

Beyond Histology: Translating Tumor Genotypes into Clinically Effective Targeted Therapies

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Abstract

Increased understanding of intertumoral 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 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. *Clin Cancer Res*; 20(9); 2264–75. ©2014 AACR.

Introduction

The disease "cancer" is in reality a multitude of disease entities. When restricted to a single anatomic site of origin, tumors exhibit a high degree of clinical and histopathologic 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 with 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 histologic 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 intratumoral heterogeneity as well as heterogeneity among multiple lesions within 1 patient (a type

of intertumoral 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 (Fig. 1; ref. 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; ref. 9), the task of distinguishing "driver" (causative) versus "passenger" (nonfunctional) 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.

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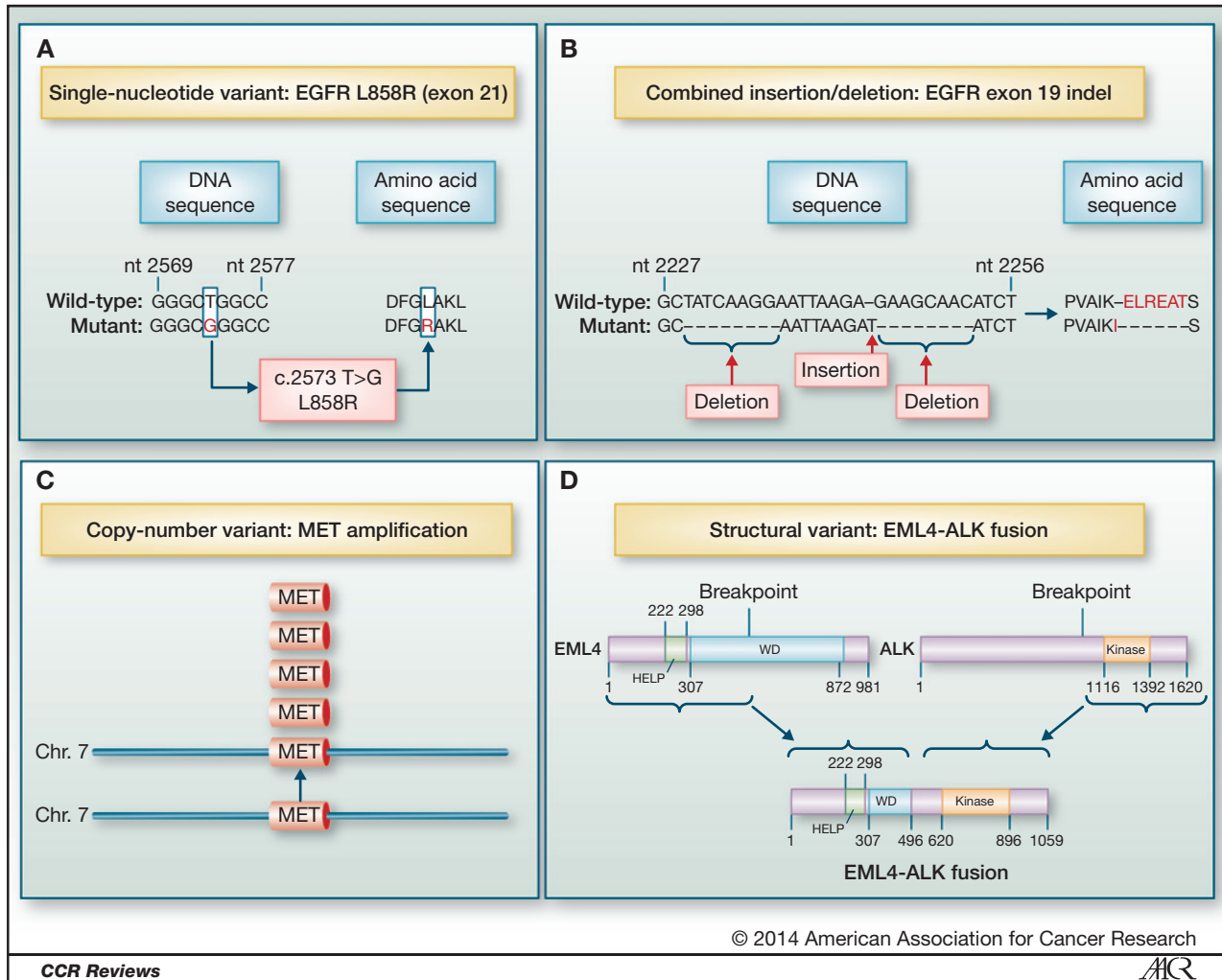


Figure 1. Examples of types of "driver" genomic alterations found in cancer. Schematic representations of mutations known to occur in NSCLC, including point mutations, insertions/deletions, CNVs, and structural variants. A, SNV in exon 21 of *EGFR* (c.2573 T>G) encoding a substitution of arginine for leucine at codon 858 (L858R). B, combined insertion/deletion (indel) in exon 19 of *EGFR* confer sensitivity to EGFR TKIs. Red, a nucleotide or amino acid that has been altered in the mutant form. Amplification of *MET* (C) and structural variants resulting in *EML4-ALK* fusions confer sensitivity to crizotinib (D).

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 biologic importance. One such example is The Cancer Genome Atlas (TCGA), which is an initiative sponsored by the NIH that aims to catalog systematically genetic changes occurring in more than 20 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 multiplatform 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 reimburse-

ment 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 (SNV), copy-number variants (CNV), and structural variants (SV) 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.

Table 1. Types of clinical molecular tests and variants detected

Molecular methodology	Variant types			
	SNVs	Small duplications, insertions, deletions, indels	Exon duplications, deletions, or gene copy-number changes	SVs
Allele-specific PCR	✓			
PCR and Sanger dideoxy sequencing	✓	✓		a
PCR and pyrosequencing	✓	•		
PCR and MS	✓	•		
PCR and single-base extension	✓			
MLPA	✓		✓	
FISH			b	✓
NGS—custom panels (amplicon capture)	✓	✓		
NGS—custom panels (hybridization capture)	✓	✓	✓	•
NGS—WES	✓	✓	✓	•
NGS—WGS	✓	✓	✓	✓

✓, Variant detected; •, variant detected with difficulty; a, variant detected if fusion RNA is extracted first; b, variant in gene copy number only.

Abbreviations: Indels, mutations including both insertions and deletions; MS, mass spectrometry. Adapted from Vnencak-Jones et al. "Types of Molecular Testing." My Cancer Genome, <http://www.mycancergenome.org/content/other/molecular-medicine/types-of-molecular-tumor-testing>. © Copyright 2013 Vanderbilt University.

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% to 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 resequencing 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 resequencing 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.

Although 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 about 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 patients with cancer. 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 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 under way 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 (Nashville, TN) 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 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 approximately 40 mutations in 6 to 9 genes, have been used to inform treatment decisions for nearly 2,000 patients with cancer at Vanderbilt (as of August 2013). The data generated from these panels are 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 and 2013, 424 harbored known driver mutations, including *KRAS* (22.9%), *EGFR* (14.8%), *PIK3CA* (2.1%), *BRAF* (1.9%), *ERBB2* (0.9%), *MEK1* (0.8%), *NRAS* (0.5%), *AKT1* (0.3%), and *PTEN* (0.2%; Fig. 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 anaplastic lymphoma kinase (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 subclassified regarding their likelihood of response to pharmacologic inhibition of their activated pathways.

However, even among molecular subsets defined for NSCLCs, heterogeneity exists (Fig. 2B). For example, mutations in *EGFR* are known to be present in 10% to 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* tyrosine kinase inhibitors (TKIs) erlotinib, gefitinib, and afatinib (22–25; Table 2). The majority of these activating mutations in *EGFR* occur either as multinucleotide 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; ref. 26; Fig. 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 approximately 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 MAP-ERK kinase inhibitor, selumetinib, to docetaxel increased progression-free survival by 3.2 months compared with 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 subclassifications according to "driver" mutations (Fig. 2C; ref. 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, V600R, and V600G substitutions (all less than 5%; ref. 32; Fig. 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

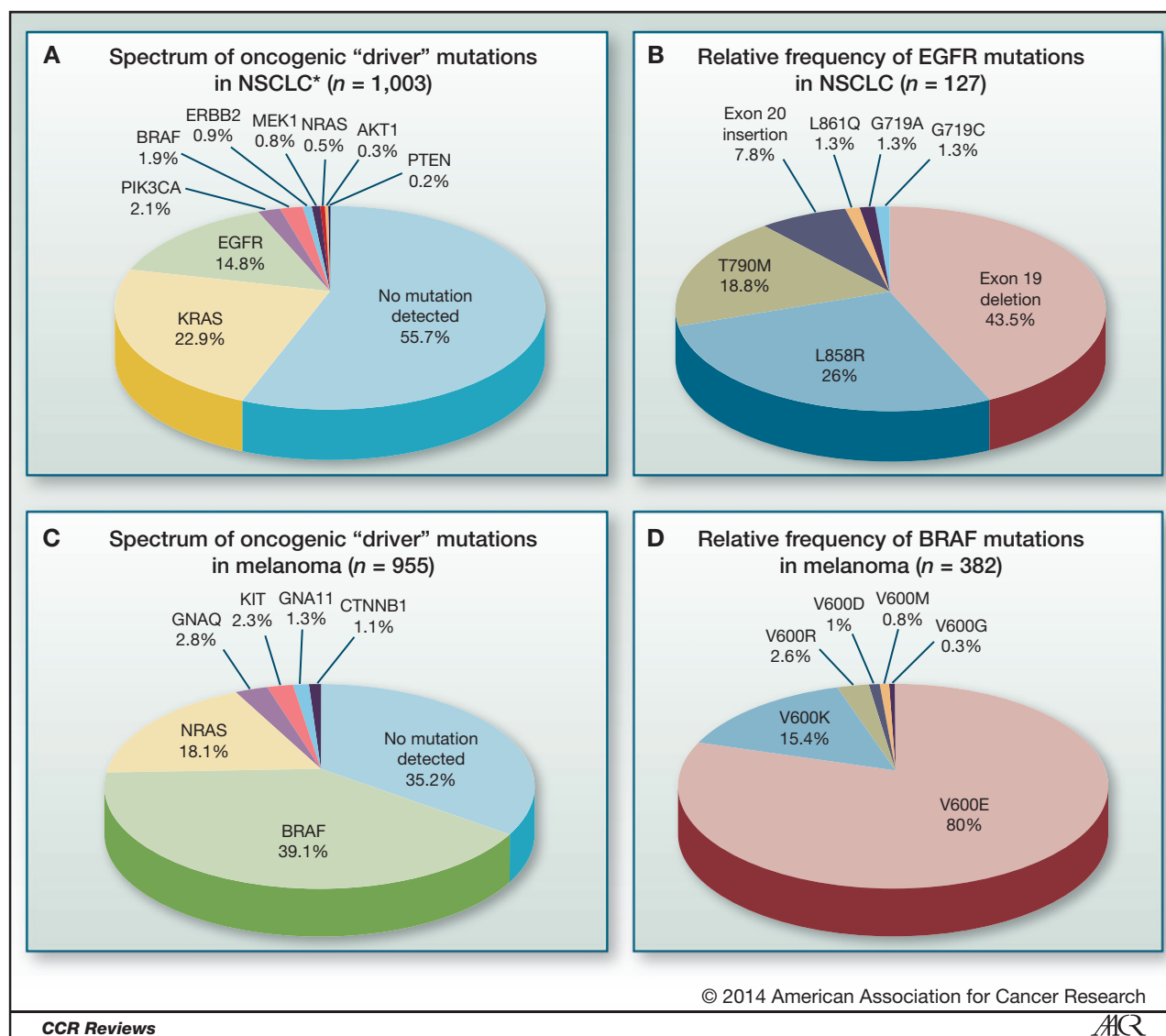


Figure 2. Routine molecular subtyping of NSCLCs and melanomas. Frequency of mutations identified by SNaPshot genotyping through Vanderbilt's PCMI from July 2010 to August 2013. A, frequency of lung cancer-associated mutations. 34 lung tumor specimens contained multiple mutations in the genes listed. *, 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. A total of 23 melanoma specimens contained multiple mutations in the genes listed. D, spectrum of mutations identified in *BRAF*. One 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.

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 and 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%; ref. 32; Fig. 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 about 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

Table 2. Alterations in signaling enzymes known to be associated with sensitivity to available targeted therapies

Oncogene		Targeted therapy	Level of evidence	Associated publications
Kinase-targeted therapies in NSCLC				
ALK fusions		Crizotinib	Phase III trial	Shaw et al. (36)
		IPI-504	Phase II trial	Sequist et al. (52)
		Ganetespib	Phase II trial	Socinski et al. (79)
		LDK378	Phase I trial	Shaw et al. (77)
		AP26113	Phase I trial	Camidge et al. (78)
		CH5424802	Phase I/II trial	Seto et al. (84)
ALK	Fusion + G1269A	Ganetespib	Case report	Sang et al. (49)
BRAF	Y472C	Dasatinib	Case report	Sen et al. (37)
	V600E	Vemurafenib	Case report	Gautschi et al. (53)
		Dabrafenib	Case report	Rudin et al. (38)
DDR2	S768R	Dasatinib	Case report	Pitini et al. (39)
		Dasatinib + erlotinib	Case report	Hammerman et al. (54)
EGFR	Exon19del/L858R	Gefitinib	Phase III trial	Mok et al. (3)
				Mitsudomi et al. (40)
				Maemondo et al. (80)
		Erlotinib	Phase III trial	Zhou et al. (4)
				Rosell et al. (41)
		Afatinib	Phase III trial	Sequist et al. (25)
		Dacomitinib	Phase II trial	Ramalingam et al. (42)
	Exon 19ins	Erlotinib	Retrospective analysis	He et al. (51)
		Gefitinib		
		Afatinib		
	G719A/S	Neratinib	Phase II trial	Sequist et al. (43)
	L861Q	Gefitinib	Case reports	Yeh et al. (50)
		Erlotinib		
	T790M	CO-1686	Phase I trial	Sequist et al. (86)
		AZD9291	Phase I trial	Ranson et al. (29)
ERBB2	Exon20ins/G776L	Afatinib	Phase II trial	De Greve et al. (55)
		Trastuzumab + paclitaxel	Case report	Cappuzzo et al. (56)
		Trastuzumab + chemo	Retrospective analysis	Mazieres et al. (57)
FGFR1	Amplification	BGJ398	Case report	Malchers et al. (81)
KRAS	^a G12A/R/D/C/S/V	Selumetinib + docetaxel	Phase II trial	Janne et al. (31)
	^a G13D			
MEK1	K57N	Selumetinib	Preclinical data	Marks et al. (58)
MET	Amplification	Crizotinib	Case report	Ou et al. (59)
NRAS	^b Q61L/R/K	Selumetinib	Preclinical data	Ohashi et al. (60)
		Trametinib		
PIK3CA	H1047R	BKM120	Phase I trial	Bendell et al. (61)
RET fusions		Cabozantinib	Phase II trial	Drilon et al. (62)
		Vandetinib	Case report	Gautschi et al. (63)
		Ganestespib	Preclinical data	Sang et al. (49)
ROS1 fusions		Crizotinib	Case report	Bergethon et al. (64)
			Case report	Davies et al. (65)
		Ganestespib	Preclinical data	Sang et al. (49)
Kinase-targeted therapies in melanoma				
BRAF	L597S	TAK-733	Case report	Dahlman et al. (66)
	L597R	Vemurafenib	Case report	Bahadoran et al. (67)
	L597V	Trametinib	Phase I/II trial	Falchook et al. (45)

(Continued on the following page)

Table 2. Alterations in signaling enzymes known to be associated with sensitivity to available targeted therapies (Cont'd)

Oncogene	Targeted therapy	Level of evidence	Associated publications	
KIT	°V600E/K/M/R/D	Vemurafenib	Phase III trial	Chapman et al. (1)
		Dabrafenib	Phase III trial	Hauschild et al. (35)
		Trametinib	Phase III trial	Flaherty et al. (46)
		Dabrafenib + trametinib	Phase I/II trial	Flaherty et al. (68)
	K601E	Trametinib	Case report	Kim et al. (82)
	L576P	Imatinib	Phase II trial	Carvajal et al. (70)
	K642E		Phase II trial	Hodi et al. (85)
	V559A	Imatinib	Case report	Terheyden et al. (71)
	W557R	Imatinib	Preclinical data	Beadling et al. (72)
	V559D			
	L576P	Dasatinib	Case report	Woodman et al. (73)
	L576P	Sunitinib	Retrospective analysis	Minor et al. (74)
	W557G			
V560D	Sorafenib	Case report	Quintas-Cardama et al. (75)	
L576P	Nilotinib	Phase II Trial	Cho et al. (83)	
V559A				
NRAS	°Q61L/K/R	MEK162	Phase II trial	Ascierto et al. (76)
		Selumetinib	Phase I trial	Adjei et al. (47)
		Trametinib + PD-0332991	Preclinical data	Kwong et al. (48)

^aSpecific 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.

^bSpecific 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.

^cV600M/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).

^dSpecific 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.

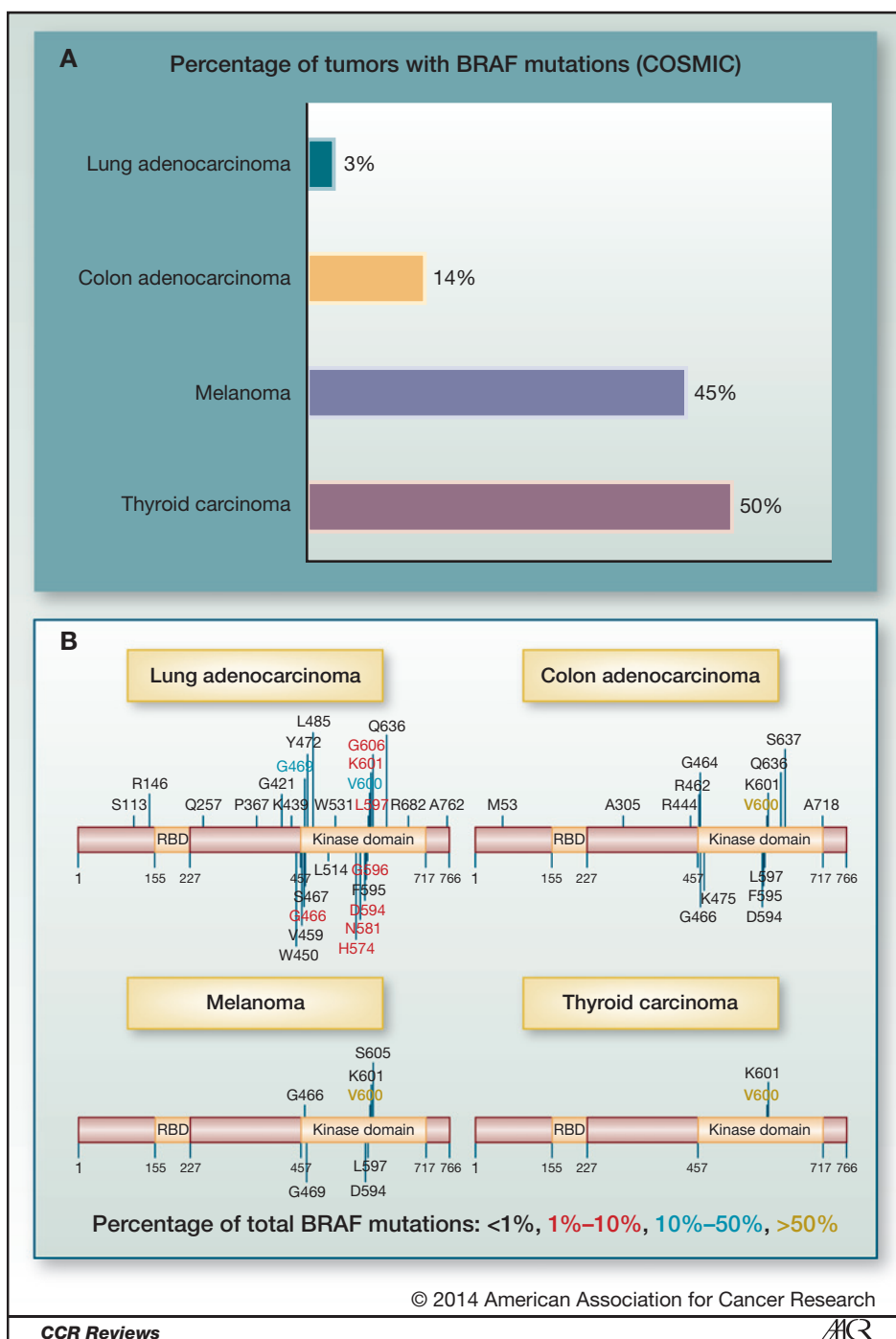
technology advances for routine tumor genotyping in the clinic, resources like MCG will be critical to facilitate mainstream assimilation of personalized cancer medicine worldwide.

BRAF-omas and ALK-omas: Characterizing Cancer Based on Driver Mutations Rather Than Histology or Site of Origin

Despite significant progress in the molecular subclassification 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, colon adenocarcinomas, melanomas, and papillary thyroid cancers (PTC) are all known to harbor BRAF mutations in approximately 3%, 14%, 45%, and 50% of cases, respectively (Fig. 3A). However, the response of these "BRAF-omas" to vemurafenib varies substantially based on tissue of origin (Table 2). Although 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; ref. 90), the efficacy of these inhibitors seems significantly lower in BRAF-mutant colon cancer (91, 92). In addition, although V600 substitutions are the most common BRAF alterations found in melanoma, colon cancer, and PTC, BRAF mutations in lung cancer commonly occur at non-V600 locations, such as G466, G469, D594, and L597 (93, 94; Fig. 3B). The response rate of lung tumors harboring

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



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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; ref. 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 *ALK*. *ALK* fusions are found in 2% to 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 proved 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. 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 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 with 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 U.S. Food and Drug Administration 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 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. Although 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 nontrivial 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 of 5% versus 25% or progression-free survival (PFS) of 15% versus 35% at 6 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 pregenomic 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 anticancer 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 to 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 posttranscriptional 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.

Disclosure of Potential Conflicts of Interest

C.M. Lovly reports receiving speakers bureau honoraria from Abbott Molecular and Qiagen and is a consultant/advisory board member for Pfizer. L. Horn reports receiving a commercial research grant from Astellas; is a consultant/advisory board member for Bristol-Myers Squibb, Clovis, and PUMA (uncompensated); and has provided expert testimony for Boehringer Ingelheim. W. Pao reports receiving commercial research grants from AstraZeneca, Bristol-Myers Squibb, Clovis, Enzon, Symphogen, and Xcovery; is a consultant/advisory board member for AstraZeneca, Bristol-Myers Squibb, Clariant, Clovis, Exelixis, MolecularMD, and Symphony Evolution; and has ownership interest in MolecularMD. No potential conflicts of interest were disclosed by the other authors.

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