# Mutation analysis of $\mathbf{2 4}$ known cancer genes in the NCI-60 cell line set 

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#### 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. However, no systematic mutation analysis of genes causally implicated in oncogenesis has been reported. This study reports the sequence analysis of 24 known cancer genes in the NCI-60 and an assessment of 4 of the 24 genes for homozygous deletions. One hundred thirty-seven oncogenic mutations were identified in 14 (APC, BRAF, CDKN2, CTNNB1, HRAS, KRAS, NRAS, SMAD4, PIK3CA, PTEN, RB1, STK11, TP53, and VHL) of the 24 genes. All lines have at least one mutation among the cancer genes examined, with most lines ( $73 \%$ ) having more than one. Identification of those cancer genes mutated in the NCI-60, in combination with pharmacologic and molecular profiles of the cells, will allow for more informed interpretation of anticancer agent screening and will enhance the use of the NCI-60 cell lines for molecularly targeted screens.


## Introduction

The NCI-60 cell lines were assembled by the National Cancer Institute as an in vitro anticancer drug screen (1-3), which went into operation in 1990. The panel comprises 60 human cancer cell lines representing nine tissue of origin types: breast, colon, central nervous system, renal, lung, melanoma, ovarian, prostate, and hematogenous. More than 100,000 compounds have been screened for anticancer activity against the NCI-60 (chemosensitivity profiles of the NCI-60 cell lines ${ }^{4}$ and more refined but less extensive

[^0]activity data sets ${ }^{5}$ can be accessed online). The resulting data have proved rich in information about the mechanisms of action and resistance of those compounds (4-6). The cells have also been profiled more extensively at the DNA, RNA, protein, chromosomal, and functional levels than any other set of cells (7). For example, DNA copy number changes have been assessed by array-based comparative genomic hybridization $(8,9)$ and chromosomal aberrations have been catalogued by spectral karyotyping (10). At the DNA sequence level, five known cancer genes have previously been analyzed: $T P 53$ (11), $K R A S$, $N R A S$, and $H R A S$ (12), and PIK3CA (13). RNA expression has been studied on various array-based platforms, and protein expression has been analyzed by two-dimensional gel electrophoresis and by reverse-phase lysate array (7). The various data are being integrated and analyzed, resulting in several leads with possible therapeutic implications (14, 14a). This article and two others in the current issue (14a, 14b) inaugurate Molecular Cancer Therapeutics' "Spotlight on Molecular Profiling" series. The data sets have been incorporated into "CellMiner," a searchable relational database for integrative analysis. ${ }^{5}$

Genes encoding protein kinase domains are the most frequently mutated in human cancer (15) and are tractable candidates for therapeutic intervention. The kinase inhibitor imatinib was developed to target the BCR-ABL tyrosine kinase fusion protein in treatment of chronic myelogenous leukemia (16). Response to two other kinase inhibitors, gefitinib and erlotinib, has been linked to activating mutations in the epidermal growth factor receptor ( $E G F R$ ) gene in patients with lung adenocarcinoma (17). There are also promising results from kinase inhibitors targeting the FLT3 tyrosine kinase receptor in acute myelogenous leukemia (18), in which the gene is frequently mutationally activated. In each of those examples, the acquired mutation renders the cancer cells carrying it more sensitive to the inhibitor. In addition, we have previously identified frequent mutations of the $B R A F$ kinase gene in malignant melanoma and other cancers (19), providing the impetus to pursue development of small-molecule inhibitors (20). With respect to nonkinase genes, restoration of wild-type tumor suppressor function is being investigated, as exemplified by the recent use of smallmolecule inhibitors of MDM2, a negative modulator of the transcriptional activity and stability of TP53, to restore function to the TP53 pathway (21). However, restoration of tumor suppressor gene function when the gene is inactivated through mutation remains very challenging. It is therefore becoming increasingly clear that understanding the genetics of cancer is key to the further development of targeted therapeutics. Hence, characterization of the genetic abnormalities found in the NCI-60 panel will improve its potential for use in the discovery of new therapies.

Although cancer cell lines are limited, in some instances, with respect to representation of the histopathologic diversity of any given cancer type and may have acquired further genetic events in vitro, they are mainstays in drug development programs. As a component of the large-scale systematic sequencing studies being carried out to identify mutations in human cancer by the Wellcome Trust Sanger Institute Cancer Genome Project, we report here the results of sequencing the NCI-60 cell lines for the coding exons and splice junctions of 24 genes causally implicated in oncogenesis. We also report assessment of 4 of the 24 genes for homozygous deletions.

## Materials and Methods

## Cell Lines

Fifty-nine of the 60 NCI-60 cell lines were kindly provided by the Developmental Therapeutics Program at the National Cancer Institute (Bethesda, MD; Table 1). MDA-N, an

[^1]ERBB2 transfectant of MDA-MB-435, was not available at the time of the study because its use was 'restricted' according to the Developmental Therapeutics Program. The cells were cultured in RPMI 1640 supplemented with $10 \%$ fetal bovine serum and $5 \mathrm{mmol} / \mathrm{L}_{\mathrm{L}}$ glutamine. Genomic DNA was extracted using the Qiagen (Hilden, Germany) genomic DNA purification kit.

## PCR and Sequencing

PCR primers were designed to amplify the exons and flanking intronic sequences of the 24 cancer genes (Table 2). PCR products were $\sim 500 \mathrm{bp}$ in length, with multiple overlapping amplimers for larger exons (Supplementary Table S1). ${ }^{6}$ In total, the coding sequences of the 24 genes covered $\sim 70 \mathrm{~kb}$ with PCR amplimers successfully designed for, and sequencing attempted on, 66 kb of the total. PCR amplification of genomic DNA templates and direct sequencing were done as described previously (22).

## Sequence Analysis and Confirmation of Putative Variants

Sequence traces were analyzed using a combination of software (Mutation Surveyor and inhouse bespoke software) and manual analysis. All putative disease-causing mutations were confirmed by bidirectional sequencing of a second independently amplified PCR product.

## Classification of Sequencing Results

The 24 genes screened are commonly mutated in cancer through small intragenic somatic mutations or somatic homozygous deletions ${ }^{7}$ or represent plausible drug targets. There are no matched normal DNA samples for the NCI-60 with which to determine the somatic or germ-line nature of the observed variants. We have classified sequence variants into four strata: likely oncogenic mutations, tentative oncogenic variants, variants of unknown significance, and single-nucleotide polymorphisms (SNP). For designation as likely oncogenic mutations, conservative criteria were applied; only those sequence changes that had previously been shown to be somatic mutations in human cancer and/or those consistent with the position and type of mutations for a given gene were included. This class also included homozygous deletions in tumor suppressor genes. Tentative oncogenic variants were those which, though located similarly to known cancer mutations, are different from those previously reported or are present as heterozygous variants in tumor suppressor genes other than missense mutations in TP53. All other sequence changes were deemed variants of unknown significance if they were not clearly previously reported SNPs.

## Detection of Homozygous Deletions

Exon deletions in CDKN2A, PTEN, RB1, and SMAD4 (MADH4) were identified by multiplex PCR. Briefly, PCR primers were designed to amplify exons 1,2 , and 3 of $C D K N 2 A$ together with exon 1 of $A R F$, all 9 exons of PTEN, 27 exons of $R B 1$, and exons 1 and 3 to 13 of $M A D H 4$. Control PCR amplimers were designed for $\beta$-actin and random intergenic genomic sequences (Supplementary Table S2). ${ }^{6}$ PCR products were resolved on $2 \%$ agarose gels. All multiplex PCR experiments were done in duplicate.

## SNP Genotyping

Cell lines were genotyped for $\sim 10,000$ single SNPs using the Affymetrix (Santa Clara, CA) 10 K SNP array as described previously (23). The genotype of each cell line was compared with those of the other NCI-60 lines, and a percentage identity score was calculated for each pair of genotypes. ${ }^{8}$

[^2]
## Results/Discussion

More than 60 genes are causally implicated in cancer through the acquisition of somatic small intragenic mutations (15). Twenty-four of those genes were selected for sequence analysis based on mutation frequency and biological interest. In total, 3.9 Mbp of sequence were screened in the 24 genes. Four of the genes are also known to be inactivated frequently by homozygous deletions (CDKN2A, $73 \%$; RB1, 13\%; SMAD4, 48\%; and PTEN, 35\%). ${ }^{9}$ Therefore, those four genes were also assessed for homozygous deletions. Taking into account point mutations, small insertions/deletions, and homozygous deletions, 14 of 24 cancer genes were found to have likely oncogenic mutations in at least one cell line ( $A P C$, BRAF, CDKN2, CTNNB1, HRAS, KRAS, NRAS, SMAD4, PIK3CA, PTEN, RB1, STK11, $T P 53$, and $V H L$ ). Without matched normal DNA from the same individuals, it was not possible to ascertain whether the mutations were somatic, although it is likely that the majority are of somatic origin.

A total of 137 oncogenic mutations were found in the 14 genes (Table 3). ${ }^{10}$ TP53, the gene most commonly mutated in cancer, had likely oncogenic mutations in $64 \%$ ( 38 of 59 ) of the cell lines (Table 3). Included was the previously reported large homozygous deletion in HL-60 (24) confirmed via genomic PCR (data not shown). CDKN2A single-exon or multiple-exon deletions/point mutations were observed in $56 \%$ ( 33 of 59) of the NCI-60 cell lines. Conversely, mutations were detected only once each in the $H R A S$ and $C T N N B 1$ genes. The number of analyzed cancer genes with likely oncogenic mutations ranged from five in the microsatellite-stable colorectal cancer line HT-29 (APC, BRAF, SMAD4, PIK3CA, and TP53) to one (TP53) in several other lines: the ovarian cancer cell lines OVCAR-3 and OVCAR-4, the lung adenocarcinoma line NCI-H522, and the glioma lines SN12C and SNB-75.

Previously published data on mutations in KRAS, NRAS, HRAS (12), and PIK3CA (13) for the NCI-60 cell lines are consistent with those in this study. However, with respect to the previously published TP53 sequence analysis by O'Connor (11), we obtained different results for 9 of the 59 cell lines. Some are annotation differences in the TP53 data: HS578T has a p.V157F mutation here but p.D157E reported, RPMI-8226 is p.E285K here but has a previous annotation of p.E285L, and SK-MEL-28 is p.L145R here rather than p.C145V (7). In addition, in our analysis, MOLT-4 has a heterozygous TP53 nonsense mutation (p.R306X) in genomic DNA but no detectable mutation at the cDNA level in the previous study. It is plausible that the mutant TP53 transcript in MOLT-4 undergoes nonsensemediated decay and therefore is not detectable in cDNA.

An additional 19 tentative oncogenic variants were identified, including missense variants in the receptor tyrosine kinase genes $E G F R, E R B B 2$, and $F L T 3$. In addition, a putative splicing mutation in PDGFRA was identified in the chronic myelogenous leukemia line K562. The remainder of this class consisted of heterozygous frameshift mutations in tumor suppressor genes found primarily in microsatellite-unstable lines. ${ }^{11}$ Of particular interest among these were two different heterozygous frameshift mutations in BRCA2 in the HCT-15 colorectal cancer cell line. BRCA2 has not been previously reported to be a target for mutation in microsatellite-unstable cancers. Also included in this category are three heterozygous TP53 truncating variants and a heterozygous truncating $A P C$ variant in the KM12 colorectal line. It is likely that a substantial proportion of these heterozygous truncating tumor suppressor

[^3]gene variants are actually disease causing and that the second allele of the tumor suppressor gene has been inactivated in accordance with the two-hit genetic model. It is possible, for example, that alterations in the second allele have not been detected, given the classes of genetic change that we have not directly addressed (e.g., large rearrangements and promoter methylation) and the fact that it was not possible to sequence every exon in every cell line. All additional data on variants of unknown significance and single SNPs are available in Supplementary Table S3 ${ }^{6}$ and online. ${ }^{12}$

Based on the resequencing results and genotyping data we generated using Affymetrix 10K SNP Mapping Arrays, there are three pairs among the 59 cell lines analyzed that seem to be derived from the same individuals. ${ }^{13}$ Thus, a total of 56 independent cell lines is analyzed in this report. The synonymous pairs are the following: (a) "breast" cancer line NCI/ADR-RES and the ovarian cancer OVCAR-8 (9), which have identical TP53 and ERBB2 variants and $99 \%$ genotype similarity; (b) the melanoma line M14 and the "breast" cancer line MDA-MB-435, which have identical BRAF, CDKN2A, and TP53 variants and $97 \%$ genotype similarity, with this combination of mutations strongly indicating that MDA-MB-435 is a melanoma (25); and (c) two glioma lines SNB-19 and U251, which have identical TP53, CDKN2A, and PTEN variants and 96\% genotype similarity. There is evidence that, though identical at the mutation and genotypic level, those two lines have diverged karyotypically (10).

With respect to the use of the NCI-60 for informing on commonly mutated cancer genes as drug targets, $>50 \%$ of the NCI-60 are TP53 mutant. Whereas restoring p53 pathway function in TP53 wild-type cancer cells continues to be a focus of intensive drug development efforts (26), restoring TP53 function in cells with mutant TP53 remains challenging. The NCI-60 lines also reflect the mutation patterns seen in the $K R A S$ and $N R A S$ genes in primary tumors. To date, direct inhibition of activated RAS and hence its downstream effectors has not been effective in cancer therapy. Downstream of RAS there are several BRAF mutations in the NCI-60. Recently, the BRAF mutant lines of the NCI-60 have been found to be sensitive to kinase inhibitors of the downstream $B R A F$ effector/signaling target mitogenactivated protein/extracellular signal-regulated kinase kinase (14). The mutations in PIK3CA and PTEN suggest that the panel may be of value for analysis of compounds that target the phosphatidylinositol 3-kinase pathway. PIK3CA, a lipid kinase, is a clear target for therapeutic development $(13,27)$.

Other likely oncogenic mutations of interest included a homozygous STK11 (LKB1) 5-bp deletion in the DU145 prostate cancer cell. Although previous work has implicated STK11 inactivation in non-small cell lung cancer (28), to our knowledge this is the first report of a mutation in STK11 in prostate cancer. It will be of interest to extend that observation to a set of primary prostate cancers to determine the prevalence of STK11 inactivation in this common tumor type.

The receptor tyrosine kinases are perhaps the most successfully exploited set of molecular targets in cancer to date. Several family members (EGFR, ERBB2, FLT3, KIT, MET, and $P D G F R A$ ) were included in the set of 24 genes assessed. No mutations identical to those most frequently reported (17) were seen. However, several interesting variants were identified. In $E G F R$, two amino acid substitutions, p.P753S in the SK-MEL-28 melanoma and p.T751I in the RPMI-8226 myeloma line, were identified within the region of the kinase domain frequently affected by in-frame deletions. Both residues are subject to missense substitution as part of more complex deletion/substitution mutations in lung

[^4]adenocarcinoma. ${ }^{14}$ Further investigation of those lines for sensitivity to EGFR inhibitors and the potential role of $E G F R$ mutations in a subset of melanoma and myeloma are warranted. An $E R B B 2$ p.G776V variant was detected in the ovarian cancer line OVCAR-8 (and NCI/ADR-RES). Gly ${ }^{776}$ and the adjacent $\mathrm{Val}^{777}$ are somatically mutated in gastric, lung, and colon cancers $(29,30)$. Recently, an ERBB2 Gly ${ }^{776}$ mutant non-small cell lung cancer cell line and transformed mouse cells were shown to exhibit in vitro sensitivity to a small-molecule ERBB2 kinase inhibitor (31), and there has been a report of clinical response to trastuzumab in a patient with $E R B B 2$ mutant lung cancer refractory to other treatments (32). Finally, a p.A627T variant in FLT3 was detected in the CCRF-CEM acute lymphoblastic leukemia cell line. Ala ${ }^{627}$ is just adjacent to the G-loop ATP-binding motif within the kinase domain and is very highly conserved. Internal tandem duplications and point mutations of FLT3 are frequent in acute myelogenous leukemia. ${ }^{9}$

Sequencing over 1 Mb /case of coding sequence in a series of primary tumors suggests that there are tens of amino acid-changing somatic mutations in most tumors spread across thousands of genes (22). Therefore, every tumor is likely to have a unique somatic mutation pattern in addition to any germ-line variation. Hence, the NCI-60 panel can only contain a small subset of the gene/mutation combinations found in primary tumors. We are continuing our systematic analysis of known cancer genes for mutations in the NCI-60 panel. The work presented here defining the mutation profiles of 24 known cancer genes in the NCI- 60 will inform drug development programs and contribute to the growing amount of molecular data on the NCI-60. The data can be analyzed and combined to identify active compounds for further investigation and potential development.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Table 1

NCI-60 cell lines

| Cell line | Tumor type |
| :---: | :---: |
| 786-0 | renal cell carcinoma |
| A498 | renal cell carcinoma |
| A549 | lung carcinoma |
| ACHN | renal cell carcinoma |
| BT-549 | breast carcinoma |
| CAKI-1 | renal cell carcinoma |
| CCRF-CEM | acute lymphoblastic leukaemia |
| COLO205 | colorectal carcinoma |
| DU-145 | prostate carcinoma |
| EKVX | lung adenocarcinoma |
| HCC-2998 | colorectal carcinoma |
| HCT-116 | colorectal carcinoma |
| HCT-15 | colorectal carcinoma |
| HL-60 | acute myeloid leukaemia |
| HOP62 | lung adenocarcinoma |
| HOP-92 | lung large cell carcinoma |
| HS578T | breast carcinoma |
| HT29 | colorectal carcinoma |
| IGROV1 | ovarian carcinoma |
| K-562 | chronic myeloid leukaemia |
| KM12 | colorectal carcinoma |
| LOXIMVI | melanoma |
| M14 | melanoma |
| MALME-3M | melanoma |
| MCF7 | breast carcinoma |
| MDA-MB-231 | breast carcinoma |
| MDA-MB-435 | melanoma (see text) |
| MOLT-4 | acute lymphoblastic leukaemia |
| NCI/ADR-RES | ovarian carcinoma (see text) |
| NCI-H226 | lung squamous cell carcinoma |
| NCI-H23 | lung adenocarcinoma |
| NCI-H322M | lung bronchoalveolar carcinoma |
| NCI-H460 | lung large cell carcinoma |
| NCI-H522 | lung adenocarcinoma |
| OVCAR3 | ovarian carcinoma |
| OVCAR-4 | ovarian carcinoma |
| OVCAR-5 | ovarian carcinoma |
| OVCAR-8 | ovarian carcinoma |
| PC-3 | prostate carcinoma |


| Cell line | Tumor type |
| :--- | :--- |
| RPMI-8226 | myeloma |
| RXF393 | renal cell carcinoma |
| SF-268 | glioma |
| SF-295 | glioma |
| SF-539 | glioma |
| SK-MEL-2 | melanoma |
| SK-MEL-28 | melanoma |
| SK-MEL-5 | melanoma |
| SK-OV-3 | ovarian carcinoma |
| SN12C | renal cell carcinoma |
| SNB19 | glioma (see text) |
| SNB-75 | glioma |
| SR | non Hodgkin lymphoma |
| SW620 | colorectal carcinoma |
| T47D | breast carcinoma |
| TK-10 | renal cell carcinoma |
| U251 | glioma |
| UACC-257 | melanoma |
| UACC-62 | melanoma |
| UO-31 | renal cell carcinoma |

Cancer genes analyzed

| Gene symbol | Gene name | National Center for Biotechnology Information gene ID | Likely oncogenic mutations* |
| :---: | :---: | :---: | :---: |
| APC | Adenomatous polyposis coli | 324 | 8 |
| BRAF | v-raf murine sarcoma viral oncogene homologue B1 | 673 | 10 |
| BRCA1 | Familial breast/ovarian cancer gene 1 | 672 | 0 |
| BRCA2 | Familial breast/ovarian cancer gene 2 | 675 | 0 |
| CDKN2A | Cyclin-dependent kinase inhibitor 2A, p16 | 1029 | 33 |
| CTNNB1 | Catenin (cadherin associated protein) $\beta 1$ | 1499 | 1 |
| ERBB2 | v-erb-b2 erythroblastic leukemia viral oncogene homologue 2 | 2064 | 0 |
| HRAS | v-Ha-ras Harvey rat sarcoma viral oncogene homologue | 3265 | 1 |
| EGFR | Epidermal growth factor receptor | 1956 | 0 |
| FLT3 | fms-related tyrosine kinase 3 | 2322 | 0 |
| KIT | v-KITHardy-Zuckerman 4 feline sarcoma viral oncogene homologue | 3815 | 0 |
| KRAS | v -Ki-ras2 Kirsten rat sarcoma viral oncogene homologue | 3845 | 11 |
| MAP2K4 | Mitogen-activated protein kinase kinase 4 | 6416 | 0 |
| MET | met proto-oncogene | 4233 | 0 |
| NRAS | Neuroblastoma RAS viral (v-ras) oncogene homologue | 4893 | 3 |
| PDGFRA | Platelet-derived growth factor receptor, a polypeptide | 5156 | 0 |
| PIK3CA | Phosphoinositide-3-kinase, catalytic, a polypeptide | 5290 | 7 |
| PTEN | Phosphatase and tensin homologue | 5728 | 11 |
| RB1 | Retinoblastoma 1 | 5925 | 3 |
| RET | ret proto-oncogene | 5979 | 0 |
| SMAD4 | SMAD, mothers against DPP homologue 4 (MADH4) | 4089 | 2 |
| STK11 | Serine/threonine kinase 11/LKB1 (Peutz-Jehgers syndrome) | 6794 | 4 |
| TP53 | Tumor protein p53 (Li-Fraumeni syndrome) | 7157 | 41 |
| VHL | von Hippel-Lindau tumor suppressor | 7428 | 2 |

[^6]Mutations/variants identified in 24 cancer genes in the NCI-60

| Cell line | Variants identified |
| :---: | :---: |
| 786-0 | CDKN2A Hom c.1_150 del 150, p.? LOM; PTEN Hom c.445C $>$ T, p.Q149X LOM; TP53 Het c.832C>G, P278A c.A560-2A>G, p.? LOM; VHL Hom c.311delG p.G105fsX55 LOM |
| A498 | CDKN2A Hom c.1_471 del 471, p.? LOM; VHL Hom c.426_429delTGAC p.G144fsX14 LOM |
| A549 | CDKN2A Hom c.1_471 del 471, p.? LOM; KRAS Hom c.34G>A, p.G12S LOM; STK11 Hom c. $109 \mathrm{C}>\mathrm{T}$, p.Q37X LOM |
| ACHN | CDKN2A Hom c.1_471 del 471, p.? LOM |
| BT-549 | RB1 Hom c.265_607 del 343, p.? LOM; TP53Hom c.747G>C, p.R249S LOM |
| CAKI-1 | CDKN2A Hom c.1_471 del 471, p.? LOM |
| CCRF-CEM | CDKN2A Hom c.1_471 del 471, p.? LOM; KRAS Het c.35G>A, p.G12D LOM; PTENHom c. del 80-492, p.? LOM; TP53 Het c. $743 \mathrm{G}>\mathrm{A}, \mathrm{p}$. R248Q c. $524 \mathrm{G}>\mathrm{A}, \mathrm{p} . \mathrm{R} 175 \mathrm{H}$ LOM; FLT3 Het c. $1879 \mathrm{G}>\mathrm{A}$ p.A627T TOV |
| COLO-205 | APC Hom c.4666_4667insA p.T1556fsX3 LOM; BRAFHet c.1799T>A, p.V600E LOM; SMAD4Hom del exon1-6 LOM; TP53 Hom c.308_333>TA, p.Y103fsX37 LOM |
| DU145 | CDKN2A Hom c.250G>T p.D84Y LOM; RB1 Hom c.2143A>T, p.K715X LOM; STK11 Hom c.532_536delAAGCC p.K178fsX86 LOM; TP53 Het c.820G>T, p.V274F LOM |
| EKVX | TP53 Hom c.609_610GG>TT, p.E204X LOM |
| HCC2998 | APC Het c.1994T>A, p.L665X c.4348C>T, R1450X LOM; RB1 Het c.409G>T, p.E137X TOV; TP53 Het c.637C>T, p.R213X TOV |
| HCT-116 | CDKN2A Het c.68_69insG p.R24fsX20 c.220delG p.E74fsX15 (p14) LOM; CTNNB1 c.133_135 del TCT, p.S45 del LOM; $K R A S$ Het c.38G>A, p.G13D LOM; PIK3CA Het c.3140A>G, p.H1047R LOM; BRCA2 Het c.8021_8022insA p.I2675fsX6 TOV |
| HCT-15 | APC Het c.6496C>T,p.R2166X Hom c.4248delC p.I1417fsX2 LOM; KRAS Het c.38G>A, p.G13D LOM; PIK3CA Het c. 1633G>A p. E545K LOM; TP53 Het c.C1101-2A>C, p.? C722T, S241F LOM; BRCA2 Het c.3599_3600delGT p.C1200fsX1 c.5351delA p.N1784fsX7 TOV |
| HL-60 | CDKN2A Hom c.238C>T p.R80X LOM; NRAS Het c.182A>T, p.Q61L LOM; TP53Hom deletion LOM |
| HOP62 | CDKN2A Hom c.1_471 del 471, p.? LOM; KRAS Het c.34G>T, p.G12C LOM; TP53 Hom c.G673-2A>G, p.? LOM |
| HOP-92 | CDKN2A Hom c.1_471 del 471, p.? LOM; TP53 Hom c.524G>T, p.R175L LOM |
| Hs-578-T | CDKN2A Hom c.1_471 del 471, p.? LOM; HRAS Het c.35G>A p.G12D LOM; TP53 Hom c. $469 \mathrm{G}>\mathrm{T}$, p.V157F LOM |
| HT-29 | APCHet c.2557G>T p.E853X c.4666_4667insA p.T1556fsX3 LOM; BRAFHet c.1799T>A, p.V600E LOM; SMAD4Hom c.931C>T, p.Q311X LOM; PIK3CA Het c.1345C>A p.P449T LOM; TP53 Hom c.818G>A, p.R273H LOM |
| IGROV-1 | TP53 Het c.377A>G, p.Y126C LOM; BRCA/ Het c.1961delA p.K654fsX47 TOV; SMAD4 Het c.692delG p.G231fsX10 TOV; PTENHet c.955_958delACTT p.T319fsX1 TOV |
| K-562 | CDKN2A Hom c.1_471 del 471, p.? LOM; TP53 Hom c.406_407insC p.Q136fsX13 LOM; |

Cell line Variants identified

| Cell line | Variants identified |
| :---: | :---: |
|  | PDGFRA Het Exon $10+1 \mathrm{G}>\mathrm{A}$ p.? TOV |
| KM12 | PTENHet c.385G>T, p.G129X c. 800 del A, p.K267fsX9 LOM; APC Het c. $5454-5455$ ins A, p.N1819fsX7 TOV; TP53 Het c.215delG, p.R72fsX51 TOV; BRCA2 Het c.5351delA p.N1784fsX7 TOV |
| LOXIMVI | BRAFHet c.1799T>A, p.V600E LOM; CDKN2A Hom c.1_471 del 471, p.? LOM |
| M14/MDA-MB-435 | BRAFHet c. 1799T>A, p.V600E LOM; CDKN2A Het c. $150+2 \mathrm{~T}>\mathrm{C}$ p.? c. 456-+24 AGgtgaggactgatgatctgagaatt >C p.? LOM; TP53 Het c.797G>A, p.G266E LOM |
| MALME-3M | BRAFHet c.1799T>A, p.V600E LOM; CDKN2A Hom c.1_471 del 471, p.? LOM |
| MCF7 | CDKN2A Hom c.1_471 del 471, p.? LOM; PIK3CA Het c.1633G>A p.E545K LOM |
| MDA-MB-231 | BRAFHet c.1391G>T, p.G464V LOM; CDKN2A Hom c.1_471 del 471, p.? LOM; KRAS Het c.38G>A p.G13D LOM; TP53 Hom c.839G>A, p.R280K LOM |
| MOLT-4 | CDKN2A Hom c.1_471 del 471, p.? LOM; NRASHet c.34G>T p.G12C LOM; PTENHom c.800delA p.K267fsX9 LOM; STK11 Het c.640C>T p.Q214X TOV; TP53 Het c.916C>T, p.R306X TOV |
| NCI-H226 | CDKN2A Hom c.1_150 del 150, p.? LOM |
| NCI-H23 | KRASHet c.34G>T, p.G12C LOM; STK11 Hom c.996G>A, p.W332X LOM; TP53 Hom c.738G>C, p.M246I LOM |
| NCI-H322M | TP53Hom c. $743 \mathrm{G}>$ T, p.R248L LOM |
| NCI-H460 | CDKN2A Hom del 1_457 del 457, p.? LOM; KRASHom c.183A>T, p.Q61H LOM; PIK3CA Het c.1633G>A p.E545K LOM; STK11 Hom c.109C>T p. Q37X LOM |
| NCI-H522 | TP53 Hom 572delC, p.P191fsX56 LOM |
| OVCAR-3 | TP53Hom c.743G>A, p.R248Q LOM |
| OVCAR-4 | TP53Hom c.388C>G, p.L130V LOM |
| OVCAR-5 | CDKN2A Hom c.1_471 del 471, p.? LOM; KRAS Hom c.35G>T, p.G12V LOM |
| OVCAR-8/NCI/ADR-RES | TP53 Hom c. 376 -1G>A, p.? LOM; ERBB2 Het c. $2327 \mathrm{G}>\mathrm{T}$ p.G776V TOV |
| PC-3 | PTENHom c.165-1026 del 862, p.? LOM; TP53 Hom c.414delC p.K139fsX31 LOM |
| RPMI-8226 | KRASHet c.35G>C, p.G12A LOM; TP53 Hom c.853G>A, p.E285K LOM; EGFR Het c. $2252 \mathrm{C}>\mathrm{T}$ p.T751I TOV |
| RXF393 | CDKN2A Hom c.1_471 del 471, p.? LOM; PTENHom c.1_164 del 164, p.? LOM; TP53 Hom c. $524 \mathrm{G}>\mathrm{A}, \mathrm{p} . \mathrm{R} 175 \mathrm{H}$ LOM |
| SF-268 | CDKN2A Hom c.1_471 del 471, p.? LOM; TP53 Hom c.818G>A, p.R273H LOM |
| SF-295 | CDKN2A Hom c.1_471 del 471, p.? LOM; PTENHom c.697C>T, p.R233X LOM; TP53 Hom c.743G>A, p.R248Q LOM |
| SF539 | RBI Hom c.346_349delACTT p.T116fsX8 LOM; TP53 Hom c.1024delC p.R342fsX3 LOM; PTENHom c.1-1026 del 1026, p.? LOM |
| SK-MEL-2 | NRAS Hom c. 182A>G, p.Q61R LOM; TP53 Het c.733G>A, p.G245S LOM |
| SK-MEL-28 | BRAFHom c.1799T>A, p.V600E LOM; TP53Hom c.435_436G>GT, p.L145R LOM; EGFR Hom c. $2257 \mathrm{C}>\mathrm{T}$ p.P753S TOV |

Variants identified

| Cell line | Variants identified |
| :---: | :---: |
| SK-MEL-5 | $B R A F$ Het c.1799T>A, p.V600E LOM; CDKN2A Hom c.1_471 del 471, p.? LOM |
| SK-OV-3 | CDKN2A Hom del 1_457 del 457, p.? LOM; PIK3CA Het c.3140A>G, p.H1047R LOM; TP53 Hom c. del267C p.S90fsX33 LOM; APC Het c.4666delA p.T1556fsX9 TOV |
| SN12C | TP53 Hom c.1006G>T, p.E336X LOM |
| SNB-75 | TP53 Hom c.772G>A, p.E258K LOM |
| SR | $C D K N 2 A$ Hom c.1_471 del 471, p.? LOM |
| SW620 | KRAS Hom c.35G>T, p.G12V LOM; TP53 Hom c.818G>A, p.R273H C925T, p.P309S LOM; $A P C$ Hom c.4012C>T, p.Q1338X LOM |
| T47D | PIK3CA Het c.3140A>G, p.H1047R LOM; TP53 Hom c.580C> T, p.L194F LOM |
| TK10 | TP53 Het c.791T>G, p.L264R LOM |
| U251/SNB-19 | CDKN2A Hom c.1_471 del 471, p.? LOM; PTENHom c.723_724insTT p.E242fsX15 LOM; TP53 Hom c.818G>A, p.R273H LOM |
| UACC-257 | $B R A F$ Het c. $1799 \mathrm{~T}>\mathrm{A}$, p.V600E LOM |
| UACC-62 | BRAFHom c.1799T>A, p.V600E LOM; CDKN2A Hom c.1_471 del 471, p.? LOM; PTENHom c.741_742insA p.P248fsX5 LOM |
| UO-31 | $C D K N 2 A$ Hom c.1_471 del 471, p.? LOM |


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    $4_{\text {http://dtp.nci.nih.gov/docs/cancer/cancer_data.html. }}$

[^1]:    5http://discover.nci.nih.gov.

[^2]:    ${ }^{6}$ Supplementary materials for this article are available at Molecular Cancer Therapeutics Online (http://mct.aacrjournals.org/).
    $7_{\text {http://www.sanger.ac.uk/genetics/CGP/Census/. }}$

[^3]:    $8_{\text {http://www.sanger.ac.uk/genetics/CGP/Genotyping/. }}$
    9 http ///www.sanger.ac.uk/genetics/CGP/cosmic/.
    $10_{\text {http://www.sanger.ac.uk/genetics/CGP/CellLines/. }}$
    $11_{\text {http://www.sanger.ac.uk/genetics/CGP/MSI/table1.shtml. }}$

[^4]:    $12_{\text {http://www.sanger.ac.uk/CGP. }}$
    13 http://www.sanger.ac.uk/genetics/CGP/Genotyping/nci60.shtml.

[^5]:    $14_{\text {http://www.sanger.ac.uk/CGP/COSMIC. }}$

[^6]:    *Mutations seen in common ancestor lines counted once.

