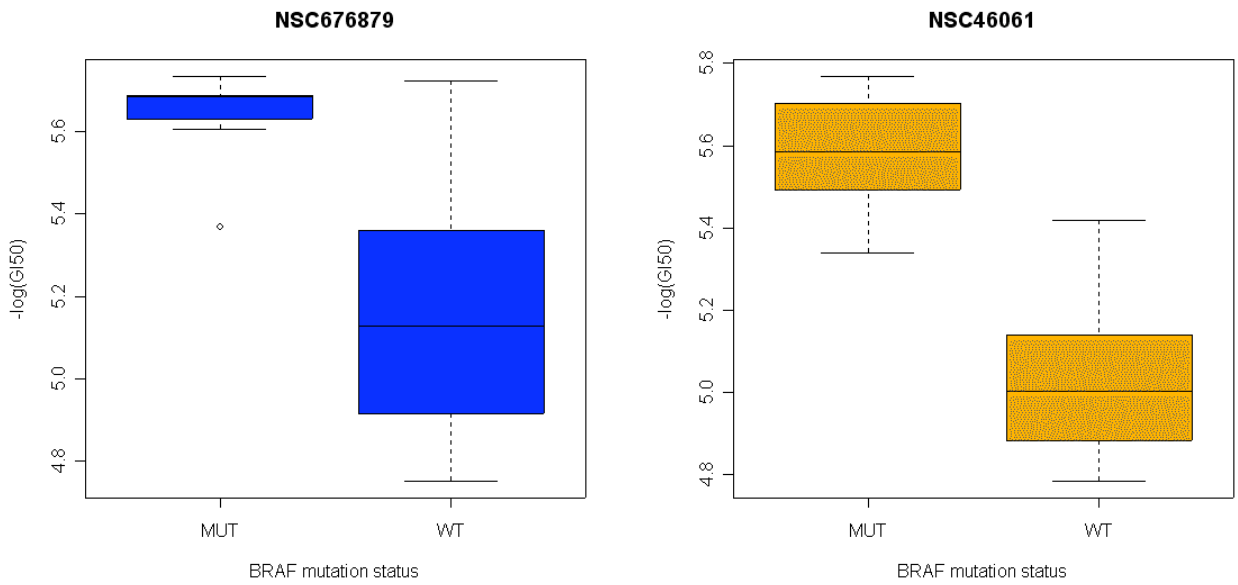

Figure 4-2: Box and whisker plots of the – log_{10} (GI50) values of phenothiazine compounds- NSC 676879 and NSC 46061- tested in BRAF mutant (MUT) and wild type (WT) cell lines of the NCI-60. Larger –log_{10} (GI50) values indicate increased sensitivity to the drug compound.

The results of the NCI-60 screen demonstrate that there is an approximately six-fold difference in the median (-log(GI50)) values between BRAF mutant and wild type cell lines treated with phenothiazine compounds NSC 676879 and NSC 46061 (Figure 4-2). However, the differential sensitivity may be a melanoma-specific phenomenon, as eight of the ten V600E BRAF mutants are melanomas.

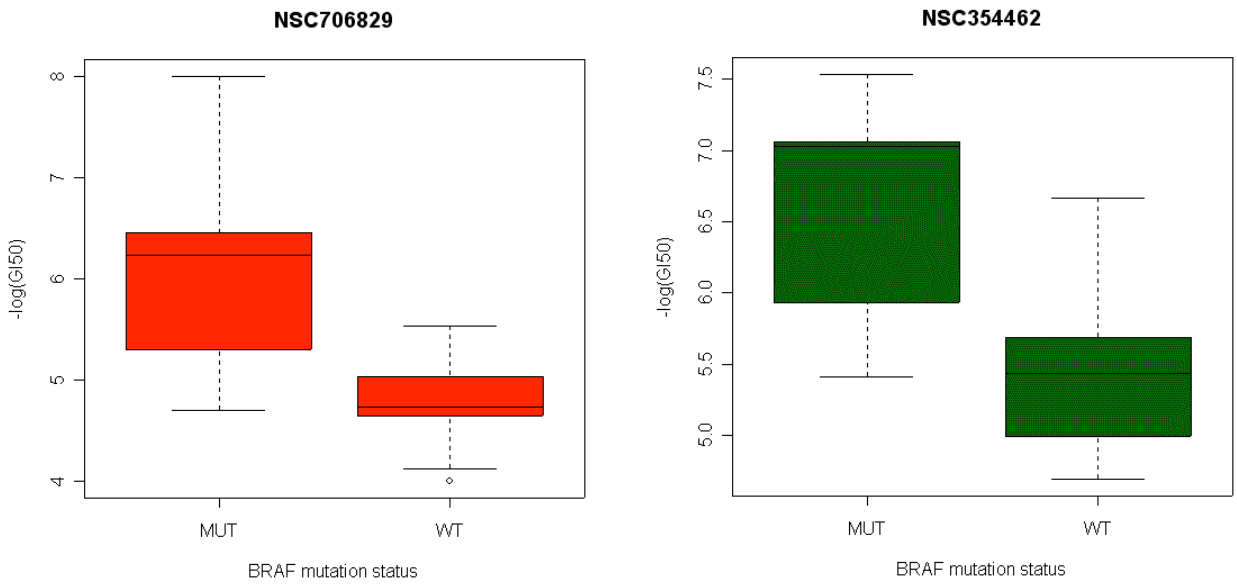

Figure 4-3. Box and whisker plots of the – log (GI50) values of MEK inhibitors-NSC 706829 and NSC 354462- tested in BRAF mutant (MUT) and wild type (WT) cell lines of the NCI-60. Larger –log (GI50) values indicate increased sensitivity to the drug compound. MUT=mutant, WT=wild type.

The results of the NCI-60 cell line screen demonstrate that there is at least a ten-fold difference in the median (-log(GI50)) values between BRAF mutant and wild type cell lines treated with MEK inhibitor compounds, NSC 706829 and NSC 35462 (Figure 4-3). That finding was recently confirmed using a different MEK inhibitor, CI-1040, by *in vitro* cell viability assays (Solit et al. 2006). The effect size of MEK inhibitor activity between BRAF mutant and wild type lines is larger than that of phenothiazine acitivity.

The strongest statistical correlation that I observed was that between the BRAF mutation and activity of phenothiazines. The MEK inhibitor effect

had been reported by others (Solit et al. 2006). Therefore I decided to follow up the association of BRAF mutation with the phenothiazine compounds.

I identified multiple phenothiazine compounds significantly associated with BRAF mutation. Fourteen out of the 7794 compounds are phenothiazines. However, I observed that of the top 50 compounds associated with BRAF mutation, six are phenothiazine compounds, suggesting that phenothiazines as a class may be significantly associated with BRAF mutation.

A display of the GI_{50} measures of the top three (NSC 676879, NSC 46061, NSC 17474) phenothiazine compounds tested in the NCI-60 shows that BRAF mutant cell lines were most sensitive to inhibition (Figures 4-4, 4-5, 4-6). The G464V BRAF mutant line was not as sensitive as the V600E BRAF mutants to inhibition by phenothiazine compounds NSC 676879 and NSC 17474. However the G464V mutant appeared to be as sensitive as the V600E BRAF mutants to inhibition by phenothiazine NSC 46061 (Figure 4-5). I also observed that the melanoma cell line panel was the most sensitive to growth inhibition. There is a strong correlation between melanoma status and BRAF mutation status, with eight of nine melanoma cell lines of the NCI-60 harboring V600E BRAF mutations. Therefore, it is not clear at this stage whether the sensitivity is due to V600E BRAF mutation or melanoma status. However, the only melanoma cell line (SKMEL-2), that is wild type for BRAF (and is a RAS mutant), was less sensitive to all three phenothiazine compounds than were the rest of the melanoma cell lines in the panel.

Furthermore, the tendency of increased sensitivity of BRAF mutant cell lines to phenothiazines was also observed in colorectal cancers. The GI_{50} values of the two V600E BRAF mutant colorectal cancer cell lines, HT29 and COLO205, showed a similar trend of increased sensitivity to phenothiazines. The RAS mutant colorectal cancer cell lines, SW620, HCC-2998, HCT-116 and HCT-15, overall had higher GI_{50} values for the phenothiazines than did the V600E BRAF mutant colorectal lines.

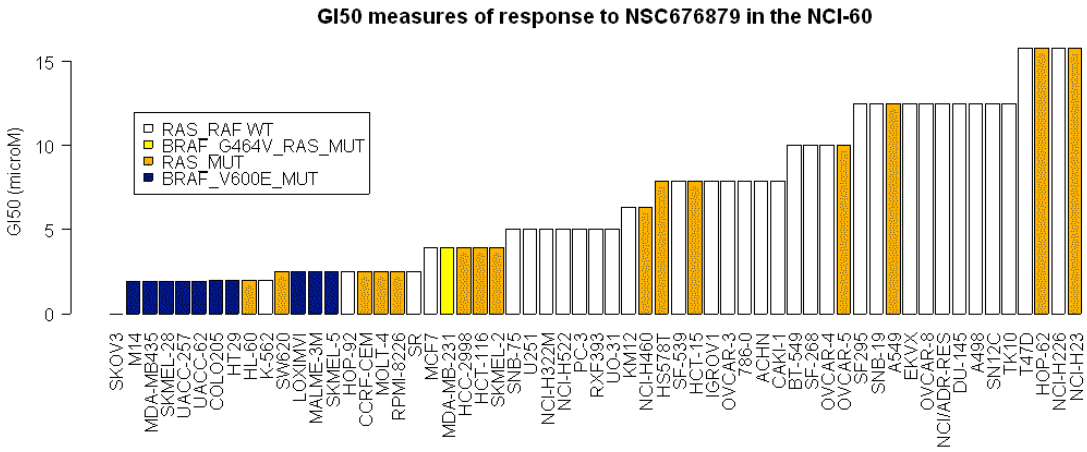

Figure 4-4. GI_{50} values of NSC 676879 in the NCI-60 cell lines. Cell lines are colored according to BRAF and RAS mutation status. SKOV3 was not tested. Results adapted from Developmental Therapeutics Program website (www.dtp.nci.nih.gov)

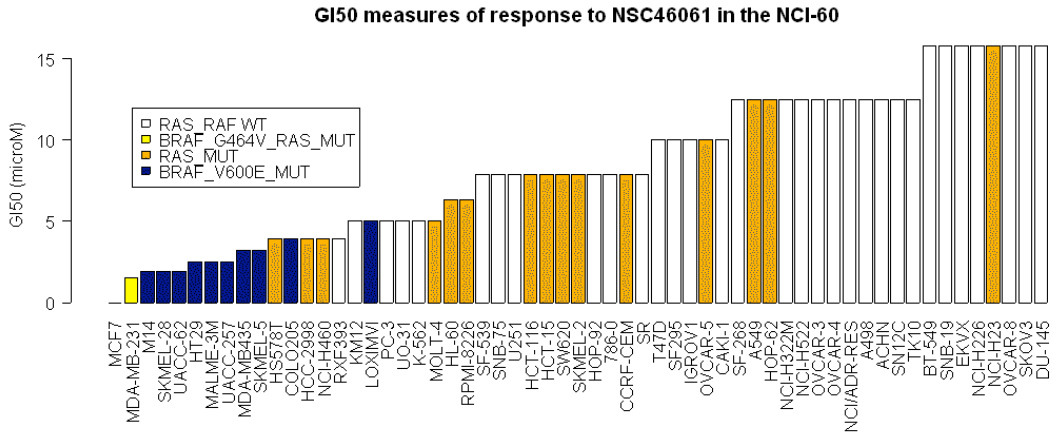

Figure 4-5. GI_{50} values of NSC 46061 in the NCI-60 cell lines. Cell lines are colored according to BRAF and RAS mutation status. MCF7 was not tested. Results adapted from Developmental Therapeutics Program website (www.dtp.nci.nih.gov)

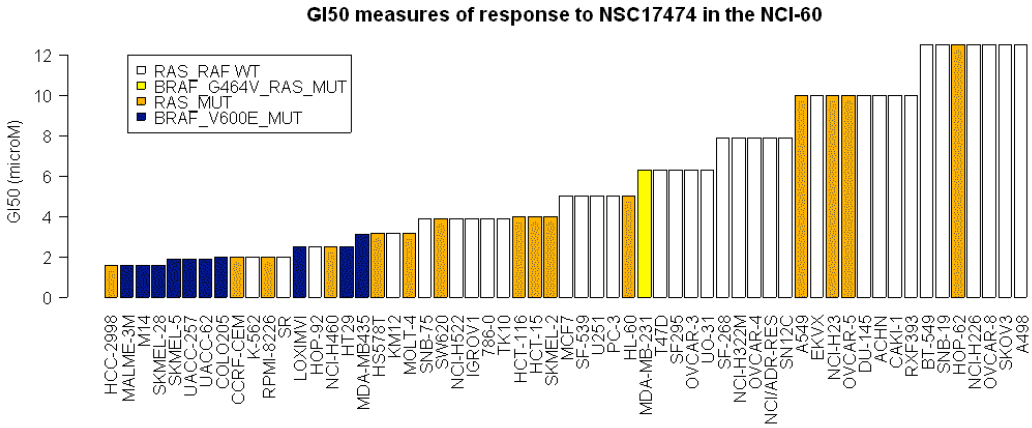

Figure 4-6. GI_{50} values of NSC 17474 in the NCI-60 cell lines. Cell lines are colored according to BRAF and RAS mutation status. Results adapted from Developmental Therapeutics Program website (www.dtp.nci.nih.gov)

The phenothiazines are planar three-ring heterocyclic compounds with the molecular formula- $C_{12}H_9NS$ (Figure 4-7). Phenothiazines fall into three groups: aliphatic, piperidine, piperazine (Figure 4-8). The three groups differ in chemical structure and pharmacological effects. I observed that the top three phenothiazines identified as statistically associated with BRAF mutation, belong to the piperazine group (Figure 4-9). Overall, however, piperazine and aliphatic phenothiazine compounds were represented among the six phenothiazines statistically significantly associated with BRAF mutation.

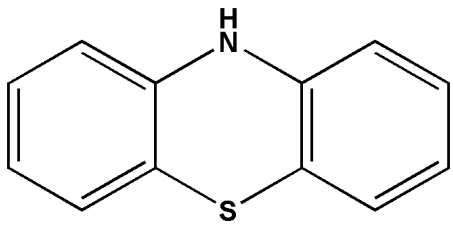

Figure 4-7. General chemical structure of phenothiazines

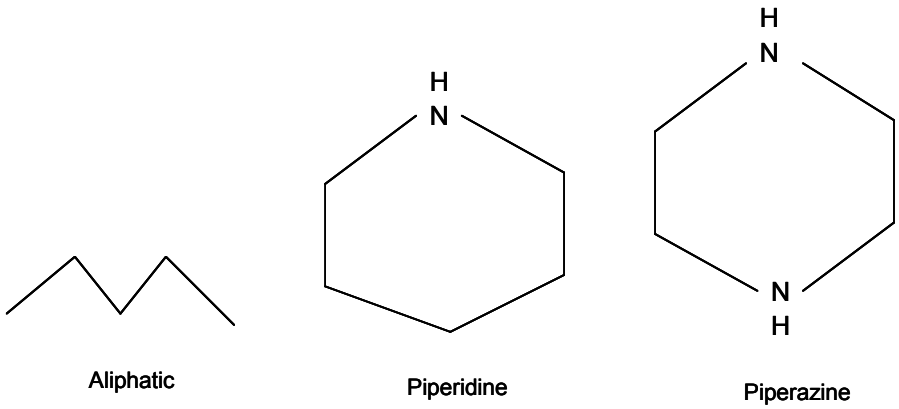

Figure 4-8. General structures of aliphatic, piperidine, and piperazine side chains.

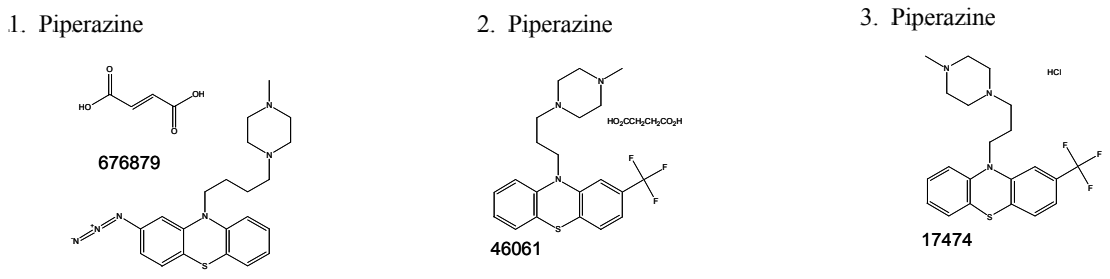

Figure 4-9. Chemical structures of the top three phenothiazines identified as statistically associated with BRAF mutation. Compounds are shown in descending order of statistical significance and labeled to show which phenothiazine structural class they belong to.

4.3 Discussion

I have identified compounds for which sensitivity of response is statistically significantly associated with mutations of *CDKN2A* and *BRAF,* and of combinations of genes representing pathways in which these genes reside *(RB1* and/or *CDKN2A),* and *(RAS* and/or *BRAF).* However, I did not identify compounds statistically significantly (FDR adjusted p-value < 0.05) associated with the remaining thirteen gene mutation categories. In some cases, (e.g., with mutations of *APC, RB1, NRAS, STK11*, and *MADH4)* perhaps I lack the statistical power to make adequate comparisons between mutant and wild type response to compounds. There is, however, reasonable statistical power for *CDKN2A* and *TP53* and I do find some compounds statistically significantly associated with mutation of *CDKN2A*. However, I do not find any compounds statistically significantly associated with mutation of *TP53*.

I hypothesized that grouping cell lines with mutation of genes in the same pathway may aid in identifying compounds acting on that particular pathway. However, applying this approach to the analysis of the APC-CTNNB1, RAS-PIK3CA, PIK3CA-PTEN, and RAS-PIK3CA-PTEN pathways, did not yield more statistically significant associations between mutation in those gene combinations and drug activity. I did, however, identify statistically significant associations between pathway mutations of RB1 and/or CDKN2A and mutations of RAS and/or BRAF that had previously been identified through analysis of CDKN2A and BRAF independently.

The lack of effectiveness of the pathway approach for RAS and BRAF deserves particular note. There is evidence that RAS can signal through BRAF. That might lead us to expect that compounds showing particular effectiveness in cell lines with BRAF mutations might show similar effectiveness in RAS mutant lines. That does not seem to be the case, either for RAS mutant lines separately or combined with BRAF. The reasons are unclear. It may be that there are several compensatory outlets of mutant RAS signaling and abrogation of the MEK-ERK-MAPKinase pathway therefore has little effect. It may also be that mutant RAS predominantly signals through CRAF and that the compounds that have an effect with BRAF mutations do not influence pathways modulated by CRAF.

BRAF yielded the most statistically significant associations between mutations and drug activity. Phenothiazine compound NSC 676879 was the most significant compound associated with mutation of *BRAF*. The activity

pattern of three phenothiazine compounds in the NCI-60 showed that BRAF mutant cell lines were more sensitive to growth inhibition than the RAS mutant and BRAF wild type cell lines. I therefore prioritized the statistical association between increased phenothiazine activity and presence of BRAF mutation for follow-up experimental studies.

In a broader sense, my analysis demonstrates the difficulty of finding associations between molecular genetic profiles in cancers and drug activity. A limitation of my study, in some cases, was a lack of statistical power to make associations between mutation of cancer gene(s) and drug activity. However, in some instances where I had reasonable statistical power to detect associations I did not identify differential activity of compounds between the mutant and wild type cells. Those results could be a function of the set-up of the drug screen. Perhaps a GI_{50} value may not be an appropriate measure of drug activity for all compounds. Another statistical limitation of the screen is the fact that the drug set and cell lines do not represent samples drawn at random from assumed underlying populations. In the same vein, perhaps expanding the number of cell lines in each tissue category of the NCI-60 screening panel would aid in identifying more associations between mutation and drug activity. There are only nine tissue types represented in the NCI-60 panel. In addition to increasing the numbers of cell lines in each tissue panel, adding more tissue types to the panel might increase the yield of drug-gene associations.

Another limitation is that I have sequenced only 24 cancer genes. Perhaps mutation analysis of more genes in the NCI-60 would identify compounds associated with those mutations. My results may also point to a fact that a majority of differential drug sensitivities will not be due to a single mutated gene product or even a combination of two or three mutated gene products. For example, perhaps the biological complexity of the TP53 mutation and the myriad interactions it is involved in makes it difficult to assume that cells mutant for TP53 are similar genetically. Perhaps different types of TP53 mutations confer a particular genetic profile. Therefore, I may be losing possible drug effects by combining all TP53 mutations into the same group. Nevertheless, the analysis presented here of the relationship between mutations of cancer genes and drug activity is a step toward empowering molecularly targeted drug screens.