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# Prospective Enterprise-Level Molecular Genotyping of a Cohort of Cancer Patients

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Ongoing cancer genome characterization studies continue to elucidate the spectrum of genomic abnormalities that drive many cancers, and in the clinical arena assessment of the driver genetic alterations in patients is playing an increasingly important diagnostic and/or prognostic role for many cancer types. However, the landscape of genomic abnormalities is still unknown for less common cancers, and the influence of specific genotypes on clinical behavior is often still unclear. To address some of these deficiencies, we developed Profile, a prospective cohort study to obtain genomic information on all patients at a large tertiary care medical center for cancer-related care. We enrolled patients with any cancer diagnosis, and, for each patient (unselected for cancer site or type) we applied mass spectrometric genotyping (OncoMap) of 471 common recurrent mutations in 41 cancer-related genes. We report the results of the first 5000 patients, of which 26% exhibited potentially actionable somatic mutations. These observations indicate the utility of genotyping in advancing the field of precision oncology. *(J Mol Diagn 2014, 16: 660–672; http://dx.doi.org/10.1016/j.jmoldx.2014.06.004)*

Within the past decade the application of genome interrogation technologies to patient samples has greatly expanded our understanding of the spectrum of genomic alterations that underpin cancer initiation and progression and those events that contribute to the evolution of cancer and the emergence of resistance to targeted therapies. Studies such as the Cancer Genome Atlas (*http://cancergenome.nih.gov*) and the International Cancer Genome Consortium[1] have comprehensively characterized >20 cancer types. Such studies have confirmed the incidence of many known oncogenes and tumor suppressor genes but have also identified hitherto unrecognized genes and pathways recurrently altered in cancers.

In parallel with research endeavors, information gleaned from these studies has been translated to the molecular diagnostics arena to develop clinical tests that can detect somatic alterations in specific cancer types. Often, these clinical tests take the form of a gene- or alteration-targeted approach and can be used for diagnostic purposes (eg, *BRAF* testing to distinguish between subtypes of thyroid papillary carcinoma), prognostic indications (*NPM1* and *FLT3* testing in acute myeloid leukemia),[2,3] predicting response to a targeted therapy (*EGFR* mutation analysis as an indicator for therapeutic response in metastatic non-small cell lung cancer), or detecting resistance to a targeted agent (*ABL1* kinase domain mutational analysis for imatinib (Gleevec)-resistance in patients with chronic myelogenous leukemia). Moreover, clinical guidelines for some cancers encourage a sequential testing process, as exemplified by the

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testing guidelines for non-small cell lung cancer by the College of American Pathologists, International Association for the Study of Lung Cancer, and the Association for Molecular Pathology.[4] Completing these tests is not necessarily a cost-effective exercise and uses substantial amounts of nucleic acid material, which may be limiting for many patients with cancer. In addition, expanding catalogs of cancer mutations challenge that these events are tissue specific or occur in isolation. For example, activating *BRAF* mutations have been described in >50% of papillary thyroid carcinomas[5] and cutaneous melanomas but also at a lower frequency in lung cancer,[6] colorectal adenocarcinoma,[7] pediatric low-grade glioma,[7] and multiple myeloma.[8] These mutant BRAF proteins are potential targets for RAF inhibitors,[9] and clinical trials have confirmed the utility of targeted therapies in some of these instances.[10,11] An ever-expanding number of other targetable proteins, including phosphatidylinositol-4, 5-bisphosphate 3-kinase, catalytic subunit α (PIK3CA), epidermal growth factor receptor (EGFR), and v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 2 (ERBB2), are aberrant in multiple cancer types.[7,12,13] Moreover, interrogating the mutational status of multiple genes has found clinical utility in some settings; for example, acquired resistance to EGFR tyrosine kinase inhibitors (TKIs) in non-small cell lung cancer can be due to the *EGFR* T790M mutation, *MET* gene amplification, or mutation of *PIK3CA*.[14]

A more rational approach to individualized cancer treatment, therefore, would be the application of multigene testing, using a small amount of DNA, to generate a more comprehensive assessment of mutations in several genes concurrently, in a more clinically relevant time frame. Although several challenges need to be overcome to implement such a paradigm,[14–18] such as generating high-quality genomic data from archival [eg, formalin-fixed, paraffin-embedded (FFPE)] tumor material, this information can inform a precision or individualized approach to clinical decision making, particularly in selecting an appropriate targeted therapy for a patient. Furthermore, the ability to combine multiple common targetable alterations in one assay greatly enhances the ability to identify patients who might be suitable candidates for clinical trials of investigational therapies and to implement such testing on all patients in the cancer center facilitates the implementation of basket trials that extend beyond specific anatomically defined cancer types.

To this end, in the past few years we have developed a panel-based test that allows more broad screening of genomic alterations known to be informative in cancer. We and others have used mass spectrometric genotyping[7] or allele-specific PCR technologies[19] to establish personalized cancer medicine initiatives.[7,19–25] More recently, advances in next-generation sequencing technologies have enabled even more comprehensive genomic characterization in a massively parallel fashion and in a relatively short time frame, allowing the assessment of many types of genomic alterations (mutations, insertions and deletions, copy number alterations, structural rearrangements, and epigenetic changes) in hundreds or thousands of genes (targeted sequencing and whole-exome sequencing), whole-genome sequencing, transcriptome sequencing (RNASeq), and epigenetic interrogations (methyl-Seq and ChipSeq). Pilot studies that apply massively parallel sequencing technologies and/or integrative analyses in focused clinical settings for a few patients have also been reported.[26,27]

We implemented a prospective genomic characterization study called Profile that aimed to apply genomic technologies to advance the field of precision oncology by addressing some of the challenges described above. We obtained consent from >12,000 patients with cancer who came to Dana-Farber Cancer Institute or Brigham and Women's Hospital between August 2011 and June 2013, and OncoMap,[7,28] a mass spectrometric genotyping assay that detects 471 unique mutations in 41 cancer genes, was performed in a laboratory certified by Clinical Laboratory Improvement Amendments. Test results were reviewed by laboratory staff and interpreted and reported by board-certified pathologists. Here, we report initial findings from profiles on >5000 patients with cancer.

## Materials and Methods

### Patients and Tumor Tissue Collection

Patients gave consent to the institutional review board–approved protocol 11 to 104 from the Dana-Farber Cancer Institute Office for the Protection of Research Subjects. Tumor specimens were obtained from Dana-Farber Cancer Institute and the Department of Pathology at Brigham and Women's Hospital. Patient charts were reviewed, and appropriate specimens for testing were selected with the following criteria: ≥30% viable tumor content (initially 50% tumor was required but this was decreased over time because performance metrics were comparable) and sufficient area (>3 mm in greatest linear diameter) for DNA extraction. Specimen types profiled included FFPE, fresh/frozen, and blood/bone marrow.

### DNA Extraction and Preparation

For solid tumor specimens, tissue was sectioned and slides stained with hematoxylin and eosin were obtained. Tumor-rich areas of FFPE were manually dissected from unstained slides or whole FFPE blocks; fresh tissues were grossly minced and digested overnight with Proteinase K. DNA was extracted manually or by using an automated protocol (QiaSymphony) with Qiagen reagents (Qiagen, Valencia, CA). Blood or marrow samples with mononuclear hematological malignancies were enriched by Ficoll gradient before DNA extraction. Total DNA of 200 ng was required to proceed with OncoMap testing. DNA (100 ng) extracted from FFPE samples was whole genome amplified (GenomePlex Complete Whole Genome Amplification kit; Sigma-Aldrich, St. Louis, MO) for iPLEX analysis (see

**Table 1** Forty-One Genes and 471 Mutations Interrogated in OncoMap Version 4

| Gene | AA change | Gene | AA change | Gene | AA change | Gene | AA change | Gene | AA change |
|---|---|---|---|---|---|---|---|---|---|
| *ABL1* | M244V | *CSF1R* | Y969* | *FGFR2* | N549K | *KRAS* | Q61R | *PIK3R1* | N564K |
| *ABL1* | L248V | *CTNNB1* | A13T | *FGFR3* | R248C | *KRAS* | A146T | *PIK3R1* | R574fs*27 |
| *ABL1* | G250E | *CTNNB1* | A21T | *FGFR3* | S249C | *MAP2K1* | Q56P | *PIK3R1* | T576del |
| *ABL1* | Q252H | *CTNNB1* | V22A | *FGFR3* | G370C | *MAP2K1* | K57N | *PIK3R1* | W583del |
| *ABL1* | Q252H | *CTNNB1* | V22_G38del | *FGFR3* | S371C | *MAP2K1* | K57N | *PTEN* | K6fs*4 |
| *ABL1* | Y253F | *CTNNB1* | W25_D32del | *FGFR3* | Y373C | *MAP2K1* | D67N | *PTEN* | R130G |
| *ABL1* | Y253H | *CTNNB1* | D32A | *FGFR3* | K650Q | *MAP2K1* | C121S | *PTEN* | R130Q |
| *ABL1* | E255K | *CTNNB1* | D32G | *FGFR3* | G697C | *MAP2K1* | C121S | *PTEN* | R130* |
| *ABL1* | E255V | *CTNNB1* | D32H | *FGFR3* | L794fs*23 | *MAP2K1* | P124L | *PTEN* | R130fs*4 |
| *ABL1* | D276G | *CTNNB1* | D32N | *FLT3* | Y572C | *MET* | T1010I | *PTEN* | R173C |
| *ABL1* | T315I | *CTNNB1* | D32V | *FLT3* | D835del | *MET* | H1112R | *PTEN* | R173H |
| *ABL1* | F317L | *CTNNB1* | D32Y | *FLT3* | D835E | *MET* | H1112Y | *PTEN* | R233* |
| *ABL1* | M351T | *CTNNB1* | S33C | *FLT3* | D835E | *MET* | Y1248C | *PTEN* | P248fs*5 |
| *ABL1* | E355G | *CTNNB1* | S33F | *FLT3* | D835H | *MET* | Y1248H | *PTEN* | P248fs*5 |
| *ABL1* | F359V | *CTNNB1* | S33Y | *FLT3* | D835V | *MET* | M1268T | *PTEN* | K267fs*9 |
| *ABL1* | H396R | *CTNNB1* | G34E | *FLT3* | D835Y | *MLH1* | V384D | *PTEN* | V317fs*3 |
| *AKT1* | E17K | *CTNNB1* | G34R | *FLT3* | I836del | *MYC* | P57S | *PTEN* | N323fs*2 |
| *AKT2* | S302G | *CTNNB1* | G34R | *FLT3* | I836M | *MYC* | A59V | *PTEN* | N323fs*21 |
| *AKT2* | R371H | *CTNNB1* | G34V | *GNA11* | Q209L | *MYC* | T73I | *PTEN* | R335* |
| *APC* | R876* | *CTNNB1* | S37A | *GNA11* | Q209P | *MYC* | S77F | *RB1* | E137* |
| *APC* | R1114* | *CTNNB1* | S37C | *GNAQ* | Q209L | *MYC* | N101T | *RB1* | L199* |
| *APC* | E1306* | *CTNNB1* | S37F | *GNAQ* | Q209L | *MYC* | P260A | *RB1* | R320* |
| *APC* | E1309fs*4 | *CTNNB1* | S37P | *GNAQ* | Q209P | *NPM1* | W288fs*12 | *RB1* | R358* |
| *APC* | E1309fs*6 | *CTNNB1* | S37Y | *GNAS* | R201C | *NPM1* | W288fs*12 | *RB1* | R455* |
| *APC* | Q1338* | *CTNNB1* | T41A | *GNAS* | R201H | *NPM1* | W288fs*12 | *RB1* | R552* |
| *APC* | Q1367* | *CTNNB1* | T41I | *GNAS* | Q227L | *NRAS* | G12A | *RB1* | R556* |
| *APC* | Q1378* | *CTNNB1* | T41P | *HRAS* | G12C | *NRAS* | G12C | *RB1* | R579* |
| *APC* | E1379* | *CTNNB1* | T41S | *HRAS* | G12D | *NRAS* | G12D | *RB1* | L660fs*2 |
| *APC* | Q1429* | *CTNNB1* | T41S | *HRAS* | G12R | *NRAS* | G12R | *RB1* | C706F |
| *APC* | R1450* | *CTNNB1* | S45A | *HRAS* | G12V | *NRAS* | G12S | *RB1* | E748* |
| *APC* | S1465fs*3 | *CTNNB1* | S45C | *HRAS* | G13C | *NRAS* | G12V | *RET* | F612_C620del |
| *APC* | T1556fs*3 | *CTNNB1* | S45F | *HRAS* | G13R | *NRAS* | G13A | *RET* | D631G |
| *BRAF* | R444W | *CTNNB1* | S45P | *HRAS* | G13S | *NRAS* | G13C | *RET* | D631_L633>E |
| *BRAF* | G464E | *CTNNB1* | S45Y | *HRAS* | G13V | *NRAS* | G13D | *RET* | E632_L633del |
| *BRAF* | G464R | *EGFR* | G719A | *HRAS* | Q61H | *NRAS* | G13R | *RET* | E632_L633>V |
| *BRAF* | G464V | *EGFR* | G719C | *HRAS* | Q61H | *NRAS* | G13S | *RET* | E632_A640>VRP |
| *BRAF* | G466A | *EGFR* | G719D | *HRAS* | Q61K | *NRAS* | G13V | *RET* | C634R |
| *BRAF* | G466E | *EGFR* | G719S | *HRAS* | Q61L | *NRAS* | A18T | *RET* | C634W |
| *BRAF* | G466R | *EGFR* | L730F | *HRAS* | Q61P | *NRAS* | Q61E | *RET* | C634Y |
| *BRAF* | G466V | *EGFR* | W731* | *HRAS* | Q61R | *NRAS* | Q61H | *RET* | E768D |
| *BRAF* | G469A | *EGFR* | P733L | *HRAS* | Q61R | *NRAS* | Q61H | *RET* | A883F |
| *BRAF* | G469E | *EGFR* | E734K | *HRAS* | Q61R | *NRAS* | Q61K | *RET* | A883F |
| *BRAF* | G469R | *EGFR* | G735S | *IDH1* | R132C | *NRAS* | Q61L | *RET* | D898_E901del |
| *BRAF* | G469R | *EGFR* | V742A | *IDH1* | R132H | *NRAS* | Q61L | *RET* | M918T |
| *BRAF* | G469S | *EGFR* | K745R | *IDH1* | R132S | *NRAS* | Q61P | *SRC* | Q531* |
| *BRAF* | G469S | *EGFR* | E746K | *IDH2* | R140Q | *NRAS* | Q61R | *STK11* | Q37* |
| *BRAF* | G469V | *EGFR* | E746_A750del | *IDH2* | R172K | *NRAS* | Q61R | *STK11* | E57fs*7 |
| *BRAF* | V471F | *EGFR* | E746_A750del | *JAK2* | V617F | *PDGFRA* | V561D | *STK11* | Q170* |
| *BRAF* | N581S | *EGFR* | E746_A750>V | *JAK3* | P132T | *PDGFRA* | S566_E571>K | *STK11* | D194N |
| *BRAF* | E586K | *EGFR* | E746_T751del | *JAK3* | A572V | *PDGFRA* | S566_E571>R | *STK11* | D194V |
| *BRAF* | D587A | *EGFR* | E746_T751>A | *JAK3* | V722I | *PDGFRA* | S566_E571>R | *STK11* | G196V |
| *BRAF* | D587E | *EGFR* | E746_S752>A | *KIT* | D52N | *PDGFRA* | R841_D842del | *STK11* | E199K |
| *BRAF* | D587E | *EGFR* | E746_S752>V | *KIT* | Y503_F504insAY | *PDGFRA* | D842I | *STK11* | E199* |
| *BRAF* | I592M | *EGFR* | L747_R748>FP | *KIT* | K550_K558del | *PDGFRA* | D842V | *STK11* | F264fs*22 |
| *BRAF* | I592V | *EGFR* | L747_E749del | *KIT* | W557G | *PDGFRA* | D842Y | *STK11* | P281L |
| *BRAF* | D594E | *EGFR* | L747_A750>P | *KIT* | W557R | *PDGFRA* | D842Y | *STK11* | P281fs*6 |
| *BRAF* | D594G | *EGFR* | L747_A750>P | *KIT* | W557R | *PDGFRA* | D842_M844del | *STK11* | W332* |

(*table continues*)

**Table 1** (*continued*)

| Gene | AA change | Gene | AA change | Gene | AA change | Gene | AA change | Gene | AA change |
|---|---|---|---|---|---|---|---|---|---|
| *BRAF* | D594V | *EGFR* | L747_T751del | *KIT* | K558_V560del | *PDGFRA* | D842_H845del | *TP53* | R175H |
| *BRAF* | F595L | *EGFR* | L747_T751>P | *KIT* | K558_E562del | *PDGFRA* | D842_H845>V | *TP53* | G245S |
| *BRAF* | F595S | *EGFR* | L747_T751>S | *KIT* | V559A | *PDGFRA* | D842_D846>E | *TP53* | R248Q |
| *BRAF* | G596R | *EGFR* | L747_S752del | *KIT* | V559D | *PDGFRA* | D842_D846>G | *TP53* | R248W |
| *BRAF* | L597Q | *EGFR* | L747_P753>Q | *KIT* | V559del | *PDGFRA* | D842_D846>N | *TP53* | R273C |
| *BRAF* | L597R | *EGFR* | L747_P753>S | *KIT* | V559G | *PDGFRA* | D842_S847>EA | *TP53* | R273H |
| *BRAF* | L597S | *EGFR* | A750P | *KIT* | V559I | *PDGFRA* | I843_D846del | *TP53* | R306* |
| *BRAF* | L597V | *EGFR* | S752Y | *KIT* | V560D | *PDGFRA* | I843_S847>T | *VHL* | P81S |
| *BRAF* | T599I | *EGFR* | S752_I759del | *KIT* | V560G | *PDGFRA* | H845_N848>P | *VHL* | L85P |
| *BRAF* | T599_V600insTT | *EGFR* | P753S | *KIT* | L576P | *PDGFRA* | D846Y | *VHL* | L89H |
| *BRAF* | V600A | *EGFR* | D761N | *KIT* | P585P | *PIK3CA* | R88Q | *VHL* | F148fs*11 |
| *BRAF* | V600D | *EGFR* | D761Y | *KIT* | K642E | *PIK3CA* | N345K | *VHL* | L158Q |
| *BRAF* | V600E | *EGFR* | S768I | *KIT* | V654A | *PIK3CA* | C420R | *VHL* | R161* |
| *BRAF* | V600E | *EGFR* | V769_D770insASV | *KIT* | T670I | *PIK3CA* | P539R | *VHL* | R167W |
| *BRAF* | V600K | *EGFR* | V769_D770insASV | *KIT* | D816H | *PIK3CA* | E542K | | |
| *BRAF* | V600L | *EGFR* | D770_N771insN | *KIT* | D816V | *PIK3CA* | E542Q | | |
| *BRAF* | V600L | *EGFR* | N771_P772>SVDNR | *KIT* | D816Y | *PIK3CA* | E545A | | |
| *BRAF* | V600M | *EGFR* | P772_H773insV | *KIT* | N822K | *PIK3CA* | E545D | | |
| *BRAF* | V600R | *EGFR* | H773R | *KIT* | N822K | *PIK3CA* | E545D | | |
| *BRAF* | K601del | *EGFR* | I744_A750>VK | *KIT* | V825A | *PIK3CA* | E545G | | |
| *BRAF* | K601E | *EGFR* | T790M | *KIT* | E839K | *PIK3CA* | E545K | | |
| *BRAF* | K601N | *EGFR* | G810D | *KRAS* | G12A | *PIK3CA* | E545Q | | |
| *BRAF* | K601N | *EGFR* | G810S | *KRAS* | G12C | *PIK3CA* | Q546K | | |
| *BRAF* | S605F | *EGFR* | L858M | *KRAS* | G12D | *PIK3CA* | H701P | | |
| *BRAF* | S605N | *EGFR* | L858R | *KRAS* | G12R | *PIK3CA* | Y1021C | | |
| *CDK4* | R24H | *EGFR* | L858R | *KRAS* | G12S | *PIK3CA* | M1043I | | |
| *CDKN2A* | R58* | *EGFR* | L858R | *KRAS* | G12V | *PIK3CA* | M1043I | | |
| *CDKN2A* | E61* | *EGFR* | L861Q | *KRAS* | G13A | *PIK3CA* | H1047L | | |
| *CDKN2A* | E69* | *ERBB2* | L755P | *KRAS* | G13C | *PIK3CA* | H1047R | | |
| *CDKN2A* | R80* | *ERBB2* | L755S | *KRAS* | G13D | *PIK3CA* | H1047Y | | |
| *CDKN2A* | H83Y | *ERBB2* | D769H | *KRAS* | G13R | *PIK3CA* | G1049R | | |
| *CDKN2A* | D84Y | *ERBB2* | Y772_A775dup | *KRAS* | G13S | *PIK3CA* | G1049S | | |
| *CDKN2A* | E88* | *ERBB2* | A775_G776insYVMA | *KRAS* | G13V | *PIK3CA* | N1068fs*4 | | |
| *CDKN2A* | D108Y | *ERBB2* | G776S | *KRAS* | L19F | *PIK3R1* | G376R | | |
| *CDKN2A* | W110* | *ERBB2* | G776VC | *KRAS* | L19F | *PIK3R1* | G376R | | |
| *CDKN2A* | W110* | *ERBB2* | V777L | *KRAS* | Q22K | *PIK3R1* | E439del | | |
| *CDKN2A* | P114L | *FGFR1* | S125L | *KRAS* | A59T | *PIK3R1* | K459_S460>N | | |
| *CSF1R* | L301S | *FGFR1* | P252T | *KRAS* | Q61E | *PIK3R1* | R461* | | |
| *CSF1R* | L301* | *FGFR2* | S252W | *KRAS* | Q61H | *PIK3R1* | R557_K561>Q | | |
| *CSF1R* | Y969C | *FGFR2* | K310R | *KRAS* | Q61H | *PIK3R1* | D560Y | | |
| *CSF1R* | Y969F | *FGFR2* | S372C | *KRAS* | Q61K | *PIK3R1* | D560_S565del | | |
| *CSF1R* | Y969H | *FGFR2* | Y375C | *KRAS* | Q61L | *PIK3R1* | N564D | | |
| *CSF1R* | Y969* | *FGFR2* | C382R | *KRAS* | Q61P | *PIK3R1* | N564K | | |

immediately below), whereas fresh frozen and blood DNA was processed with unamplified DNA. Confirmation of mutations was performed on unamplified genomic DNA by using homogenous mass-extend chemistry.[29]

## OncoMap Assay Design and Genotyping

Selection of cancer gene mutations for assay design and mass spectrometric genotyping was performed as previously described.[7] Specimens were genotyped with OncoMap version 4, which assays 471 unique mutations in 41 cancer-related genes (Table 1). OncoMap version 4 identifies mutations in a high-throughput manner such that 48 to 96 patient samples are processed in parallel and consists of two chemistries (high complexity iPLEX[5] and homogenous mass-extend) and a manual review step, as previously described.[7] Genomic profiling was performed in an environment certified by Clinical Laboratory Improvement Amendments.

## OncoMap Validation

Validation studies were performed to determine precision, accuracy, sensitivity, specificity, and limit of detection by using blood, fresh frozen, and FFPE samples that had existing genomic characterization by using an orthogonal clinical test (eg, pyrosequencing, Sanger sequencing, PCR/electrophoresis,

real-time PCR). Thirty samples with known mutations in *KRAS, EGFR, BRAF, TP53, AKT*, and *PIK3CA* were selected. Within this sample set, additional mutations identified in *APC, P53, CTNNB1*, and *JAK3* were also assessed for a total of 28 genetic variants detectable by 53 individual assays. Normal (noncancerous) liver was included to verify detection of wild-type loci. To obtain sufficient quantities of DNA, several isolations were performed from each FFPE sample, pooled to ensure sample homogeneity, and then divided into aliquots to produce nine replicates. OncoMap was performed in triplicate across three experiments to determine intra- and inter-run precision.

A total of 114 samples (41 wild-type and 73 mutant) were analyzed by reference methods (pyrosequencing, PCR/CE fragment analysis, Sanger sequencing, and allele-specific PCR) at the Center for Advanced Molecular Diagnostics, Brigham and Women's Hospital, and were subsequently analyzed in OncoMap to determine accuracy and concordance with gold standard methods for variants at the following loci: *KRAS* G12 and G13; *NRAS* G12, G13, and Q61; *EGFR* exon 19 (deletion); *JAK2* V617F; *EGFR* L858R; and *KIT* exon 11 (deletion).

To assess our ability to detect all 471 mutations in 439 assays, we designed synthetic oligonucleotides that harbored each of the genetic variants listed in Table 1 (IDT, Coralville, IA). These were pooled into groups of three nonoverlapping variants and spiked into normal liver DNA isolated from FFPE tissue such that the ratio of wild-type to variant was 1:1 and were analyzed with OncoMap. The limit of detection was determined by mixing genomic DNAs isolated from the following cell lines: THP-1 (*NRAS* G12D; ATCC, Rockville, MD), PC9-2 (*EGFR* E746-A750del), H1975 (*EGFR* L858R and T790M), and A-549-2 (*KRAS* G12S; gift from Dr. Pasi Janne, Dana-Farber Cancer Institute). Six replicates were prepared at defined ratios representing 0%, 5%, 7.5%, 10%, and 25% allele frequencies for five known variants and assessed in OncoMap. Ninety-five percent confidence intervals were calculated with R version 2.15.0 (R Project for Statistical Computing, Vienna, Austria; *http://www.r-project.org*) by using the binconf function in the Hmisc package. The calculation method used the Wilson score interval to generate the confidence interval.

## Results

### Characteristics of Clinical Tumor Cohort

Of 9950 patients with an available specimen accessioned in the pathology laboratory, 5372 (53.9%) were estimated by a pathologist to have sufficient and appropriate material to attempt DNA extraction. Of these, 5123 patients had sufficient DNA (200 ng or more) to proceed with OncoMap, and 99.9% ($n = 5118$) of attempted tests yielded an OncoMap result. Estimated tumor content exceeded 30% in all specimens as determined by pathological review (hematoxylin and eosin evaluation).

The distribution of cancer types assayed in our cohort is depicted in Table 2, and consists of 24 main cancer types. This distribution of cases reflected the population of cancers for which the test was ordered and performed and was not necessarily reflective of the incidence of cancer types seen at our institutions; the distribution of cases was likely skewed by both differences in the availability of appropriate materials/specimens for testing and differences in ordering habits of the involved physicians. Specimens ($n = 5118$) yielded an OncoMap result. Of these, 451 specimens were obtained from frozen tissue, 74 from blood, 189 from bone marrow, and 4404 (86%) from FFPE blocks. Specimens ($n = 2182$; 42.6%) harbored one or more mutations. Specimens with mutations (74.4%) had one mutation identified by OncoMap, approximately one-third of reportable cases. Twenty percent of specimens with mutations (8.5% of reportable cases) had two mutations; the remainder had between three and five events. All specimens with four or more mutations ($n = 22$) were identified as either colorectal or endometrial adenocarcinoma.

### Performance of OncoMap

To facilitate cancer gene mutation profiling in clinical tumor specimens, we used OncoMap version 4, a panel of genotyping assays that assessed the status of 471 mutations, across 41 cancer-related genes. The complete mutation profiling algorithm, including iPLEX chemistry, automated

**Table 2** Distribution of Cancer Types Assayed in Our Cohort of Patient Samples, Consisting of 24 Major Cancer Types

| Cancer site or type | Cases (*n*) |
|---|---|
| Acute leukemia | 144 |
| Brain tumor | 317 |
| Breast carcinoma | 638 |
| Cervical carcinoma | 39 |
| Chronic leukemia | 51 |
| Endocrine organ | 36 |
| Endometrial carcinoma | 531 |
| Female genital tract | 157 |
| Genitourinary tract | 218 |
| Head and neck carcinoma | 149 |
| Kidney carcinoma | 153 |
| Liver carcinoma | 29 |
| Lower GI tract | 392 |
| Lung carcinoma | 532 |
| Lymphoma | 168 |
| Myelodysplastic/myeloproliferative disease | 55 |
| Other/miscellaneous | 54 |
| Ovary carcinoma | 280 |
| Pancreatobiliary tract | 143 |
| Pleural tumor | 127 |
| Prostate carcinoma | 207 |
| Sarcoma | 387 |
| Skin cancer | 143 |
| Upper GI tract | 168 |
| Total | 5118 |

GI, gastrointestinal.

calling, manual review, validation by using homogenous mass-extend chemistry, and manual review by laboratory personnel and a pathologist, has been previously reported[7]; a schematic overview of the process, including the time taken for each step, is indicated in Figure 1.

Validation studies have found 100% intra- and inter-assay precision (95% CI, 99.2%−100%), because all 28 genetic variants (see *Materials and Methods*) were detected in nine of nine replicates in the expected 53 assays. Concordance studies that compared OncoMap with various validated methods for single gene testing reported 98.3% sensitivity (95% CI, 94.13%−99.54%) and 100% specificity (95% CI, 98.1%−100%) for *KRAS, NRAS, BRAF, JAK2, KIT*, and *EGFR*. Two samples gave a false negative OncoMap result for *JAK2* V617F, both with allele frequency <1%. Further evaluation of all 471 genetic variants that used synthetic oligonucleotides found the expected mutation in 432 of 439 assays; 7 assays displayed poor performance and failed to detect the expected mutation. For each of the seven failing assays, at least one additional complementary assay (ie, the opposite strand) detected the variant in question, with no false positives, resulting in an overall sensitivity and specificity of 100% (95% CI, 99.19%−100%). Limit of detection experiments performed on cell lines with known genetic variants mixed in defined ratios to produce allele frequencies that varied between 0% and 25% found successful detection (100%; 95% CI, 88.65%−100%) to 7.5% mutant allele frequency for each of the five mutations monitored (*EGFR* T790M, *EGFR* L858R, *EGFR* E746-A750del, *NRAS* G12D, and *KRAS* G12S); *EGFR* T790M and *EGFR* L858R were also detected (100%; 95% CI, 75.75%−100%) at 5% allele frequency.

## Expected Mutations in Well-Characterized Cancer Types

In total, 2890 mutations were identified in our cohort. The spectrum of mutations by gene is shown in Figure 2. As expected, *KRAS* was the most commonly mutated gene in our cancer population, occurring in 538 samples (10% of all samples) or almost 20% of mutated samples. The next most commonly mutated gene was *PIK3CA* (497 instances; 17% of mutations), followed by *TP53* (326 instances; 11%) and *BRAF* (202 instances corresponding to 7% of mutations). The *TP53* mutation frequency was less than might be expected but may be explained by the ability of a genotyping technology to detect only specific, predetermined mutations incorporated into the assay design. This was a limitation when interrogating tumor suppressor genes such as *TP53*, which may have many loss-of-function mutations scattered throughout the gene (eg, this platform can detect only approximately 20% of the known *TP53* mutations, by frequency, in the Catalogue of Somatic Mutations in Cancer[30] database). The full landscape of mutations by cancer type is indicated in Figure 3. *CDK4*, *CSF1R*, *FGFR1*, and *SRC* were not detectably mutated in any of the cases.

## Interpretation of Mutations

A tiering approach was designed to assess the import of genomic alterations in specific cancer types. Each mutation was assigned one of three tiers. In tier 1, the alteration has

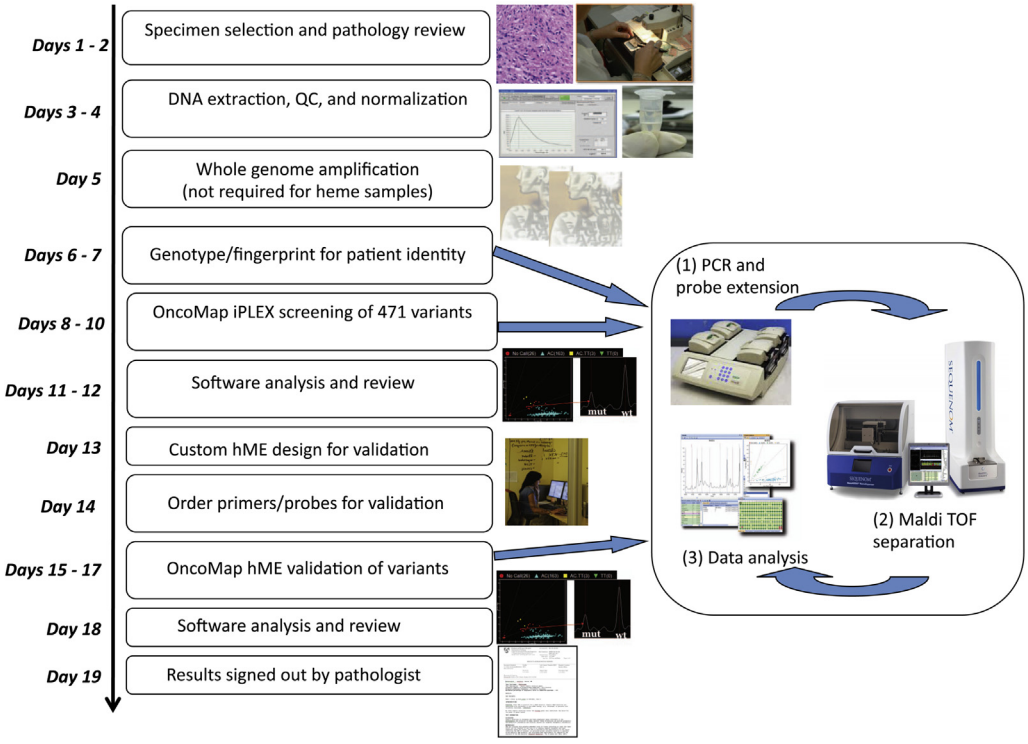


**Figure 1** Technical and bioinformatics steps in the clinical diagnostics pipeline. The timeline from receipt of specimen to generation of a report is 3 to 4 weeks. hMe, homogenous mass-extend; QC, quality control.

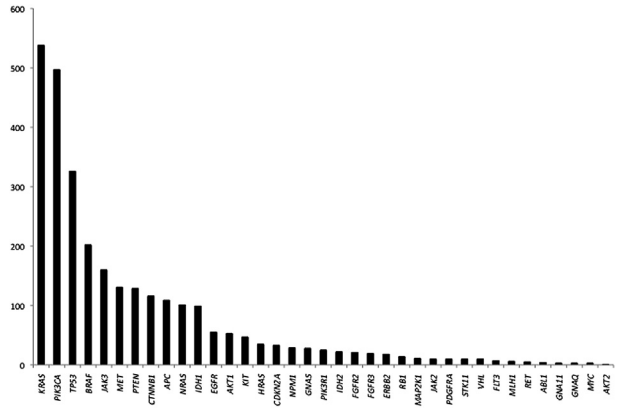


**Figure 2** Incidence of mutations by gene in our cancer cohort. Of the 2890 mutations detected in 5118 patients, approximately 19% were *KRAS* mutations, followed by *PIK3CA* (17% of mutations), *TP53* (11%), and *BRAF* (7%).

well-established published evidence to confirm clinical utility in this tumor type, in at least one of the following contexts: predicting response to treatment with a therapy approved by the Food and Drug Administration, assessing prognosis, establishing a definitive diagnosis, or conferring an inherited increased risk of cancer to this patient and family. In tier 2, the alteration may have clinical utility in at least one of the following contexts: selection of an investigational therapy in clinical trials for this cancer type; limited evidence of prognostic association; supportive of a specific diagnosis; proven association of response to treatment with a therapy approved by the Food and Drug Administration in a different type of cancer; or similar to a different mutation with a proven association with response to treatment with a therapy approved by the Food and Drug Administration in this type of cancer. In tier 3, the alteration is of uncertain clinical utility but may have a role as suggested by at least one of the following: demonstration of association with response to treatment in this cancer type in preclinical studies (eg, *in vitro* studies or animal models); alteration in a biochemical pathway that has other known, therapeutically targetable alterations; alteration in a highly conserved region of the protein predicted, *in silico*, to alter protein function; or selection of an investigational therapy for a different cancer type. Of all specimens tested, 26% have at least one tier 1 (10%) or tier 2 (16%) mutation, which may directly affect clinical decision making.

As previously described, we identified known driver mutations in well-characterized cancers, *KRAS* mutations in colorectal cancer, endometrial cancer, and lung cancer; *BRAF* mutations in melanoma, papillary thyroid carcinoma, and colorectal adenocarcinoma; *PIK3CA* mutations in breast, lung, and endometrial cancers; *EGFR* and *KRAS* mutations in lung adenocarcinoma; and *IDH1* mutations in gliomas. As expected, the distribution of mutations reflected patterns previously observed in human tumors, although the frequency of tumor suppressor mutations was lower (reflective of the reduced coverage of such mutations by OncoMap).

## Mutations Predicting Response/Resistance to Targeted Therapies

### Receptor Tyrosine Kinases EGFR, ERBB2, KIT, PDGFRA

Our genotyping test robustly detected mutations that constitute established markers of response to targeted therapies. *EGFR* mutations predictive of response to erlotinib and gefitinib were identified at 8.6% frequency in non-small cell lung cancer, which is a little lower than expected. This is because of the inability of a genotyping approach to detect each of the possible, variable, *EGFR* exon 19 deletions. Of note, we identified two lung adenocarcinomas with co-occurring *EGFR* L858R and T790M mutations. L858R indicated sensitivity to a TKI therapy, and the presence of a T790M (usually) indicated that resistance to a TKI has emerged. In one case, the specimen tested was a post-TKI relapse specimen, with the L858R allele present at approximately 40% to 50% as determined by relative peak heights of Sequenom assays, and the T790M mutation present in approximately 2% of alleles. Interestingly, in the second case, the patient had a history of multifocal lung adenocarcinoma, and two specimens tested (one from 2008, one from 2012) were genomically distinct; the more recent tumor contained a baseline *de novo* T790M, which, although rare, has

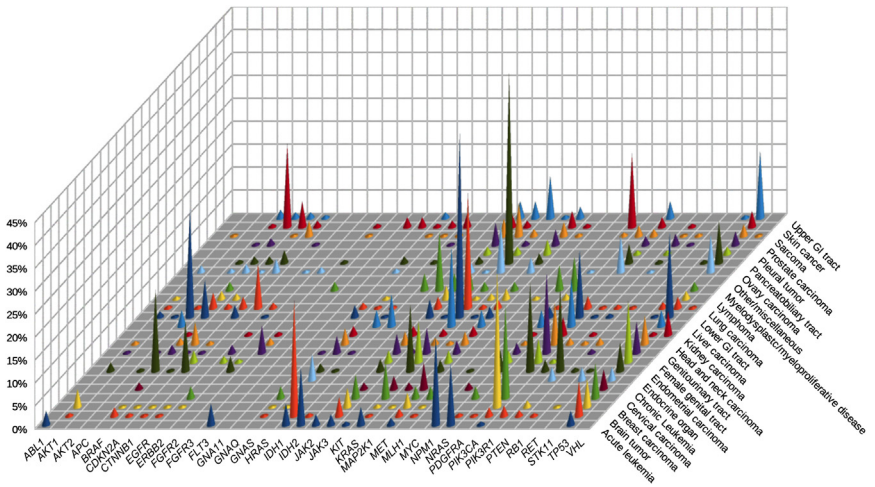


**Figure 3** Landscape of mutations by gene in our cancer cohort. The frequency of gene mutation (normalized by the number of samples in each category) is indicated on the *y* axis, genes mutated on the *x* axis, and cancer type on the *z* axis. GI, gastrointestinal.

been previously reported in large series and predicts a poor response to EGFR TKIs.[31] In this case, the allele fraction (as determined by peak height) of the L858R allele was also higher (10% to 15%) than the T790M allele (approximately 5%).

Activating *ERBB2* mutations were seen in 18 cases, 5 cases of lung adenocarcinomas, 4 cases of bladder cancer, 4 cases of female genital tract cancer, 3 cases of breast cancer, 1 case of colon cancer, and 1 case of kidney cancer. Interestingly, in three cases *ERBB2* mutations co-occurred with canonical *PIK3CA* mutations, and in another two instances (one colon, one ovarian) an *ERBB2* mutation co-occurred with a *KRAS* G12 or G13 mutation.

Fifty-six samples harbored canonical *KIT* or *PDGFRA* mutations. Although 79% of these were gastrointestinal stromal tumors, and an additional 3.5% were noncutaneous melanomas, we also observed targetable mutations in mastocytosis ($n = 3$), germ cell tumors ($n = 3$), a glioblastoma, an acute leukemia, a thymus carcinoma, and an ovarian dysgerminoma. Mutation profiling also identified mutations that confer secondary resistance to targeted therapies (eg, resistance alleles arising during the course of targeted therapy). Six instances of *PDGFRA* mutation D842V or D842Y were identified in five gastrointestinal stromal tumors and one glioblastoma, and these alterations are predictive of resistance to imatinib in gastrointestinal stromal tumors[32]; recent *in vitro* data indicates potential response to newer inhibitors of platelet-derived growth factor receptor, α polypeptide (PDGFRA) such as crenolanib.[33]

### RAS/RAF/MEK/ERK Pathway

*BRAF* V600E mutations linked to sensitivity to inhibitors such as vemurafenib were detected in 44% of papillary thyroid cancers and 34% of melanomas. In addition, activating *BRAF* mutations were also detected in rarer cancers or at lower frequencies such as Langerhans cell histiocytosis,[34] hairy cell leukemia,[35] metanephric adenoma[36]; pancreatic breast, ovarian, and prostate adenocarcinoma,[37] indicating the utility of exploring a targeted inhibitor[38] for these specific patients. Interestingly, we observed two cases of lung adenocarcinoma and one urinary bladder cancer with co-occurring *BRAF* and *KRAS* mutations; in each case the *BRAF* alterations were non-V600E mutations (G464E, G466E, L597V). One instance in a colorectal adenocarcinoma exhibited *BRAF* V600E and a *KRAS* G12D mutation. With mutations that confer heightened sensitivity to targeted therapies, OncoMap robustly detected mutations associated with resistance to several agents. Established examples include *KRAS* mutations in lung cancer (23%), colorectal cancer (42%), and endometrial cancer (20%) that confer resistance to erlotinib, gefitinib (lung cancer), or cetuximab (colorectal cancer).[39–41] *HRAS* mutations were identified in 2 of 10 adrenal gland pheochromocytomas, as recently reported.[42]

Similarly, we identified *MEK1* (*MAP2K1*) mutations in 11 specimens, four lung adenocarcinomas, one oral squamous cell carcinoma, three gastrointestinal tract adenocarcinomas, a breast cancer, a thymoma, and a hairy cell leukemia. *MEK1* mutations have previously been identified in malignant melanomas[43] whereby they often occur with *BRAF* or *NRAS* mutations; there is evidence that some *MEK1* mutations may confer resistance to MEK [mitogen activated protein (MAP) extracellular signal-related kinase (ERK) kinase]/RAF inhibitors in melanoma.

### PI3K/AKT/Mammalian Target of Rapamycin Pathway

Inhibitors of the PI3K/AKT/mammalian target of rapamycin pathway have found promise in preclinical and clinical trials in multiple cancer types.[44] We identified gain-of-function *AKT1* E17K mutations in several meningiomas (as recently identified by our group with the use of whole-genome and whole-exome sequencing[45]), an oral squamous cell carcinoma, a liposarcoma, and the more common events in breast, colorectal, ovarian,[46] endometrial,[7] and lung[6] adenocarcinomas. This mutation may predict resistance to PI3K inhibition (and conceivably receptor tyrosine kinase inhibition) in some contexts.[46] Three hundred sixty-nine additional samples (7% of all patients tested) across all cancer types (predominantly breast) harbored mutations in *PIK3CA*, *PIK3R1*, *PTEN*, or a combination thereof. These mutations might be expected to enrich for tumors responsive to the PI3K inhibitors currently in development.

### Metabolic and Other Signaling Pathways

Several tumors harbored mutations that may have prognostic and therapeutic relevance. For example, *IDH1* and *IDH2* gain-of-function mutations have been identified in leukemias[47] and glioblastomas[48]; in our cohort we identified *IDH1* mutations in these cancers, less commonly in melanoma[49] ($n = 4$), chondrosarcoma,[50] cholangiocarcinoma,[51] and prostate cancer[52] but also in previously unreported cancers such as lung, colorectal, and endometrial adenocarcinomas and a urinary bladder carcinoma.

We identified 28 samples with *GNAS* mutations across many cancer types, some known (lung, pancreatic, and colorectal adenocarcinoma) but also in breast, ovarian, and cervical cancers. All but one of the *GNAS* mutations were codon 201 in exon 8; there was one instance of codon 227 mutation in exon 9. Clinically, *GNAS* mutations in pituitary neoplasms have been associated with increased sensitivity to octreotide (somatostatin agonist) in some studies.[53]

## Noncanonical Mutations in Potentially Actionable Genes

Although genotyping assumes an *a priori* knowledge of specific regions in a gene that may be mutated, the mass spectrometric genotyping assay described here can be designed to incorporate additional sites in genes that may be known to be mutated at a lesser frequency. Although not as comprehensive as full-length sequencing of a gene, OncoMap nonetheless provides more information for some genes than current gold standard clinical tests such as pyrosequencing. For example, somatic mutations of *BRAF* occur at high frequency in numerous human cancers,[54] and the *BRAF* V600E mutation (resulting in increased kinase activity) accounts for >90% of described mutations;

pyrosequencing is often used to detect mutations in amino acids 599 to 601 only. In our cohort, of 202 *BRAF* mutations identified, 34 (17%) were non-V600 mutations, and 8 were indels at/near the V600 locus that were not the canonical c.1799T>A nucleotide change. Although we do not yet know the full implication of all these alterations, we know that V600E- or V600K-mutant tumors may indicate better response to targeted therapies than patients with wild-type tumors,[55] and *BRAF* L597 mutations (seen in a colorectal adenocarcinoma and a bladder carcinoma in our cohort) may indicate sensitivity to MEK inhibitors in melanoma,[56] indicating the utility of using a more comprehensive assay when performing molecular profiles of patients' tumors.

In addition, *BRAF* N581S in a bone marrow myeloproliferative neoplasm was identified; this mutation has been seen rarely in several solid tumors,[14,16,57] but to our knowledge this is the first *BRAF* mutation in myeloproliferative neoplasm.

### Missense Mutations in *MET* and *JAK3* May Be Somatic or Germline

*MET* T1010I (also known as T992I) mutations were observed in a reasonable frequency of our cohort (2.55% of cases). There is conflicting evidence in the literature about the transforming ability of this alteration[58,59]; it has also been identified as a heterozygous single nucleotide polymorphism in a normal (noncancer) population at a frequency of 2.49% (European American population; Exome Variant Server; National Heart, Lung, and Blood Institute Grand Opportunity Exome Sequencing Project, Seattle, WA; *http://evs.gs.washington.edu/EVS*, last accessed November 2013; $P = 0.999$, $\chi^2$ test, no significant difference). Because our OncoMap tests were performed on tumor specimens and not matched germline samples, we cannot determine whether these represent somatic or germline events or a mix of both. [However, the allele frequencies (based on peak heights as the expected locations) for the T1010I allele (expected 50% if heterozygous single nucleotide polymorphism in diploid genome) ranged from 23% to 99% (mean, 46.5%; median, 46.4%), further supporting the likelihood this is a germline variant.] Similarly, evidence exists for the transforming ability of *JAK3* alleles P132T and V722I,[60] but both are also found in normal populations. The frequency of *JAK3* V722I alterations is 2.67% in our cohort, compared with 2.56% in the Exome Sequencing Project database ($P = 0.985$), and *JAK3* P132T occurs at a frequency of 23 of 5118 cases (0.45%), compared with an Exome Sequencing Project frequency of 0.05% ($P = 0.0043$). The difference in *JAK3* P132T incidence in our cancer cohort and a normal cohort may be due to differences in ancestral populations (we used European American numbers as representative of our cohort) or might indicate that (in some cases) the single nucleotide polymorphisms might represent cancer susceptibility single nucleotide polymorphisms; thorough analysis of normal (noncancer) specimen would be necessary to support this.

### Cancers with Co-Occurring Actionable Mutations

The presence of co-occurring mutations in known cancer genes may modify the clinical response to single-agent targeted therapy. In our cohort, 435 patient samples had two mutations, 101 had three mutations, and 22 had four or more mutations (Table 3). Of the 536 cases with two or three mutations, *PIK3CA* was the most frequently co-occurring mutated gene, with 204 specimens harboring mutant *PIK3CA* with another gene (most often *TP53*). Twelve specimens had two mutations within *PIK3CA*. Samples ($n = 206$) had a *KRAS* mutation and another mutation; 134 samples had a *TP53* mutation with another mutation; and 61 specimens (mostly endometrial, breast, lung, and ovarian adenocarcinomas) harbored *KRAS* and *PIK3CA* mutations. As we previously noted, coincident mutations in these genes have been reported in cancers of the large intestine,[61] but they have typically exhibited a mutually exclusive pattern of occurrence in endometrial cancer.[55] Fifty-nine cases had *KRAS* and *APC* mutations.

## Discussion

One of the goals of precision cancer medicine is to combine genetic, genomic, and molecular characterization of a tumor with contextual information on anatomical site and other histological criteria to generate a more accurate diagnosis, prognosis, and/or choice of therapy for a patient. In contrast to many existing clinical tests that focus on one or a small number of gene alterations, newer technologies allow the simultaneous interrogation of many cancer genes. We previously reported the adaptation of genotyping-based mutation profiling for the characterization of both frozen and FFPE-derived tumor specimens in a research setting.[7] The intent of the Profile study was to initiate an enterprise-level genomic characterization study wherein the logistical and scientific barriers to implementation of a precision cancer medicine approach could be identified and resolved. Although Profile testing generates clinical-grade results in a laboratory certified by Clinical Laboratory Improvement Amendments, most genotyping results from such a broad panel have no known clinical meaning for most patients with cancer. Therefore, we initially considered this to be a research test and developed a consenting process for patients.

Of patients who consented, approximately 25% had a specimen at an outside hospital that was not available for testing. Of the 9950 that had consent and material available in our department for testing, >50% were estimated by a pathologist to have sufficient material to test, and, of these, approximately 95% yielded an OncoMap result.

The success rate of generating a profile for a patient who gave consent can be enhanced by improving access to material at other institutions/pathology departments and by using a platform that requires less input DNA (eg, our experience with next-generation sequencing technologies is that they require less than half the amount of input DNA needed for OncoMap).

**Table 3** Samples with Co-Occurring Mutations in Our Cancer Cohort

| Combination | Count | Combination | Count | Combination | Count | Combination | Count |
|---|---|---|---|---|---|---|---|
| *ABL1-ABL1** | 1 | *CDKN2A-HRAS* | 1 | *FLT3-NPM1* | 2 | *KRAS-PIK3CA* | 34 |
| *AKT1-BRAF* | 1 | *CDKN2A-IDH1* | 1 | *GNAS-KRAS* | 9 | *KRAS-PIK3CA-PIK3CA* | 1 |
| *AKT1-CTNNB1* | 3 | *CDKN2A-IDH1-NRAS* | 1 | *GNAS-KRAS-PIK3CA* | 1 | *KRAS-PIK3CA-PTEN* | 5 |
| *AKT1-CTNNB1-PTEN* | 1 | *CDKN2A-JAK3* | 2 | *GNAS-KRAS-TP53* | 3 | *KRAS-PIK3CA-TP53* | 4 |
| *AKT1-JAK3* | 3 | *CDKN2A-KRAS* | 2 | *GNAS-MET* | 1 | *KRAS-PIK3R1* | 1 |
| *AKT1-JAK3-TP53* | 1 | *CDKN2A-KRAS-MET* | 1 | *GNAS-TP53* | 1 | *KRAS-PIK3R1-PTEN* | 1 |
| *AKT1-KRAS* | 3 | *CDKN2A-KRAS-TP53* | 1 | *HRAS-JAK3* | 2 | *KRAS-PTEN* | 8 |
| *AKT1-KRAS-PTEN* | 2 | *CDKN2A-NRAS* | 1 | *HRAS-MET* | 1 | *KRAS-STK11* | 3 |
| *AKT1-NRAS-PIK3CA* | 1 | *CDKN2A-PIK3CA* | 1 | *HRAS-PIK3CA* | 5 | *KRAS-TP53* | 27 |
| *AKT1-PIK3CA* | 1 | *CDKN2A-PIK3CA-TP53* | 1 | *IDH1-JAK3* | 4 | *MAP2K1-PIK3CA* | 1 |
| *AKT1-TP53* | 2 | *CDKN2A-TP53* | 3 | *IDH1-KRAS* | 1 | *MET-NRAS* | 2 |
| *APC-APC** | 1 | *CTNNB1-EGFR* | 3 | *IDH1-MET* | 1 | *MET-PIK3CA* | 7 |
| *APC-APC-KRAS* | 6 | *CTNNB1-EGFR-EGFR* | 1 | *IDH1-NPM1* | 1 | *MET-PIK3CA-PTEN* | 1 |
| *APC-BRAF* | 1 | *CTNNB1-EGFR-JAK3* | 1 | *IDH1-NRAS* | 2 | *MET-PIK3CA-TP53* | 1 |
| *APC-BRAF-TP53* | 1 | *CTNNB1-FGFR2-PTEN* | 1 | *IDH1-PIK3CA-PTEN* | 1 | *MET-RB1* | 1 |
| *APC-CDKN2A-KRAS* | 1 | *CTNNB1-FGFR3* | 1 | *IDH1-PIK3R1* | 2 | *MET-TP53* | 9 |
| *APC-GNAS-KRAS* | 1 | *CTNNB1-GNAS-KRAS* | 1 | *IDH1-TP53* | 14 | *MYC-PTEN* | 1 |
| *APC-JAK3-KRAS* | 1 | *CTNNB1-JAK3-KRAS* | 1 | *IDH2-JAK3-MET* | 1 | *NPM1-NPM1** | 1 |
| *APC-KRAS* | 24 | *CTNNB1-KRAS* | 10 | *IDH2-KIT* | 1 | *NPM1-NRAS* | 3 |
| *APC-KRAS-MET* | 1 | *CTNNB1-KRAS-PIK3CA* | 1 | *IDH2-NPM1* | 3 | *NRAS-NRAS** | 1 |
| *APC-KRAS-PIK3CA* | 11 | *CTNNB1-KRAS-PTEN* | 2 | *IDH2-NPM1-NRAS* | 2 | *NRAS-PIK3CA* | 1 |
| *APC-KRAS-PTEN* | 1 | *CTNNB1-MET* | 1 | *IDH2-NRAS* | 2 | *NRAS-TP53* | 1 |
| *APC-KRAS-TP53* | 7 | *CTNNB1-PIK3CA* | 23 | *JAK2-KIT* | 1 | *PDGFRA-TP53* | 1 |
| *APC-MET* | 1 | *CTNNB1-PIK3CA-PIK3CA* | 1 | *JAK3-KRAS* | 5 | *PIK3CA-CTNNB1* | 1 |
| *APC-NRAS* | 2 | *CTNNB1-PIK3CA-PTEN* | 3 | *JAK3-KRAS-PIK3CA* | 2 | *PIK3CA-HRAS* | 1 |
| *APC-PIK3CA* | 3 | *CTNNB1-PIK3CA-TP53* | 1 | *JAK3-KRAS-PTEN* | 2 | *PIK3CA-KRAS* | 1 |
| *APC-PIK3CA-PTEN* | 1 | *CTNNB1-PIK3R1* | 4 | *JAK3-MAP2K1* | 1 | *PIK3CA-PIK3CA** | 9 |
| *APC-PTEN* | 4 | *CTNNB1-PTEN* | 5 | *JAK3-MET* | 4 | *PIK3CA-PIK3R1* | 1 |
| *APC-RB1* | 1 | *CTNNB1-TP53* | 2 | *JAK3-NRAS* | 3 | *PIK3CA-PIK3R1-PTEN* | 1 |
| *APC-TP53* | 6 | *EGFR-EGFR** | 2 | *JAK3-PIK3CA* | 12 | *PIK3CA-PTEN* | 10 |
| *BRAF-CDKN2A* | 2 | *EGFR-MLH1-PIK3CA* | 1 | *JAK3-PIK3CA-PIK3CA* | 1 | *PIK3CA-PTEN-PTEN* | 1 |
| *BRAF-GNAS* | 1 | *EGFR-PIK3CA* | 1 | *JAK3-PIK3R1* | 2 | *PIK3CA-RB1-TP53* | 1 |
| *BRAF-GNAS-PIK3CA* | 1 | *EGFR-TP53* | 2 | *JAK3-PTEN* | 2 | *PIK3CA-RET* | 1 |
| *BRAF-HRAS* | 1 | *ERBB2-KRAS* | 2 | *JAK3-TP53* | 6 | *PIK3CA-TP53* | 19 |
| *BRAF-IDH1* | 2 | *ERBB2-PIK3CA* | 3 | *KIT-KIT** | 3 | *PIK3CA-TP53-TP53* | 1 |
| *BRAF-JAK3* | 3 | *FGFR2-KRAS-PIK3CA* | 1 | *KIT-KIT-PIK3CA* | 1 | *PIK3R1-PTEN* | 2 |
| *BRAF-JAK3-MET* | 1 | *FGFR2-PIK3CA* | 7 | *KIT-KRAS* | 1 | *PIK3R1-PTEN-PTEN* | 1 |
| *BRAF-JAK3-PIK3CA* | 1 | *FGFR2-PIK3CA-PTEN* | 1 | *KIT-PIK3CA-TP53* | 1 | *PTEN-PTEN** | 2 |
| *BRAF-KRAS* | 4 | *FGFR2-PIK3CA-TP53* | 1 | *KIT-TP53* | 1 | *PTEN-PTEN-TP53* | 1 |
| *BRAF-MET* | 2 | *FGFR2-PTEN* | 3 | *KRAS-KRAS** | 2 | *PTEN-RB1* | 1 |
| *BRAF-NRAS* | 1 | *FGFR2-TP53* | 1 | *KRAS-MET* | 6 | *PTEN-RB1-EGFR* | 2 |
| *BRAF-PIK3CA* | 8 | *FGFR3-KRAS* | 1 | *KRAS-MET-STK11* | 1 | *PTEN-TP53* | 2 |
| *BRAF-PIK3CA-STK11* | 1 | *FGFR3-PIK3CA* | 4 | *KRAS-MYC-PTEN* | 1 | *RB1-TP53* | 3 |
| *BRAF-PTEN* | 1 | *FGFR3-PTEN* | 1 | *KRAS-NPM1-NRAS* | 1 | *TP53-CTNNB1* | 1 |
| *BRAF-TP53* | 6 | *FGFR3-TP53-TP53* | 1 | *KRAS-NRAS* | 2 | *TP53-TP53** | 1 |

More than 10% of specimens had two or three mutations. Combinations of gene mutations are indicated by underline.
*Co-occurring mutation within the same gene.

A key performance characteristic is robust performance in samples derived from FFPE and/or archival tumor material, using a relatively small amount of DNA. Of patients with sufficient material to test, approximately 95% yielded an OncoMap result. Of all samples tested, <0.1% failed genotyping, indicating the utility of a robust platform to screen for cancer-driving mutations. Moreover, a variety of specimen types (solid, blood, bone marrow), fixation method (frozen, fresh, FFPE), specimen age (0 to 10 years), and quality performed well with this platform. OncoMap achieved 98.3% overall sensitivity (95% CI, 94.13%—99.54%) and 100% specificity (95% CI, 98.1%—100%), using clinically validated reference tests as a benchmark, in both fresh/frozen and FFPE-derived tumor DNA, indicating that false positive mutation calls are likely to be relatively rare. The sensitivity of OncoMap, here determined as 5% to 10%, is less than real-time PCR, comparable with pyrosequencing, and exceeds Sanger sequencing, all of which are common cancer molecular

diagnostic technologies. As previously noted,[7] however, achieving this level of specificity requires the implementation of an analytical algorithm in which genotyping data are subjected to automated and manual review of candidate mutations and validation of all candidates by using alternative genotyping chemistries. Thus, clinical implementation of this particular platform requires both genomic data generation and bioinformatic analysis in a molecular pathology or clinical diagnostic setting. The resultant 3- to 4-week turnaround time from specimen receipt to report generation (Figure 1), however, is less than ideal for some clinical cases.

Advances in our understanding of biological driver events for some cancers, coupled with improvements in technologies used to detect somatic cancer alterations, have led to the establishment of personalized cancer medicine programs at several cancer centers in the United States.[19–25,27,41,52] Most of these programs use some form of genotyping to profile patient samples for alterations in a panel of potentially actionable or drugable gene mutations that may inform a therapeutic paradigm for patients.

In this study, we report the clinical implementation of an updated panel of assays interrogating 471 unique sites in 41 known cancer genes. More than 5000 OncoMap profiles were generated over 2 years, from patients with cancer who gave consent, spanning all cancer types across both solid tumors and hematological malignancies. With the use of this panel, we robustly detected mutations in more than one-third of patients tested. Many of these mutations (26%) directly affect clinical use (tier 1 and 2 alterations) and/or predict resistance to existing agents such as TKIs (eg, *EGFR* and *KRAS* mutations) or investigational therapies currently in clinical trials. We also identified multiple gene mutations that may guide the use of emerging agents in specific cancer types, and we found the value of applying this OncoMap platform across a large cohort of patients with cancer. Some recent findings in rarer cancers (such as meningiomas) that were originally identified by large-scale whole-genome or whole-exome sequencing approaches were recapitulated by using genotyping, resulting in the description of mutations that may inform molecular classification and new therapeutic avenues. Finally, we determined that in approximately 10% of cases examined tumor specimens harbor expected and unexpected combinations of gene mutations, thus reinforcing that a broad profile of cancer-driving mutations is informative and may help to further elucidate differential patient responses to targeted therapies or why a long tail of clinical response is seen in patient populations selected for response to a particular therapy.

Although this study indicates the clinical utility of a high-throughput, cost-effective approach to simultaneously detect mutations in multiple cancer genes, we acknowledge the technical limitations of genotyping, which restricts both the number of genes and fraction of base pairs interrogated, the type of alteration investigated (mostly single nucleotide substitutions and small insertions/deletions), the amount of input DNA required (high compared with some other molecular assays), and the labor-intensive and time-consuming nature of a two-chemistry process. In the past decade, major advances in massively parallel sequencing technologies will allow much more comprehensive assessment of the full spectrum of genomic alterations (eg, mutations, indels, copy number changes, structural rearrangements, and epigenetic changes), contributing to individual cancers. Initial reports that use such technologies capable of reading multifaceted genomic information in an efficient, timely, and cost-effective manner have found the utility of this approach for tumor mutation profiling and individualized cancer treatment.[26,27]

Our study represents the first large-scale, enterprise-level application of the OncoMap platform for mutation profiling of all types of cancer in a clinical laboratory. The proven effect of using mutation assessment in the selection of patients for targeted therapies (eg, in BRAF- and ALK-inhibitor phase 1 trials[9,62]) reiterates the need for molecular stratification of patients with cancer. The results of our study highlight several examples of informative oncogene mutations missed by standard single-gene clinical assays and describe a rational framework for enterprise-level tumor profiling to be used as a standard means to guide patient stratification and enrollment for targeted cancer therapies.

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## References

1. International Cancer Genome Consortium, Hudson TJ, Anderson W, Artez A, Barker AD, Bell C, et al: International network of cancer genome projects. Nature 2010, 464:993–998
2. Thiede C, Koch S, Creutzig E, Steudel C, Illmer T, Schaich M, Ehninger G: Prevalence and prognostic impact of NPM1 mutations in 1485 adult patients with acute myeloid leukemia (AML). Blood 2006, 107:4011–4020
3. Falini B, Mecucci C, Tiacci E, Alcalay M, Rosati R, Pasqualucci L, La Starza R, Diverio D, Colombo E, Santucci A, Bigerna B, Pacini R, Pucciarini A, Liso A, Vignetti M, Fazi P, Meani N, Pettirossi V, Saglio G, Mandelli F, Lo-Coco F, Pelicci PG, Martelli MF; GIMEMA Acute Leukemia Working Party: Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. N Engl J Med 2005, 352:254–266
4. Lindeman NI, Cagle PT, Beasley MB, Chitale DA, Dacic S, Giaccone G, Jenkins RB, Kwiatkowski DJ, Saldivar JS, Squire J, Thunnissen E, Ladanyi M; College of American Pathologists International Association for the Study of Lung Cancer and Association for Molecular Pathology: Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology. J Mol Diagn 2013, 15:415–453

5. Gabriel S, Ziaugra L, Tabbaa D: SNP genotyping using the Sequenom MassARRAY iPLEX platform. Curr Protoc Hum Genet 2009. Chapter 2:Unit 2.12
6. Ding L, Getz G, Wheeler DA, Mardis ER, McLellan MD, Cibulskis K, et al: Somatic mutations affect key pathways in lung adenocarcinoma. Nature 2008, 455:1069–1075
7. MacConaill LE, Campbell CD, Kehoe SM, Bass AJ, Hatton C, Niu L, Davis M, Yao K, Hanna M, Mondal C, Luongo L, Emery CM, Baker AC, Philips J, Goff DJ, Fiorentino M, Rubin MA, Polyak K, Chan J, Wang Y, Fletcher JA, Santagata S, Corso G, Roviello F, Shivdasani R, Kieran MW, Ligon KL, Stiles CD, Hahn WC, Meyerson ML, Garraway LA: Profiling critical cancer gene mutations in clinical tumor samples. PLoS One 2009, 4:e7887
8. Chapman MA, Lawrence MS, Keats JJ, Cibulskis K, Sougnez C, Schinzel AC, et al: Initial genome sequencing and analysis of multiple myeloma. Nature 2011, 471:467–472
9. Flaherty KT, Puzanov I, Kim KB, Ribas A, McArthur GA, Sosman JA, O'Dwyer PJ, Lee RJ, Grippo JF, Nolop K, Chapman PB: Inhibition of mutated, activated BRAF in metastatic melanoma. N Engl J Med 2010, 363:809–819
10. Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo N, Sunpaweravong P, Han B, Margono B, Ichinose Y, Nishiwaki Y, Ohe Y, Yang JJ, Chewaskulyong B, Jiang H, Duffield EL, Watkins CL, Armour AA, Fukuoka M: Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 2009, 361:947–957
11. Chapman PB, Hauschild A, Robert C, Haanen JB, Ascierto P, Larkin J, Dummer R, Garbe C, Testori A, Maio M, Hogg D, Lorigan P, Lebbe C, Jouary T, Schadendorf D, Ribas A, O'Day SJ, Sosman JA, Kirkwood JM, Eggermont AM, Dreno B, Nolop K, Li J, Nelson B, Hou J, Lee RJ, Flaherty KT, McArthur GA; BRIM-3 Study Group: Improved survival with vemurafenib in melanoma with BRAF V600E mutation. N Engl J Med 2011, 364:2507–2516
12. Chalhoub N, Baker SJ: PTEN and the PI3-kinase pathway in cancer. Annu Rev Pathol 2009, 4:127–150
13. Krause DS, Van Etten RA: Tyrosine kinases as targets for cancer therapy. N Engl J Med 2005, 353:172–187
14. Sequist LV, Waltman BA, Dias-Santagata D, Digumarthy S, Turke AB, Fidias P, Bergethon K, Shaw AT, Gettinger S, Cosper AK, Akhavanfard S, Heist RS, Temel J, Christensen JG, Wain JC, Lynch TJ, Vernovsky K, Mark EJ, Lanuti M, Iafrate AJ, Mino-Kenudson M, Engelman JA: Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. Sci Transl Med 2011, 3:75ra26
15. Girard N, Sima CS, Jackman DM, Sequist LV, Chen H, Yang JC, Ji H, Waltman B, Rosell R, Taron M, Zakowski MF, Ladanyi M, Riely G, Pao W: Nomogram to predict the presence of EGFR activating mutation in lung adenocarcinoma. Eur Respir J 2012, 39:366–372
16. Sequist LV, von Pawel J, Garmey EG, Akerley WL, Brugger W, Ferrari D, Chen Y, Costa DB, Gerber DE, Orlov S, Ramlau R, Arthur S, Gorbachevsky I, Schwartz B, Schiller JH: Randomized phase II study of erlotinib plus tivantinib versus erlotinib plus placebo in previously treated non-small-cell lung cancer. J Clin Oncol 2011, 29:3307–3315
17. Mak RH, Digumarthy SR, Muzikansky A, Engelman JA, Shepard JA, Choi NC, Sequist LV: Role of 18F-fluorodeoxyglucose positron emission tomography in predicting epidermal growth factor receptor mutations in non-small cell lung cancer. Oncologist 2011, 16:319–326
18. Cadranel J, Zalcman G, Sequist L: Genetic profiling and epidermal growth factor receptor-directed therapy in nonsmall cell lung cancer. Eur Respir J 2011, 37:183–193
19. Dias-Santagata D, Akhavanfard S, David SS, Vernovsky K, Kuhlmann G, Boisvert SL, Stubbs H, McDermott U, Settleman J, Kwak EL, Clark JW, Isakoff SJ, Sequist LV, Engelman JA, Lynch TJ, Haber DA, Louis DN, Ellisen LW, Borger DR, Iafrate AJ: Rapid targeted mutational analysis of human tumours: a clinical platform to guide personalized cancer medicine. EMBO Mol Med 2010, 2:146–158
20. Lau C, Ang D, Brzostowski EB, Riely GJ, Rusch VR, Zakowski MF, Kris MG, Ladanyi M: LC-MAP: a pilot study of prospective profiling of clinical tumor specimens for the presence of key mutations in targetable pathways in patients with lung adenocarcinoma and metastatic colorectal cancer using Sequenom genotyping. J Mol Diagn 2010, 12:910, Abstract ST64
21. Su Z, Dias-Santagata D, Duke M, Hutchinson K, Lin YL, Borger DR, Chung CH, Massion PP, Vnencak-Jones CL, Iafrate AJ, Pao W: A platform for rapid detection of multiple oncogenic mutations with relevance to targeted therapy in non-small-cell lung cancer. J Mol Diagn 2011, 13:74–84
22. Arcila M, Lau CY, Jhanwar SC, Zakowski MF, Kris MG, Ladanyi M: Comprehensive analysis for clinically relevant oncogenic driver mutations in 1131 consecutive lung adenocarcinomas. Mod Pathol 2011, 24:404a, Abstract 1721
23. Arcila M, Lau C, Nafa K, Ladanyi M: Detection of KRAS and BRAF mutations in colorectal carcinoma roles for high-sensitivity locked nucleic acid-PCR sequencing and broad-spectrum mass spectrometry genotyping. J Mol Diagn 2011, 13:64–73
24. Beadling C, Heinrich MC, Warrick A, Forbes EM, Nelson D, Justusson E, Levine J, Neff TL, Patterson J, Presnell A, McKinley A, Winter LJ, Dewey C, Harlow A, Barney O, Druker BJ, Schuff KG, Corless CL: Multiplex mutation screening by mass spectrometry: evaluation of 820 cases from a personalized cancer medicine registry. J Mol Diagn 2011, 13:504–513
25. Gonzalez-Angulo AM, Hennessy BT, Mills GB: Future of personalized medicine in oncology: a systems biology approach. J Clin Oncol 2010, 28:2777–2783
26. Wagle N, Berger MF, Davis MJ, Blumenstiel B, DeFelice M, Pochanard P, Ducar M, Van Hummelen P, MacConaill LE, Hahn WC, Meyerson M, Gabriel SB, Garraway LA: High-throughput detection of actionable genomic alterations in clinical tumor samples by targeted, massively parallel sequencing. Cancer Discov 2012, 2:82–93
27. Roychowdhury S, Iyer MK, Robinson DR, Lonigro RJ, Wu YM, Cao X, Kalyana-Sundaram S, Sam L, Balbin OA, Quist MJ, Barrette T, Everett J, Siddiqui J, Kunju LP, Navone N, Araujo JC, Troncoso P, Logothetis CJ, Innis JW, Smith DC, Lao CD, Kim SY, Roberts JS, Gruber SB, Pienta KJ, Talpaz M, Chinnaiyan AM: Personalized oncology through integrative high-throughput sequencing: a pilot study. Sci Transl Med 2011, 3:111ra21
28. Thomas RK, Baker AC, Debiasi RM, Winckler W, Laframboise T, Lin WM, et al: High-throughput oncogene mutation profiling in human cancer. Nat Genet 2007, 39:347–351
29. Storm N, Darnhofer-Patel B, van den Boom D, Rodi CP: MALDI-TOF mass spectrometry-based SNP genotyping. Methods Mol Biol 2003, 212:241–262
30. Forbes SA, Bhamra G, Bamford S, Dawson E, Kok C, Clements J, Menzies A, Teague JW, Futreal PA, Stratton MR: The Catalogue of Somatic Mutations in Cancer (COSMIC). Curr Protoc Hum Genet 2008. Chapter 10:Unit 10.11
31. Yu HA, Arcila ME, Hellmann MD, Kris MG, Ladanyi M, Riely GJ: Poor response to erlotinib in patients with tumors containing baseline EGFR T790M mutations found by routine clinical molecular testing. Ann Oncol 2014, 25:423–428
32. Demetri GD, von Mehren M, Blanke CD, Van den Abbeele AD, Eisenberg B, Roberts PJ, Heinrich MC, Tuveson DA, Singer S, Janicek M, Fletcher JA, Silverman SG, Silberman SL, Capdeville R, Kiese B, Peng B, Dimitrijevic S, Druker BJ, Corless C, Fletcher CD, Joensuu H: Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. N Engl J Med 2002, 347:472–480
33. Heinrich MC, Griffith D, McKinley A, Patterson J, Presnell A, Ramachandran A, Debiec-Rychter M: Crenolanib inhibits the drug-resistant PDGFRA D842V mutation associated with imatinib-resistant gastrointestinal stromal tumors. Clin Cancer Res 2012, 18:4375–4384
34. Badalian-Very G, Vergilio JA, Degar BA, MacConaill LE, Brandner B, Calicchio ML, Kuo FC, Ligon AH, Stevenson KE, Kehoe SM, Garraway LA, Hahn WC, Meyerson M, Fleming MD, Rollins BJ: Recurrent BRAF mutations in Langerhans cell histiocytosis. Blood 2010, 116:1919–1923

35. Tiacci E, Trifonov V, Schiavoni G, Holmes A, Kern W, Martelli MP, et al: BRAF mutations in hairy-cell leukemia. N Engl J Med 2011, 364:2305−2315
36. Choueiri TK, Cheville J, Palescandolo E, Fay AP, Kantoff PW, Atkins MB, McKenney JK, Brown V, Lampron ME, Zhou M, Hirsch MS, Signoretti S: BRAF mutations in metanephric adenoma of the kidney. Eur Urol 2012, 62:917−922
37. Cho NY, Choi M, Kim BH, Cho YM, Moon KC, Kang GH: BRAF and KRAS mutations in prostatic adenocarcinoma. Int J Cancer 2006, 119:1858−1862
38. Flaherty K, Puzanov I, Sosman J, Kim K, Ribas A, McArthur G, Lee RJ, Grippo JF, Nolop K, Chapman P: Phase I study of PLX4032: proof of concept for V600E BRAF mutation as a therapeutic target in human cancer. J Clin Oncol 2009, 27:15s, Abstract 9000
39. Khambata-Ford S, Garrett CR, Meropol NJ, Basik M, Harbison CT, Wu S, Wong TW, Huang X, Takimoto CH, Godwin AK, Tan BR, Krishnamurthi SS, Burris HA 3rd, Poplin EA, Hidalgo M, Baselga J, Clark EA, Mauro DJ: Expression of epiregulin and amphiregulin and K-ras mutation status predict disease control in metastatic colorectal cancer patients treated with cetuximab. J Clin Oncol 2007, 25: 3230−3237
40. Lievre A, Samalin E, Mitry E, Assenat E, Boyer-Gestin C, Lepere C, Bachet JB, Portales F, Vaillant JN, Ychou M, Rougier P: Bevacizumab plus FOLFIRI or FOLFOX in chemotherapy-refractory patients with metastatic colorectal cancer: a retrospective study. BMC Cancer 2009, 9:347
41. Ogino S, Meyerhardt JA, Cantor M, Brahmandam M, Clark JW, Namgyal C, Kawasaki T, Kinsella K, Michelini AL, Enzinger PC, Kulke MH, Ryan DP, Loda M, Fuchs CS: Molecular alterations in tumors and response to combination chemotherapy with gefitinib for advanced colorectal cancer. Clin Cancer Res 2005, 11:6650−6656
42. Crona J, Delgado Verdugo A, Maharjan R, Stalberg P, Granberg D, Hellman P, Bjorklund P: Somatic mutations in H-RAS in sporadic pheochromocytoma and paraganglioma identified by exome sequencing. J Clin Endocrinol Metab 2013, 98:E1266−E1271
43. Nikolaev SI, Rimoldi D, Iseli C, Valsesia A, Robyr D, Gehrig C, Harshman K, Guipponi M, Bukach O, Zoete V, Michielin O, Muehlethaler K, Speiser D, Beckmann JS, Xenarios I, Halazonetis TD, Jongeneel CV, Stevenson BJ, Antonarakis SE: Exome sequencing identifies recurrent somatic MAP2K1 and MAP2K2 mutations in melanoma. Nat Genet 2012, 44:133−139
44. Janku F, Wheler JJ, Westin SN, Moulder SL, Naing A, Tsimberidou AM, Fu S, Falchook GS, Hong DS, Garrido-Laguna I, Luthra R, Lee JJ, Lu KH, Kurzrock R: PI3K/AKT/mTOR inhibitors in patients with breast and gynecologic malignancies harboring PIK3CA mutations. J Clin Oncol 2012, 30:777−782
45. Brastianos PK, Horowitz PM, Santagata S, Jones RT, McKenna A, Getz G, Ligon KL, Palescandolo E, Van Hummelen P, Ducar MD, Raza A, Sunkavalli A, Macconaill LE, Stemmer-Rachamimov AO, Louis DN, Hahn WC, Dunn IF, Beroukhim R: Genomic sequencing of meningiomas identifies oncogenic SMO and AKT1 mutations. Nat Genet 2013, 45:285−289
46. Carpten JD, Faber AL, Horn C, Donoho GP, Briggs SL, Robbins CM, Hostetter G, Boguslawski S, Moses TY, Savage S, Uhlik M, Lin A, Du J, Qian YW, Zeckner DJ, Tucker-Kellogg G, Touchman J, Patel K, Mousses S, Bittner M, Schevitz R, Lai MH, Blanchard KL, Thomas JE: A transforming mutation in the pleckstrin homology domain of AKT1 in cancer. Nature 2007, 448:439−444
47. Mardis ER, Ding L, Dooling DJ, Larson DE, McLellan MD, Chen K, et al: Recurring mutations found by sequencing an acute myeloid leukemia genome. N Engl J Med 2009, 361:1058−1066
48. Parsons DW, Jones S, Zhang X, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Siu IM, Gallia GL, Olivi A, McLendon R, Rasheed BA, Keir S, Nikolskaya T, Nikolsky Y, Busam DA, Tekleab H, Diaz LA Jr, Hartigan J, Smith DR, Strausberg RL, Marie SK, Shinjo SM, Yan H, Riggins GJ, Bigner DD, Karchin R, Papadopoulos N, Parmigiani G, Vogelstein B, Velculescu VE, Kinzler KW: An integrated genomic analysis of human glioblastoma multiforme. Science 2008, 321:1807−1812
49. Lopez GY, Reitman ZJ, Solomon D, Waldman T, Bigner DD, McLendon RE, Rosenberg SA, Samuels Y, Yan H: IDH1(R132) mutation identified in one human melanoma metastasis, but not correlated with metastases to the brain. Biochem Biophys Res Commun 2010, 398:585−587
50. Amary MF, Bacsi K, Maggiani F, Damato S, Halai D, Berisha F, Pollock R, O'Donnell P, Grigoriadis A, Diss T, Eskandarpour M, Presneau N, Hogendoorn PC, Futreal A, Tirabosco R, Flanagan AM: IDH1 and IDH2 mutations are frequent events in central chondrosarcoma and central and periosteal chondromas but not in other mesenchymal tumours. J Pathol 2011, 224:334−343
51. Borger DR, Tanabe KK, Fan KC, Lopez HU, Fantin VR, Straley KS, Schenkein DP, Hezel AF, Ancukiewicz M, Liebman HM, Kwak EL, Clark JW, Ryan DP, Deshpande V, Dias-Santagata D, Ellisen LW, Zhu AX, Iafrate AJ: Frequent mutation of isocitrate dehydrogenase (IDH)1 and IDH2 in cholangiocarcinoma identified through broad-based tumor genotyping. Oncologist 2012, 17:72−79
52. Mauzo SH, Lee M, Petros J, Hunter S, Chang CM, Shu HK, Bellail AC, Hao C, Cohen C: Immunohistochemical demonstration of isocitrate dehydrogenase 1 (IDH1) mutation in a small subset of prostatic carcinomas. Appl Immunohistochem Mol Morphol 2014, 22:284−287
53. Picard C, Silvy M, Gerard C, Buffat C, Lavaque E, Figarella-Branger D, Dufour H, Gabert J, Beckers A, Brue T, Enjalbert A, Barlier A: Gs alpha overexpression and loss of Gs alpha imprinting in human somatotroph adenomas: association with tumor size and response to pharmacologic treatment. Int J Cancer 2007, 121:1245−1252
54. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, et al: Mutations of the BRAF gene in human cancer. Nature 2002, 417: 949−954
55. Velasco A, Bussaglia E, Pallares J, Dolcet X, Llobet D, Encinas M, Llecha N, Palacios J, Prat J, Matias-Guiu X: PIK3CA gene mutations in endometrial carcinoma: correlation with PTEN and K-RAS alterations. Hum Pathol 2006, 37:1465−1472
56. Cheung HW, Du J, Boehm JS, He F, Weir BA, Wang X, Butaney M, Sequist LV, Luo B, Engelman JA, Root DE, Meyerson M, Golub TR, Janne PA, Hahn WC: Amplification of CRKL induces transformation and epidermal growth factor receptor inhibitor resistance in human non-small cell lung cancers. Cancer Discov 2011, 1:608−625
57. Pirl WF, Traeger L, Greer JA, Bemis H, Gallagher E, Lennes I, Sequist L, Heist R, Temel JS: Tumor epidermal growth factor receptor genotype and depression in stage IV non-small cell lung cancer. Oncologist 2011, 16:1299−1306
58. Tengs T, Lee JC, Paez JG, Zhao X, LaFramboise T, Giannoukos G, Thomas RK: A transforming MET mutation discovered in non-small cell lung cancer using microarray-based resequencing. Cancer Lett 2006, 239:227−233
59. Tyner JW, Fletcher LB, Wang EQ, Yang WF, Rutenberg-Schoenberg ML, Beadling C, Mori M, Heinrich MC, Deininger MW, Druker BJ, Loriaux MM: MET receptor sequence variants R970C and T992I lack transforming capacity. Cancer Res 2010, 70:6233−6237
60. Walters DK, Mercher T, Gu TL, O'Hare T, Tyner JW, Loriaux M, Goss VL, Lee KA, Eide CA, Wong MJ, Stoffregen EP, McGreevey L, Nardone J, Moore SA, Crispino J, Boggon TJ, Heinrich MC, Deininger MW, Polakiewicz RD, Gilliland DG, Druker BJ: Activating alleles of JAK3 in acute megakaryoblastic leukemia. Cancer Cell 2006, 10:65−75
61. Yeang CH, McCormick F, Levine A: Combinatorial patterns of somatic gene mutations in cancer. FASEB J 2008, 22:2605−2622
62. Kwak EL, Bang YJ, Camidge DR, Shaw AT, Solomon B, Maki RG, Ou SH, Dezube BJ, Janne PA, Costa DB, Varella-Garcia M, Kim WH, Lynch TJ, Fidias P, Stubbs H, Engelman JA, Sequist LV, Tan W, Gandhi L, Mino-Kenudson M, Wei GC, Shreeve SM, Ratain MJ, Settleman J, Christensen JG, Haber DA, Wilner K, Salgia R, Shapiro GI, Clark JW, Iafrate AJ: Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. N Engl J Med 2010, 363:1693−1703