Beyond Histology: Translating Tumor Genotypes into Clinically Effective Targeted Therapies

Catherine B. Meador¹, Christine M. Micheel²,⁵, Mia A. Levy²,³,⁵, Christine M. Lovly¹,²,⁵, Leora Horn²,⁵, Jeremy L. Warner²,³,⁵, Douglas B. Johnson²,⁵, Zhongming Zhao²,³,⁵, Ingrid A. Anderson⁵, Jeffrey A. Sosman²,⁵, Cindy L. Vnencak-Jones⁴,⁵, Kimberly B. Dahlman¹,⁵, William Pao¹,²,⁴,⁵,⁶

¹Departments of Cancer Biology, ²Medicine, ³Biomedical Informatics, ⁴Pathology, Microbiology, and Immunology, ⁵Vanderbilt-Ingram Cancer Center, Vanderbilt University School of Medicine, Nashville, Tennessee, 37232, USA

Running title: Tumor Heterogeneity and Targeted Therapies

Keywords: Lung cancer, melanoma, tumor genotyping, personalized medicine, next-generation sequencing

Financial support:

WP received support from the V Foundation and the NCI (R01-CA121210, P01-CA129243, U54-CA143798, Vanderbilt’s Specialized Program of Research Excellence in Lung Cancer grant (CA90949), and the VICC Cancer Center Core grant (P30-CA68485). CML was supported by a Damon Runyon Clinical Investigator Award. CML and DBJ were supported by a K12 grant (K12 CA 0906525). JAS received support from 5K24 CA097588-09, the Mary Hendrickson-Johnson ACS Professorship: Melanoma Professorship, and the Melanoma Research Alliance Team Science Award. ZZ received funding from RO1LM011177 and P30CA68485. CBM was supported by Public Health Service Award T32 GM07347 from the National Institute of General Medical Studies for the Vanderbilt Medical-Scientist Training Program. Additional support was
provided by the TJ Martell Foundation, the Kleberg Foundation, the James C. Bradford Family Foundation, the Joyce Family Foundation, and an anonymous donor.

To whom correspondence should be addressed:

William Pao, MD, PhD
2220 Pierce Ave 777 PRB
Nashville, Tennessee 37232
Phone: 615-343-9454
Fax: 615-343-7602
william.pao@vanderbilt.edu

Conflicts of interest:

CML: Consulting/Advisory Board: Pfizer; Honoraria from Speakers Bureau: Qiagen, Abbott Molecular.

LH: Consultant/advisory board: Bristol-Meyers Squibb and Clovis (compensated) and PUMA (uncompensated); Honoraria: Boehringer Ingelheim; Commercial research funding: Astellas.

JAS: Commercial research grant: Novartis and Bristol-Myers Squibb; Consultant/Advisory board: Amgen, GlaxoSmithKline, Roche.

WP: Consultant: MolecularMD, AstraZeneca, Bristol-Myers Squibb, Symphony Evolution, Clovis Oncology, Exelixis, and Clarient; Commercial research grants: Enzon, Xcovery, AstraZeneca, Symphogen, Clovis Oncology, and Bristol-Myers Squibb; Other: Rights to EGFR T790M testing were licensed on behalf of WP and others by MSKCC to MolecularMD.

The other authors have no conflicts of interest to report.
ABSTRACT

Increased understanding of inter-tumoral heterogeneity at the genomic level has led to significant advancements in the treatment of solid tumors. Functional genomic alterations conferring sensitivity to targeted therapies can take many forms, and appropriate methods and tools are needed to detect these alterations. This review provides an update on genetic variability among solid tumors of similar histologic classification, using non-small cell lung cancer (NSCLC) and melanoma as examples. We also discuss relevant technological platforms for discovery and diagnosis of clinically actionable variants and highlight the implications of specific genomic alterations for response to targeted therapy.
Introduction

The disease ‘cancer’ is in reality a multitude of disease entities. When restricted to a single anatomical site of origin, tumors exhibit a high degree of clinical and histopathological heterogeneity. At the molecular level, such heterogeneity is even more complex. Over the past decade, classification of solid tumors has rapidly shifted from one based on histologic and anatomic characteristics to one increasingly incorporating genomic data. Compared to historical treatment with chemotherapy, targeted therapies specific for certain molecular subtypes of solid tumors have led to increased progression-free and overall survival for patients with metastatic disease, showing that genotype-directed targeted therapies hold significant promise for patients (1-4). Herein, we highlight the ways in which the depth and breadth of available genomic information has allowed for a greater understanding of the diversity of genetic profiles in human cancers. Specifically, we discuss the genetic heterogeneity that exists among large cohorts of solid tumors with common histological classifications and the implications of such variability for response to targeted therapeutics, with a focus on non-small cell lung cancer (NSCLC) and melanoma. The concept of intra-tumoral heterogeneity as well as heterogeneity among multiple lesions within one patient (a type of inter-tumoral heterogeneity) and implications for targeted therapies have been addressed elsewhere and are outside the scope of this review (5, 6).

Genetic alterations and driver mutations in solid tumors

Since the original discovery that oncogenes were mutated forms of normally expressed genes in human cells (7), somatic genomic alterations have been recognized as causative in the initiation and progression of cancer. These genetic changes can take many forms, including point mutations, insertions, deletions, combined insertions/deletions (indels), duplications, inversions, and translocations. In some cases, tumors harbor these mutations in oncogenes (in particular,
tyrosine kinases and serine-threonine kinases), which render them exquisitely sensitive to targeted small-molecule inhibitors (Figure 1) (8). Despite the enormous genetic complexity present in the tumor, these specific genomic alterations, or ‘driver mutations’, cause tumors to become ‘oncogene addicted’, or ultimately reliant on specific signaling pathways such that inhibition of those pathways results in cell death (8).

With the recent explosion of available next-generation sequencing (NGS) technologies, we are now able to detect the whole spectrum of somatic genomic alterations in cancers using a limited number of assays and minimal amounts of tissue. However, because a solid tumor may have up to 400 mutations per megabase (Mb) (9), the task of distinguishing ‘driver’ (causative) versus ‘passenger’ (non-functional) mutations from the pool of somatic mutations observed in tumor genomes is not trivial. Thus, the most challenging task in the identification of targetable oncogenic ‘drivers’ is the integration of the diverse range of available genomic data into biologically and clinically relevant information.

In order to begin to discern potentially functional genomic alterations from the myriad of mutations and structural variants present in solid tumors, large sequencing efforts have been initiated that provide greater statistical power for discovering genomic alterations of biological importance. One such example is The Cancer Genome Atlas (TCGA), which is an initiative sponsored by the National Institutes of Health (NIH) that aims to catalog systematically genetic changes occurring in more than twenty types of human cancers, including NSCLC and melanoma (10). This analysis is made possible by the availability of fresh frozen surgically resected specimens and matched blood samples, which in most cases provide more than enough tissue for multi-platform analysis of somatic alterations at the DNA, RNA, or protein level. A relevant consideration for clinical application of widespread sequencing efforts is the limited
amount and variability in quality of available tumor tissue (usually formalin-fixed and paraffin-embedded). This, along with cost of testing, issues around reimbursement policies, and the bioinformatics expertise necessary for interpretation of results are current barriers to the feasibility of translating certain genomics-based assays into the clinic.

**Diagnostic platforms for molecular classification of tumors in the clinic**

Despite the challenges, development of new and updated platforms for detection of single nucleotide variants (SNVs), copy number variants (CNVs), and structural variants (SVs) with minimal amounts of input genetic material is rapidly evolving. Emerging sequencing technologies have been thoroughly reviewed elsewhere (11-14); here, we discuss available technologies for molecular profiling of tumors for clinical decision making (**Table 1**). Notably, treating physicians need to know the strengths and limitations of the tumor profiling assays that they order for their patients.

For SNVs and small insertions, deletions, or indels, PCR followed by dideoxynucleotide sequencing remains a cost-effective, reliable method for detection of known variants. However, direct sequencing is low-throughput as well as limited in its sensitivity, detecting only variant alleles present at a frequency of at least 20-25%. By contrast, multiplexed assays such as SNaPshot and Sequenom mass ARRAY can query already known mutations in several genes at once, detecting variant alleles present at frequencies as low as 1.56% (15-18). NGS, in the form of targeted/custom panels, whole exome sequencing (WES), or whole genome sequencing (WGS) offers deep coverage (i.e. high sensitivity) and the highest possible throughput in terms of detecting many somatic SNVs, small insertions and/or deletions at once. However, the use of NGS does not necessarily imply comprehensiveness; for example, the Illumina Truseq Amplicon
Cancer Panel (TSACP), a multiplexed amplicon-based targeted re-sequencing assay that encompasses a panel of cancer-associated genes, interrogates only specific exons and may therefore miss detection of certain novel mutations in other locations. Capture-based targeted re-sequencing methods have similar drawbacks; thus, data outputs from these assays must be carefully interpreted and not assumed to be exhaustive in their detection of potentially functional genomic alterations.

While fluorescence in situ hybridization (FISH) and multiplex ligation-dependent probe amplification (MLPA) remain the clinical standard for targeted detection of CNVs, NGS technologies afford higher throughput and unbiased detection of CNVs. Finally, NGS, in particular WGS, provides a mechanism for genome-wide detection of structural variants, eliminating the need for previous knowledge of potential fusions or input of RNA (more easily degraded and thus logistically more difficult to obtain and preserve on a large scale).

Management, analysis, and reporting of NGS data back to the treating physician all remain significant hurdles to widespread adoption of NGS technologies, but algorithms for more automated and accurate variant calling are improving. A relevant consideration regarding the practicality and ethics of NGS is the question of ‘how much is too much?’ These platforms are invaluable for discovery, but adoption into routine patient care will require careful stewardship and meticulous, integrated analysis of these large datasets.

Routine molecular subtyping of solid tumors by known driver mutations

Although many tumors have a broad spectrum of genetic alterations, some of these changes already have well-understood implications for existing and emerging targeted therapies. In these cases, translation to the clinic can be achieved by the design of targeted molecular
genotyping assays that are accurate, sensitive, timely, and cost-effective for cancer patients. Indeed, many such academic, commercial, and government-sponsored targeted genotyping efforts are in progress at centers all over the world. For example, in 2009 the French National Cancer Institute (NCI) implemented a program to provide free tumor genotyping for certain cancer subtypes in 28 public hospitals across the country. The Lung Cancer Mutation Consortium (LCMC) is a similar effort at 14 academic institutions across the United States that was founded with the goal of increasing genotype-driven therapy for lung cancer while laying the foundation for widespread implementation of tumor genotyping across the country (19). Encouragingly, the multitude of clinical tumor genotyping efforts currently underway are too numerous to name, and they are generating an enormous amount of data about the genetic landscape of solid tumors while also facilitating access of patients to personalized cancer therapy.

Here, as a representative use case, we present updated results of molecular profiling from the Vanderbilt Personalized Cancer Medicine Initiative (PCMI). We implemented SNaPshot analysis at the Vanderbilt-Ingram Cancer Center in July, 2010, for routine stratification of NSCLCs and melanomas into clinically relevant molecular subtypes (15, 20). Genotyping is performed on DNA extracted from formalin fixed, paraffin-embedded (FFPE) specimens to assay for common (1% or more frequency in the Catalog of Somatic Mutations in Cancer [COSMIC]) somatic mutations across multiple cancer-associated genes. Together, these SNaPshot panels, which can detect ~40 mutations in 6-9 genes, have been used to inform treatment decisions for nearly 2,000 cancer patients at Vanderbilt (as of August 2013). The data generated from these panels is stored in structured format in the permanent electronic health record and also in the Vanderbilt Research Derivative (RD) for consented patients. The RD is a
comprehensive data warehouse of over 3,000,000 individual patient records that is updated in near-real-time, enabling automated collection and reporting of SNaPshot information.

**Using SNaPshot data to inform clinical care: NSCLC and melanoma as model systems**

NSCLCs are histologically subdivided into adenocarcinomas, squamous cell carcinomas, and large cell carcinomas. Previously lumped together clinically (21), NSCLCs have been shown to harbor recurrent alterations in multiple oncogenes. Among 1,003 NSCLC specimens (predominantly adenocarcinoma) genotyped at Vanderbilt University Medical Center (VUMC) between 2010-2013, 424 harbored known driver mutations, including \( \text{KRAS} \) (22.9\%), \( \text{EGFR} \) (14.8\%), \( \text{PIK3CA} \) (2.1\%), \( \text{BRAF} \) (1.9\%), \( \text{ERBB2} \) (0.9\%), \( \text{MEK1} \) (0.8\%), \( \text{NRAS} \) (0.5\%), \( \text{AKT1} \) (0.3\%), and \( \text{PTEN} \) (0.2\%) (Figure 2A). This breakdown is similar to what has been published in the literature for each of these mutations. Testing for fusions, such as those involving ALK and in some cases, ROS1 and RET, was performed separately. Such ‘driver’ mutations are typically mutually exclusive and serve as a mechanism by which tumors can be sub-classified regarding their likelihood of response to pharmacological inhibition of their activated pathways.

However, even among molecular subsets defined for NSCLCs, heterogeneity exists (Figure 2B). For example, mutations in \( \text{EGFR} \) are known to be present in 10-35\% of NSCLCs (most frequently in adenocarcinomas from former light smokers or never smokers—i.e., fewer than 100 cigarettes over a lifetime) and confer sensitivity to the EGFR TKIs erlotinib, gefitinib, and afatinib (22-25) (Table 2). The majority of these activating mutations in \( \text{EGFR} \) occur either as multi-nucleotide in-frame deletions in exon 19 or as single-nucleotide substitutions (L858R) in exon 21; less frequent lung cancer-associated EGFR mutations that also confer TKI sensitivity include G719A/G719C/G719S (exon 18) and L861Q (exon 21) (26) (Figure 2B). The EGFR
T790M mutation (exon 20) is present in 50% of EGFR-mutant tumors with acquired resistance to first-generation TKIs (erlotinib and gefitinib) and may be a biomarker of sensitivity to third-generation TKIs such as C0-1686 and AZD9291 (27-29).

In addition to EGFR alterations, heterogeneity is seen in other common lung cancer-associated ‘driver’ oncogenes. For example, KRAS-activating mutations, which are found in ~20% of lung adenocarcinomas, can occur at positions G12, G13, and Q61 (30). Though currently less ‘targetable’ than EGFR mutations, KRAS mutations are almost exclusively present in the setting of wild-type EGFR and are thus predictive of insensitivity to EGFR TKIs (30). Recent data from a phase II trial indicate that addition of the MEK inhibitor, selumetinib, to docetaxel increased progression-free survival by 3.2 months compared to docetaxel alone in patients with KRAS-mutant lung cancer (31). A phase III trial evaluating this combination is planned. As more promising targeted therapies become available for KRAS-mutant lung cancer, it may be necessary to elucidate clinically relevant differences among the molecular subsets stratified by location of mutations in the protein.

Recent advances have also been made in our understanding of the biology of melanoma, resulting in similar sub-classifications according to ‘driver’ mutations (Figure 2C) (32). Substitutions of various amino acids for valine 600 (V600) in the kinase domain of BRAF, the most common of such ‘driver’ mutations, are found in approximately 50% of melanomas (most often in primary tumors located on body surfaces with intermittent intense sun exposure). These BRAF V600 mutations are known to lead to activation of the mitogen activated protein kinase (MAPK) pathway (33); they have also been shown to confer sensitivity to the small molecule BRAF serine-threonine kinase inhibitors, vemurafenib and dabrafenib (1, 33-35). Of the BRAF V600 mutations identified to date in melanoma, the V600E substitution comprises approximately
80%; the remaining fraction is made up of V600K (15%), V600M, V600D, and V600R substitutions (all less than 5%) (32) (Figure 2D). Non-V600 mutations in BRAF have also been identified as a distinct molecular subtype (Table 2); specific implications for targeted therapies across the range of BRAF mutations are discussed in greater detail below. In addition to BRAF mutations, other recurrent oncogenic ‘driver’ mutations that have been identified in melanoma include mutations in GNA11, GNAQ (both predominantly found in uveal melanomas), KIT (predominantly mucosal and acral melanomas), NRAS (all sites except uveal), and CTNNB1 (32).

Among 955 melanoma specimens genotyped at Vanderbilt between 2010-2013, 610 harbored known recurrent mutations in genes including BRAF (39.1%), NRAS (18.1%), GNAQ (2.8%), GNA11 (1.3%), KIT (2.3%), and CTNNB1 (1.1%) (32) (Figure 2C). A more comprehensive list of targetable mutations in melanoma and NSCLC can be found in Table 2 (1, 3, 4, 25, 29, 31, 35-86).

Because of substantial international efforts to increase the collective knowledge base regarding the frequencies of actionable genomic alterations in solid tumors, equal efforts are necessary to curate this information such that it can be delivered in a reliable, easily-accessible format. In an attempt to address this need for more efficient translation of scientific progress to clinical application, Vanderbilt created My Cancer Genome (MCG) as an online resource for clinicians and patients worldwide. Routinely edited by field experts around the globe, MCG is a unique database that links scientific literature about known oncogenic ‘drivers’ to information about available clinical trials. As the technology advances for routine tumor genotyping in the clinic, resources like MCG will be critical to facilitate mainstream assimilation of personalized cancer medicine worldwide.
BRAF-omas and ALK-omas: characterizing cancer based on driver mutations rather than histology or site of origin

Despite significant progress in the molecular sub-classification of tumors based on integrated genomic information, the initial categorization of cancer types is still determined by tissue of origin. In other words, the current paradigm dictates that we refer to ‘EGFR-mutant lung tumors’ or ‘BRAF-mutant melanomas’, rather than classifying cancers solely based on their genetic makeup or other molecular features. This phenomenon is the result of decades of organ-centric clinical tradition. However, as large-scale genomic information is now available for many different kinds of cancers, we have seen that some ‘driver’ mutations are shared across tumor types (87). The potential implications of defining tumors by their driver mutations, e.g. “BRAF-omas”, are paradigm-shifting.

Even so, the presence of a ‘driver’ mutation such as mutant BRAF V600E does not automatically mean that tumors are sensitive to BRAF inhibition alone. According to data from COSMIC, lung adenocarcinomas, colorectal adenocarcinomas (CRCs), melanomas, and papillary thyroid cancers (PTCs) are all known to harbor BRAF mutations in approximately 3%, 14%, 45%, and 50% of cases, respectively (Figure 3A). However, the response of these ‘BRAF-omas’ to vemurafenib varies substantially based on tissue of origin (Table 2). While BRAF inhibitors vemurafenib and dabrafenib have achieved overall response rates greater than 50% in clinical trials of patients with V600-mutant BRAF (1, 35, 68, 88, 89) and show early promise in PTCs with BRAF V600E mutations (partial regression or stable disease in 3 of 3 BRAF-mutant tumors in a phase I trial) (90), the efficacy of these inhibitors appears significantly lower in BRAF-mutant CRCs (91, 92). In addition, while V600 substitutions are the most common BRAF alterations found in melanoma, CRC, and PTC, BRAF mutations in lung cancer
commonly occur at non-V600 locations, such as G466, G469, D594 and L597 (93, 94) (Figure 3B). The response rate of lung tumors harboring non-V600 BRAF mutations to BRAF and MEK targeted therapies is currently unknown; however, recent retrospective analyses suggest that NSCLCs harboring non-V600 BRAF mutations may portend a more favorable prognosis than those harboring BRAF V600 mutations (95). Metastatic melanomas harboring L597 mutations have been reported to respond to trametinib and TAK-733 (MEK inhibitors) (66) and potentially vemurafenib (67).

Another example of a targetable ‘driver’ genomic alteration that has been found in multiple different solid tumor types is the rearrangement of the anaplastic lymphoma kinase (ALK). ALK fusions are found in 2-7% of NSCLCs (96) and have also been found in other solid tumors including inflammatory myofibroblastic tumor (IMT), rhabdomyosarcoma, serous ovarian carcinoma, colorectal carcinoma, breast carcinoma, renal medullary carcinoma, renal cell carcinoma, and esophageal squamous cell carcinoma (97). ALK TKIs have proven effective at treating ALK-positive NSCLCs (96), and early clinical studies suggest potential benefit in IMT (98); however their efficacy in other solid tumors with ALK translocations is currently unknown. Furthermore, ALK fusions themselves are heterogeneous. All ALK fusions defined to date contain the entire ALK kinase domain; however, the 5’ gene fusion partner varies. In NSCLC, more than 10 different ALK fusion variants have been described to date. Whether the specific ALK fusion present within the tumor alters sensitivity to ALK TKIs remains to be determined. This heterogeneity even among tumors sharing common ‘drivers’ such as BRAF mutations and ALK rearrangements presents a challenge for the development of targeted therapeutics based on genetic characterization. Further elucidation of the range of drug sensitivities across genetic
subtypes within tumors of common and distinct anatomic locations will be necessary in order to make sense of this biologic diversity and more accurately predict proper therapies for patients.

An example of a new initiative along these lines is the so-called ‘basket trial’, in which inclusion criteria do not require a specific disease subtype but rather allow treatment of multiple cohorts of patients with cancers of different origins that share BRAF V600E mutations in common (99). In stark contrast to the traditional clinical trial methodology requiring large-scale, serially executed trials, the basket trial is designed to facilitate investigation of treatment efficacy in multiple independent cohorts at once. In addition, the trial design promotes real-time evaluation of outcomes such that treatment efficacy data combining tumor type with genetic profiling can be compiled and used to expand promising cohorts. The hope is that, for the responding cohorts, the initial basket trial will then evolve into larger trials, leading to FDA approval and clinical application with minimal turnaround time.

This kind of effort is also part of a broader initiative announced by the NCI National Clinical Trials Network (NCTN) to treat 1,000 tumors with specific targeted therapies on the basis of identified molecular alterations. Termed the NCI-MATCH (Molecular Analysis for Therapy Choice Trial), the goal of this trial is to learn more efficiently about the genetics of cancer from clinical data and to challenge the current standards for treatment indications in cancer therapy (100). Specifically, tumors from patients who have failed standard therapies will be evaluated for known ‘actionable’ genomic alterations and subsequently placed on targeted therapies for which preclinical or early clinical data suggest efficacy for the specific genotype identified. While these trials represent a promising step forward in the field of personalized cancer medicine, there are significant challenges inherent in their design, which will need to be carefully considered in both study implementation and data interpretation. Coordination among
multiple centers will be necessary to gain requisite patient accrual, especially for tumors of rare genotypes. Along those lines, obtaining sufficient statistical power for analysis and establishing robust controls for efficacy comparisons are non-trivial challenges presented by such studies. With such considerations in mind, the proposed study design for NCI-MATCH follows a Simon two-stage design within each drug-by-mutation arm with 30 patients per arm. The dual primary endpoints are proposed to be overall response rate (RR) of 5% versus 25% or PFS of 15% versus 35% at six months (100).

Moving forward, we can also learn from the past: the NCI has recently announced an initiative to analyze tissue of ‘exceptional responders’ of failed past clinical trials from the pre-genomic era, with the hope of expanding our repertoire of paired pharmacologic inhibitors and specific genomic alterations that confer sensitivity. This model further emphasizes the importance of fundamental changes in standard clinical trial design; before biomarker-driven enrollment in trials, potentially useful anti-cancer pharmacologic agents in development for solid tumors may have ‘failed’ approval because they were tested in a cohort of patients whose tumors harbored too much genetic heterogeneity to achieve any statistically significant clinical response or discern in which specific subset(s) the agent shows most efficacy. This initiative is a mechanism by which previously unrecognized tumor heterogeneity is now potentially informative in combination with current sequencing technology.

**Heterogeneity of mechanisms of acquired resistance to targeted therapies**

Despite progress in targeting molecular subsets of NSCLC and melanoma, those patients who initially experience clinical responses to targeted therapy ultimately develop progressive disease, usually within 6-12 months (101, 102). Known mechanisms of resistance to EGFR,
ALK, and BRAF kinase inhibitors have been discussed elsewhere (101-104) and are outside the scope of this review. However, the data so far suggest an equal, if not greater, amount of heterogeneity and complexity and emphasize the importance of rebiopsy for all patients at the time of disease progression to advance our understanding of resistance mechanisms.

**Conclusion**

Continued integration of large (e.g., TCGA) and small (e.g., case reports) datasets combined with advancements in sequencing and informatics technology will deepen our understanding of the heterogeneity of human cancers. Of note, this review focused primarily on heterogeneity at the DNA level, but epigenetic, transcriptional, and post-transcriptional changes may also contribute to heterogeneity among solid tumors. Ultimately, enhancement of our understanding of tumor heterogeneity will provide us with more distinct, clinically relevant molecular subsets of cancer that can be treated with increasing efficacy and lead to further improvement in patient outcomes.

**Acknowledgements**

We would like to thank Lucy Wang, Joseph Burden, Scott Sobecki, and Pam Carney for their help in creating the reporting tool to extract and visualize the Vanderbilt PCMI data reported in this manuscript. We would also like to thank Donald Hucks, Winston Tan, and the VUMC Molecular Diagnostics lab staff for technical assistance with acquisition of data.
References


76. Ascierto PA, Schadendorf D, Berking C, Agarwala SS, van Herpen CM, Queirolo P, et al. MEK162 for patients with advanced melanoma harbouring NRAS or Val600 BRAF


Table 1. Types of clinical molecular tests and variants detected.

✓ Variant detected. • Variant detected with difficulty. a Variant detected if fusion RNA is extracted first. b Variant in gene copy number only. FISH = fluorescence in situ hybridization; indels = mutations including both insertions and deletions; MLPA = multiplex ligation-dependent probe amplification; MS = mass spectrometry; NGS = next-generation sequencing; PCR = polymerase chain reaction; SNVs = single nucleotide variants; SVs = structural variants. Adapted from Vnencak-Jones et al. [97]. "Types of Molecular Testing." My Cancer Genome, http://www.mycancergenome.org/content/other/molecular-medicine/types-of-molecular-tumor-testing. © Copyright 2013 Vanderbilt University.

<table>
<thead>
<tr>
<th>Molecular Methodology</th>
<th>Variant Types</th>
<th>SNVs</th>
<th>Small duplications, insertions, deletions, indels</th>
<th>Exon duplications, deletions or gene copy number changes</th>
<th>SVs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allele-specific PCR</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCR and Sanger dideoxy sequencing</td>
<td>✓</td>
<td>✓</td>
<td>✗</td>
<td></td>
<td>a</td>
</tr>
<tr>
<td>PCR and pyrosequencing</td>
<td>✓</td>
<td>✓</td>
<td>•</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCR and MS</td>
<td>✓</td>
<td>✓</td>
<td>•</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCR and single base extension</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Method</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------------------------------</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MLPA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FISH</td>
<td></td>
<td></td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NGS – custom panels (amplicon capture)</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NGS – custom panels (hybridization capture)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NGS – whole exome sequencing</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NGS – whole genome sequencing</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Alterations in signaling enzymes known to be associated with sensitivity to available targeted therapies.

Specific mutations listed are those found in references cited. Other substitutions in KRAS codons 12, 13, and 61 have been found in NSCLC; differences in their sensitivity to selumetinib + docetaxel are unknown at this time.

Specific mutations listed are those found in references cited. Other substitutions in NRAS codons 12, 13, and 61 have been found in NSCLC; differences in their sensitivity to selumetinib and trametinib are unknown at this time.

V600M/R/D substitutions occur less frequently than V600E/K; specific outcomes for V600M/R/D are not included in these clinical trial data; sensitivity is predicted based on preclinical data and case reports (105-107).

Specific mutations listed are those found in references cited. Other substitutions in NRAS codons 12, 13, and 61 have been found in melanoma; differences in their sensitivity to MEK inhibitors are unknown at this time.

<table>
<thead>
<tr>
<th>Oncogene</th>
<th>Targeted therapy</th>
<th>Level of evidence</th>
<th>Associated publications</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALK fusions</td>
<td>Crizotinib</td>
<td>Phase III trial</td>
<td>Shaw et al. [36]</td>
</tr>
<tr>
<td></td>
<td>IPI-504</td>
<td>Phase II trial</td>
<td>Sequist et al. [52]</td>
</tr>
<tr>
<td></td>
<td>Ganetespib</td>
<td>Phase II trial</td>
<td>Socinski et al. [79]</td>
</tr>
<tr>
<td></td>
<td>LDK378</td>
<td>Phase I trial</td>
<td>Shaw et al. [77]</td>
</tr>
<tr>
<td></td>
<td>AP26113</td>
<td>Phase I trial</td>
<td>Camidge et al. [78]</td>
</tr>
<tr>
<td></td>
<td>CH5424802</td>
<td>Phase I/II trial</td>
<td>Seto et al. [84]</td>
</tr>
<tr>
<td>ALK Fusion + G1269A</td>
<td>Ganetespib</td>
<td>Case report</td>
<td>Sang et al. [49]</td>
</tr>
<tr>
<td>BRAF Y472C</td>
<td>Dasatinib</td>
<td>Case report</td>
<td>Sen et al. [37]</td>
</tr>
<tr>
<td>V600E</td>
<td>Vemurafenib</td>
<td>Case report</td>
<td>Gautschi et al. [53]</td>
</tr>
<tr>
<td></td>
<td>Dabrafenib</td>
<td>Case report</td>
<td>Rudin et al. [38]</td>
</tr>
<tr>
<td>Gene</td>
<td>Mutations</td>
<td>Treatment</td>
<td>Study Type</td>
</tr>
<tr>
<td>--------</td>
<td>-----------</td>
<td>-----------</td>
<td>-------------</td>
</tr>
<tr>
<td>DDR2</td>
<td>S768R</td>
<td>Dasatinib</td>
<td>Case report</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dasatinib + erlotinib</td>
<td>Case report</td>
</tr>
<tr>
<td>EGFR</td>
<td>Exon19del/ L858R</td>
<td>Gefitinib</td>
<td>Phase III trial</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Erlotinib</td>
<td>Phase III trial</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Afatinib</td>
<td>Phase III trial</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dacomitinib</td>
<td>Phase II trial</td>
</tr>
<tr>
<td>Exon 19ins</td>
<td>Erlotinib</td>
<td>Erlotinib + Gefitinib + Afatinib</td>
<td>Retrospective analysis</td>
</tr>
<tr>
<td>G719A/S</td>
<td>Neratinib</td>
<td>Phase II trial</td>
<td>Sequist et al. [43]</td>
</tr>
<tr>
<td>L861Q</td>
<td>Gefitinib + Erlotinib</td>
<td>Case reports</td>
<td>Yeh et al. [50]</td>
</tr>
<tr>
<td>T790M</td>
<td>CO-1686</td>
<td>Phase I trial</td>
<td>Sequist et al. [86]</td>
</tr>
<tr>
<td></td>
<td>AZD9291</td>
<td>Phase I trial</td>
<td>Ranson et al. [29]</td>
</tr>
<tr>
<td>ERBB2</td>
<td>Exon20ins/ G776L</td>
<td>Afatinib</td>
<td>Phase II trial</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Trastuzumab + paclitaxel</td>
<td>Case report</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Trastuzumab + chemo</td>
<td>Retrospective analysis</td>
</tr>
<tr>
<td>FGFR1</td>
<td>Amplification</td>
<td>BGJ398</td>
<td>Case report</td>
</tr>
<tr>
<td>KRAS</td>
<td>^G12A/R/D/C/S/V ^G13D</td>
<td>Selumetinib + docetaxel</td>
<td>Phase II trial</td>
</tr>
<tr>
<td>MEK1</td>
<td>K57N</td>
<td>Selumetinib</td>
<td>Preclinical data</td>
</tr>
<tr>
<td>MET</td>
<td>Amplification</td>
<td>Crizotinib</td>
<td>Case report</td>
</tr>
<tr>
<td>NRAS</td>
<td>^bQ61L/R/K</td>
<td>Selumetinib</td>
<td>Preclinical data</td>
</tr>
<tr>
<td>Oncogene</td>
<td>Targeted therapy</td>
<td>Level of evidence</td>
<td>Associated publications</td>
</tr>
<tr>
<td>---------------</td>
<td>------------------</td>
<td>-------------------</td>
<td>------------------------</td>
</tr>
<tr>
<td>PIK3CA H1047R</td>
<td>BKM120</td>
<td>Phase I trial</td>
<td>Bendell et al. [61]</td>
</tr>
<tr>
<td>RET fusions</td>
<td>Cabozantinib</td>
<td>Phase II trial</td>
<td>Drilon et al. [62]</td>
</tr>
<tr>
<td></td>
<td>Vandetinib</td>
<td>Case report</td>
<td>Gautschi et al. [63]</td>
</tr>
<tr>
<td></td>
<td>Ganestespib</td>
<td>Preclinical data</td>
<td>Sang et al. [49]</td>
</tr>
<tr>
<td>ROS1 fusions</td>
<td>Crizotinib</td>
<td>Case report</td>
<td>Bergethon et al. [64]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Case report</td>
<td>Davies et al. [65]</td>
</tr>
<tr>
<td></td>
<td>Ganestespib</td>
<td>Preclinical data</td>
<td>Sang et al. [49]</td>
</tr>
</tbody>
</table>

**Kinase-targeted therapies in melanoma**

<table>
<thead>
<tr>
<th>Oncogene</th>
<th>Targeted therapy</th>
<th>Level of evidence</th>
<th>Associated publications</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRAF</td>
<td>L597S</td>
<td>TAK-733</td>
<td>Case report</td>
</tr>
<tr>
<td></td>
<td>L597R</td>
<td>Vemurafenib</td>
<td>Case report</td>
</tr>
<tr>
<td></td>
<td>L597V</td>
<td>Trametinib</td>
<td>Case report</td>
</tr>
<tr>
<td></td>
<td>v600E/K/M/R/D</td>
<td>Vemurafenib</td>
<td>Phase I/II trial</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dabrafenib</td>
<td>Phase III trial</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Trametinib</td>
<td>Phase III trial</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dabrafenib + trametinib</td>
<td>Phase I/II trial</td>
</tr>
<tr>
<td></td>
<td>K601E</td>
<td>Trametinib</td>
<td>Case report</td>
</tr>
<tr>
<td>KIT</td>
<td>L576P</td>
<td>Imatinib</td>
<td>Phase II trial</td>
</tr>
<tr>
<td></td>
<td>K642E</td>
<td></td>
<td>Phase II trial</td>
</tr>
<tr>
<td></td>
<td>V559A</td>
<td>Imatinib</td>
<td>Case report</td>
</tr>
<tr>
<td></td>
<td>W557R</td>
<td>Imatinib</td>
<td>Preclinical data</td>
</tr>
<tr>
<td></td>
<td>V559D</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>L576P</td>
<td>Dasatinib</td>
<td>Case report</td>
</tr>
<tr>
<td></td>
<td>W557G</td>
<td>Sunitinib</td>
<td>Retrospective analysis</td>
</tr>
<tr>
<td></td>
<td>V560D</td>
<td>Sorafenib</td>
<td>Case report</td>
</tr>
<tr>
<td></td>
<td>L576P</td>
<td>Nilotinib</td>
<td>Phase II Trial</td>
</tr>
<tr>
<td>V559A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------</td>
<td>----------</td>
<td>----------</td>
<td></td>
</tr>
<tr>
<td>NRAS</td>
<td>&quot;Q61L/K/R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEK162</td>
<td>Phase II trial</td>
<td>Ascierto et al. [76]</td>
<td></td>
</tr>
<tr>
<td>Selumetinib</td>
<td>Phase I trial</td>
<td>Adjei et al. [47]</td>
<td></td>
</tr>
<tr>
<td>Trametinib + PD-0332991</td>
<td>Preclinical data</td>
<td>Kwong et al. [48]</td>
<td></td>
</tr>
</tbody>
</table>
Figure legends

Figure 1. Examples of types of ‘driver’ genomic alterations found in cancer. Schematic representations of mutations known to occur in NSCLC, including point mutations, insertions/deletions, copy number variants, and structural variants. A, single nucleotide variant in exon 21 of *EGFR* (c.2573 T>G) encoding a substitution of arginine for leucine at codon 858 (L858R), and B, combined insertion/deletion (indel) in exon 19 of *EGFR* confer sensitivity to EGFR TKIs. Red indicates a nucleotide or amino acid that has been altered in the mutant form. C, amplification of *MET* and D, structural variants resulting in *EML4-ALK* fusions confer sensitivity to ALK TKIs.

Figure 2. Routine molecular subtyping of non-small cell lung cancers and melanomas. Frequency of mutations identified by SNaPshot genotyping through Vanderbilt’s PCMI from July 2010 to August 2013. A, frequency of lung-cancer associated mutations. 34 lung tumor specimens contained multiple mutations in the genes listed. *Note: ALK FISH performed separately. B, spectrum of mutations identified in *EGFR*. 27 lung tumor specimens contained multiple mutations in *EGFR*. C, frequency of melanoma-associated mutations. 23 melanoma specimens contained multiple mutations in the genes listed. D, spectrum of mutations identified in *BRAF*. 1 melanoma specimen contained multiple mutations in *BRAF*. N = number of tested specimens containing at least one mutation in the genes listed. Frequencies are listed as percentages of total mutations identified.

Figure 3. “BRAF-omas”: location and relative frequency of *BRAF* mutations across multiple different solid tumors. Indications for targeted therapies differ based on anatomical
site (see text for details). A, percentage of lung adenocarcinoma, colon adenocarcinoma, melanoma, and thyroid carcinoma samples with documented $BRAF$ mutations in the Catalog of Somatic Mutations in Cancer (COSMIC) database. B, codon location of non-synonymous $BRAF$ mutations with frequencies of greater than 0.1% in COSMIC database for each tumor type listed in A.
Figure 1:

A. Single nucleotide variant: EGFR L858R (exon 21)

B. Combined insertion/deletion: EGFR exon 19 indel

C. Copy number variant: MET amplification

D. Structural variant: EML4-ALK fusion

© 2014 American Association for Cancer Research
Figure 2:

A  Spectrum of oncogenic “driver” mutations in NSCLC* (n = 1003)

- ERBB2 1.9%
- BRAF 39.1%
- MEK1 0.9%
- NRAS 0.8%
- AKT1 0.5%
- PTEN 0.3%
- PIK3CA 2.1%
- EGFR 14.8%
- KRAS 22.9%
- No mutation detected 55.7%

B  Relative frequency of EGFR mutations in NSCLC (n = 127)

- Exon 20 insertion 7.8%
- L858R 26%
- T790M 18.8%
- No mutation detected 43.5%

C  Spectrum of oncogenic “driver” mutations in melanoma (n = 955)

- KIT 2.3%
- GNAQ 2.8%
- GNA11 1.3%
- CTNNB1 1.1%
- NRAS 18.1%
- BRAF 39.1%
- No mutation detected 35.2%

D  Relative frequency of BRAF mutations in melanoma (n = 382)

- V600E 80%
- V600D 1%
- V600K 19.4%
- V600R 2.6%
- V600Q 0.8%
- V600M 0.3%
- No mutation detected 22.9%
Figure 3:

A Percentage of tumors with BRAF mutations (COSMIC)

- Lung adenocarcinoma: 3%
- Colon adenocarcinoma: 14%
- Melanoma: 45%
- Thyroid carcinoma: 50%

B Percentage of total BRAF mutations: <1%, 1%-10%, 10%-50%, >50%

- Lung adenocarcinoma
- Colon adenocarcinoma
- Melanoma
- Thyroid carcinoma
Beyond Histology: Translating Tumor Genotypes into Clinically Effective Targeted Therapies

Catherine B Meador, Christine M. Micheel, Mia A. Levy, et al.

Clin Cancer Res  Published OnlineFirst March 5, 2014.

Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-13-1591

Author Manuscript
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.