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Molecular Diagnostics for Melanoma

Methods and Protocols

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Preface

Abstract

A new taxonomy of cancers defined by molecular signatures with prognostic and therapeutic implications is emerging. Pathological assessment of melanoma that is the current standard method to determine diagnosis is improving due to additional criteria evolving from understanding of biology of melanoma. Genomics, epigenomics, and proteomics approaches have already led to molecular reclassification of melanoma in the context with pathological findings. Moreover, discovery of genetic alterations that drive melanoma progression provide the basis for the development of targeted therapies for patients with metastatic disease. Following these discoveries, the U.S. Food and Drug Administration (FDA) approved in 2011 and 2013 small molecular compounds vemurafenib and dabrafenib, respectively, that provide novel treatment options for melanoma patients. Vemurafenib and dabrafenib target mutated V600 codon of BRAF signaling molecule that is a key effector of RAS/RAF/MEK/ERK pathway. Mutations in this gene occur in over half of melanoma tumors and BRAF V600E mutations are the most common. Additional agent targeting downstream MAPK kinases including MEK1/2 inhibitor, trametinib was also approved by the FDA in 2013. Concomitantly with these drugs, companion diagnostic tests for detection of BRAF V600 mutations were also approved. These tests can identify specific subpopulations of melanoma patients with BRAF V600 codon mutations who most likely will benefit from the therapy. These examples are a prototype of a broad category of personalized treatment that uses a companion test to select patients for specific treatment.

Recent, promising approaches to improve responses in melanoma by blocking negative regulators of T cell activity, i.e., cytotoxic T lymphocyte-associated protein 4 (CTLA-4) and programmed cell death 1 (PD-1) receptors were demonstrated and anti-CTLA drug ipilimumab was approved by the FDA in 2011. While there are no approved diagnostics for immunotherapy, promising markers associated with response to ipilimumab and anti-PD1 therapy were identified. Given that the majority of patients do not respond to these drugs and drugs are highly toxic, predictive markers could potentially improve therapeutic ratio of these drugs.

The primary purpose of this volume is to provide updated information on well-characterized diagnostic and prognostic assays and assays predicting response to treatment for routine testing. The focus is also on a few emerging biomarkers categories with potential clinical validity rather than on early discovery stage. Most of chapters provide detailed protocols for markers' detection and novel technologies with potential for clinical application. Several review chapters provide an overview of the current status in diagnosis and therapy of melanoma and discuss the need to incorporate biomarkers to impact patient care. Important issues related to marker development and validation such as statistical approaches and specimen requirements are also discussed.

Introduction

The incidence of melanoma has more than doubled over the last 20 years in the United States and worldwide, accounting for the majority of skin cancer-related deaths. Traditionally, the prognosis and treatment decisions regarding surgical and adjuvant therapy for a patient with cutaneous melanoma have been based on the current AJCC/UICC (American Joint Committee on Cancer/Union for International Cancer Control) criteria, which include

histological and morphologic analysis of the tumor tissue, the anatomic site of origin, and assessment of local spread using TNM staging procedures (Chapter 17). Although high mitotic rate and ulceration are considered strong negative prognostic factors in melanoma as outlined by the AJCC/IUCC, these histopathological characteristics cannot always accurately predict who will relapse and who will remain disease free. While most cutaneous melanomas classified as early stage are cured by surgical resection, a subgroup of patients will relapse. Conversely, a subset of thick melanomas will not recur after surgical excision of the primary tumor. These observations suggest the utility of additional prognostic and predictive markers to determine the potential for metastatic relapse at the time of diagnosis and to guide therapeutic decisions in adjuvant settings even in early stage melanoma patients.

Advances in cancer biology and powerful technologies provide increased opportunities for the identification of aberrations in genes, proteins, and molecular profiles. Novel technologies including next generation (NGS) sequencing, gene expression arrays, epigenetics, and proteomics-based methods facilitate molecular discoveries and provide critical information regarding melanoma biology and the mechanism of progression. These approaches identify transcriptional changes, germline and somatic mutations, nucleic acid aberrations, and gene expression that may constitute the basis for development of molecular diagnostics. Consequently, a new molecular classification of melanoma is evolving based on chromosomal aberrations, gene mutations and signaling pathways activation that underlie biologically distinct subsets of tumors that require different clinical management. While novel approaches are increasingly explored for routine testing and evaluation of markers for diagnosis, classification, and prognosis, not many novel tests have been validated for their clinical utility and are available for routine use in melanoma. Thus traditional cancer diagnostics approaches including histopathology and immunohistochemistry (IHC) will likely remain standard tools for the future. Upcoming molecular analyses and novel technologies might be incorporated in the context of these established methods when, for example, the definite diagnosis cannot be reached (Chapters 12–16). They potentially might be systematically incorporated and lead to improvement of the AJCC/IUCC system.

When the treatment is developed for a specific biological target that characterizes only some patients, the test could be developed to identify patients with this target. Novel approaches including NGS offer more precise characterization of biologically distinct subtypes of melanoma and become utilized for personalized treatments. Determination of specific therapy target is becoming increasingly important to select melanoma patients most likely to benefit from targeted treatment, or to avoid treating patients likely to have serious adverse reactions (Chapters 1–3).

The recent approval by the FDA of several drugs for the treatment of metastatic melanoma, including vemurafenib and dabrafenib that target the BRAF V600 codon mutated kinase, provide examples of a parallel development of therapeutic treatment and diagnostic tests that both must be approved. The example of targeted drug design includes in vitro companion diagnostic codeveloped with the drug to determine patient eligibility for specific targeted therapy include cobas® 4800V600 Mutation test (Roche Molecular Diagnostics). Cobas test detects V600E mutated form of BRAF kinase to identify patients with this mutation for treatment with BRAF inhibitors. Alongside the recent approval of trametininb targeting downstream MAPK kinases including MEK1/2 the agency also approved a companion genetic test, the THxID BRAF V600 (Biomerieux) that will help determine whether a patient's melanoma cells have the V600E or V600K mutation in the BRAF gene and are eligible for treatment with trametinib and BRAF inhibitors. Many other mutation analysis of genes relevant for melanoma (e.g., NRAS, KIT, MEK) and other genomic aberrations

(e.g., PTEN loss, AKT amplification) are becoming important part of routine pathological assessment available to clinicians for guiding melanoma patients for specific targeted therapy (Chapters 8 and 9).

As molecular mechanisms underlying disease biology are increasingly understood, companion diagnostics are more likely to be developed in tandem with drug, and this principle will be used for a broader category of "personalized" treatments. This approach strongly relies on the accurate stratification of patients based on mutational analysis and potentially immune response profiling to guide drug efficacy and/or resistance using predictive markers.

Novel Technologies for Melanoma Diagnostics

Significant advancements in high-resolution genome-wide molecular techniques have greatly increased an ability to examine and understand genomic changes in melanoma and to identify new genes involved in etiology of this disease. Recent developments in technologies for whole-exome and whole-genome sequencing have already allowed for comprehensive mutation analysis and have led to the identification of a number of new genes and families of genes potentially involved in metastatic melanoma including GRIN2A, MAP3K5, ERBB4, NEDD9, BAP1, RAC1, PREX2, STK19, and others. These approaches enable identification of spectrum of mutations throughout the entire gene including suppressor genes and non-hot spot mutations and will further contribute to the identification of yet unknown rare aberrations. As they offer therapeutic insights for targetable mutations in tumor facilitating biomarker-informed therapies they are likely to be developed into companion diagnostics determining the eligibility criteria for specific targeted therapy. NGS sequencing technologies, however, might not be suitable for identification of genes that are not likely to represent targets for direct therapeutic intervention even in those tumors with identifiable mutations (e.g., 20–30 % of BRAF mutant patients do not respond to vemurafenib). Although NGS-based methods are still mainly used in experimental settings, they are exploited for their clinical utility and rapidly entering the clinical arena as predictive tests for selection of patients for appropriate therapies. High-throughput sequencing approaches to analyze a panel of specific mutations in oncogenes, suppressor genes, and signaling molecules that enable clinicians to match the most appropriate genetic tests to the patient's tumor are currently offered at specialist oncology clinics to identify therapeutic response and emerging resistance in melanoma patients' samples.

Platforms capable of measuring global changes in gene expression levels, high-resolution chromosomal copy number, changes in allelic balance through detection of single-nucleotide polymorphism (SNP), as well as quantitative protein expression, e.g., mass spectrometry or reverse phase protein array (RPPA) are more amenable to impact molecular classification, diagnosis, and prognosis of melanoma. Future pathology assessment will likely include genomic-based approaches such as Comparative genomic hybridization (CGH) and SNP-based platforms. These high-throughput platforms are already available for routine testing to determine prognosis. In addition, technical modifications including automatization, computer-assisted imaging, high-resolution multispectral imaging (Chapter 32), and introduction of multimarker and quantitative IHC-based approaches (Chapters 13 and 14) discussed in this volume support the strengths of these methods to serve the growing prognostic and predictive needs in the clinical management of melanoma.

Validation of Diagnostics

While many of these new tools and approaches proved to be useful for discovering candidate markers, validating cancer biomarkers continues to pose serious challenges. Proper validation of a biomarker is extremely important since the marker is to be used in a clinical setting for the classification of patients into subgroups within morphologically similar tumors and providing prognostic criteria as well as predictive relevance for therapeutic intervention. Marker and assay validation is critical to their clinical application, and several steps including analytical performance and evaluation of clinical validity and utility are needed to successfully demonstrate marker usefulness prior to clinical laboratory implementation. Rapid development of technologies creates specific biases that need to be considered, since strict adherence to protocols developed for each platform is required by the Clinical Laboratory Improvement Amendments (CLIA) certification for each clinical test and clinical laboratory. These technical aspects must be considered in addition to the challenges of clinical validation for the implementation of any marker within the clinical setting.

As part of analytical validation of the assay, the technical and analytical performance characteristics (e.g., stability, accuracy, and reproducibility) of the assay for a marker need to be established. Secondly, determining clinical validity of a molecular diagnostic, i.e., evidence that the marker can separate two subgroups of patients with different outcomes within a large population requires correlation of laboratory test results with clinical parameters. The limited availability of fresh frozen specimens restricted in the past the chance of identifying meaningful clinical association of diagnostics in patient cohorts of sufficient size. However, the advent of technological advances for the analysis of formalin-fixed archival specimens (FFPE) has not only made the discovery of novel markers possible but also facilitated their clinical validation in large cohorts of clinically annotated samples.

Validation studies require a sufficient sample size and independent validation sets to demonstrate prognostic and/or predictive power of molecular factors to warrant patient stratification according to risk for tumor recurrence or for specific therapies. Access to clinically annotated specimens collected from well-controlled clinical studies is an important consideration for establishing the clinical utility of the marker. Specimens collected within multisite clinical trials are especially suitable for retrospective and prospective analyses of associations between outcomes and molecular characteristics. Archived specimens from a large population of untreated patients should be adequate to estimate recurrence in marker-defined subgroups of patients. Specimens from randomized trials with a survival or progression-free survival endpoints are required to establish the clinical validity of markers deemed to be predictive of response to specific treatments. Despite these stringent requirements, it is encouraging to see that many laboratories and new technologies generate potentially clinically applicable tests to guide the treatment of melanoma patients as demonstrated in this volume.

Diagnostics for Precision Care

Historically, systemic therapy for metastatic melanoma provided very low response rates and little to no benefit in overall survival. Recently, several melanoma therapies, including targeted therapies and immunotherapy, have provided alternative treatment options to

melanoma patients. Signaling pathway inhibitors such as vemurafenib and dabrafenib, which target mutated BRAF, have conferred improved progression-free survival in patients with advanced melanoma compared to standard therapy. BRAF inhibitors are indicated only in those patients whose tumors harbor the mutated BRAF as wild-type BRAF melanoma are not likely to respond and because of the potential risk of tumor promotion (Chapters 1–3).

The BRAF gene encodes a serine/threonine kinase that is a key effector of the RAF/MEK/ERK pathway. BRAF is a member of the RAF family of kinases (ARAF, BRAF, and CRAF) and transmits intracellular signal from upstream receptor tyrosine kinases to the downstream serine/threonine kinases MEK and ERK. Activating mutations of BRAF are detected at all stages of melanocytic lesions including nevi, primary, and metastatic melanoma. The most common mutation occurs in exon 15 and results in a valine to glutamate substitution at codon 600 (BRAF V600E) in 85 % of tumors harboring mutated BRAF. Other less-frequent mutations observed in the codon 600 such as V600K and D were identified in 15–20 % of melanoma patients. The cobas® 4800 BRAF V600® molecular test detecting V600E mutation is an example of the companion diagnostic test used to determine patients' eligibility for treatment with vemurafenib in cutaneous melanoma. Another test THxID BRAF companion diagnostic will help determine whether a patient's melanoma cells have the V600E or V600K mutations in the BRAF gene and are eligible for treatment with vemurafenib and dabrafenib as well as trametinib.

Other tests that use different platforms and detect variant mutations at codon 600 including IHC-based test are being performed in diagnostic laboratories for treatment with BRAF targeting agents. However, it is important to consider that the outcome of the assay might differ depending on the platform requirements and specimen analyzed. Ultimately, clinicians must decide which test and in which circumstances should be used reliably to identify patients with BRAF mutation as discussed in Chapters 3 and 8.

The mutation frequency of NRAS in cutaneous melanoma is approximately 20 %, whereas mutations of the other RAS genes such as HRAS and KRAS are rare. The most common NRAS mutations affect residues in exon 1 (codon 12) or 2 (codon 60 and 61) and they are mutually exclusive with activating V600E mutations. Attempts to directly target NRAS have not been successful but RAS activates both the PI3K and MAPK pathways, which demonstrates the importance of RAS mutation testing in melanoma as a biomarker in predicting clinical response to inhibitors of both pathways.

Additional agents targeting downstream MAPK kinases including MEK1/2 (MAP2K1 and MAP2K2) inhibitors show promise both as single agents and in combination with BRAF inhibitors. MEK mutations are thought to be rare but approximately 10 % of melanomas harbor somatic mutations in either MEK1 or 2 which might render MEK mutated tumors for the therapy regimens including MEK inhibitors. Most common mutations that are not associated with resistance in MEK1/2 in melanoma involve exon 3 (P124S and I111S) can be tested using PCR or sequencing based methods. Recently FDA approved drug trametinib targeting downstream MAPK kinases including MEK1/2 provides therapeutic options for patients with tumors harboring MAPK pathway activating mutations including BRAF V600 mutations. Although, MEK inhibitors are designed to target BRAF mutant disease, they could also be effective in melanoma harboring activating mutations in MEK1/2 and RAS mutations.

Genomic alterations in PI3K-AKT signaling pathway are considerably less prevalent than MAPK pathway alterations in melanoma but large genetic diversity affects this pathway.

Although activating mutations in PI3K are rare, downstream effectors including PTEN and AKT are altered in majority of melanomas. AKT is a well known oncogene and AKT3 activation due point mutations or overexpression resulting from increased gene copy number may be common in melanoma. The PTEN tumor suppressor gene encodes a lipid phosphatase that regulates cell survival through PI3K/AKT signaling. Allelic loss or altered expression through epigenetic silencing or mutations of PTEN comprises 20 % and 40 % of melanomas, respectively. PTEN loss leads to activation of AKT and increase in activity of another downstream effector mTOR, and inhibitors of AKT and mTOR are explored for clinical application. Molecular profiling to identify patients whose tumors harbor alterations in PI3K-AKT pathways could provide rationale for developing diagnostics to select patients for treatments targeting this pathway.

While activating BRAF and RAS activating mutations are common in cutaneous melanoma, a much smaller subset of melanomas originating from mucosal, acral and chronic sun damage (CSD) skin demonstrate alterations in the KIT receptor tyrosine which do not coincide with BRAF and NRAS mutations. The vast majority of KIT activating point mutations is in exon 11 and they are sensitive to imatinib and other KIT inhibitors such as sunitinib and desatinib. Use of KIT inhibitors for patients with melanoma harboring KIT mutations represents another successful example of a personalized approach with the use of tumor mutational status to direct therapeutic decisions. KIT expression is also observed in nearly 80 % of cases of uveal melanoma (UM). Although amplification and over-expression of the KIT gene have also been identified in patients with melanoma using fluorescence in situ hybridization (FISH) method its mutational status correlates much better with response to imatinib. Sequencing approach to identify rare melanomas with KIT-mutation and the necessity of this particular molecular testing approach is discussed in Chapter 9.

Furthermore, kinome sequencing analysis has identified a high frequency of activating somatic mutations occurring in the receptor tyrosine kinase ERBB4 gene that is a member of the epidermal growth factor receptor (EGFR) superfamily in cutaneous melanoma. Several of these ERBB4 mutations were shown to increase kinase activity. Given the success of small molecule inhibitors of EGFR specific inhibitors of ERBB4 (e.g., gefitnib, erlotnib and lapatinib) could improve existing melanoma treatments in patients with mutated ERBB4 (Chapter 24).

Uveal melanomas also have frequent mutations in α subunit of the G-proteins GNAQ (35 %) and GNA11 (45 %) each (Chapter 21). These mutations are essentially absent from cutaneous and mucosal melanomas and involve the hot spot residues on exon 5. Mutations in GNAQ and GNA11 activate the MEK–ERK pathway and MEK targeting drugs (e.g., selumetinib) are currently in clinical trials for this disease in patients with GNAQ and GNA11 mutations. Genetic testing and gene expression testing in UM can also identify patients at high risk for development of aggressive tumors (type 2) and those with less aggressive tumors (type 1) and these tests became recently available (Chapters 22 and 23). UM tumors also harbor inactivating somatic mutations in BAP1 gene encoding BRCA1 associated protein that is associated with 84 % of type 2 uveal melanomas. This finding implicates BAP1 pathway as a potential target for therapy in these patients.

Although molecular subtypes of cancer patients selected for treatment by the presence of specific molecular targets often experience impressive responses to molecularly targeted therapy, most will suffer from subsequent recurrence and disease progression. Molecularly characterizing tumors to determine acquired alterations such as new mutations or gene copy number changes that lead to the development of drug resistance are critical for the prediction of drug responsiveness and for the combination of treatment strategies. The

discovery of multiple new genetic events that occur after patients develop resistance to BRAF inhibitors, many of which have implications for rational therapeutic approaches (MAP3K8/COT-1, PDGFRB, IGF1R, amplification or alternative splicing of BRAF) provide future treatment options for patients with these alterations. The upcoming evidence regarding these mechanisms strongly supports the testing of metastases and validating as diagnostics for clinical use (Chapter 10).

Several susceptibility genes have been identified in melanomas and screening for them can be recommended for patients with a family history of melanoma. Several tests identifying germline mutations in melanoma-associated genes, particularly CDKN2A and CDK4 are available (Chapter 20).

Diagnosis and Prognosis

There is a great need to accurately establish diagnosis, prognosis and to define the outcome of individual melanoma patients but the existing clinicopathologic prognostic factors are not always adequate. The pathologic diagnosis of melanoma remains challenging which creates an unmet need not only for the molecular diagnostics but also for improved histopathology and immunohistopathology approaches (Chapters 16 and 17). Better methods to determine melanoma progression would allow further improvements in the prognostic assessment of melanoma patients. Importantly, the accurate prognosis will benefit proper risk stratification of the early stage melanoma patients for adjuvant treatment.

Multiparameter-based approaches for prognostic biomarkers are beginning to emerge that offer a prognostic algorithms based on the combination of several individual biomarkers with the potential for translation into the clinic. Novel quantitative immunostaining platforms enable measurement of protein markers expression in different cell populations including tumors cells and immune cells as well as cellular and subcellular compartments distribution (Chapter 13). Prognostic impact in primary cutaneous melanoma using immunohistochemical assay based on expression levels of three markers was demonstrated across different tissue platforms and different scoring analysis (Chapter 14). Furthermore, emerging methods use integrated approach that includes quantitative multiplex immune phenotyping. Automated image capture integrate fully quantitative measures of protein expression and spacial relationship within the tumor enabling objective and reproducible analysis to define risk of recurrence or to guide therapeutic decision making (Chapter 32).

As patterns of genomic alterations and genomic mutational status for classification of clinical subgroups of melanoma with a high degree of accuracy emerge the methods to detect these molecular features could further advance differential diagnosis in melanoma (Chapter 12). For example, a study of gene copy number alterations in primary melanomas by array CGH has identified distinct subclasses of melanoma on the basis of anatomic site, extent of UV exposure, and mutational status of several genes, including NRAS and BRAF. Using array CGH to compare DNA copy numbers across progression of disease and in cases with challenging histology (e.g., Spitz nevi) consistent chromosomal aberrations can be characterized. High throughput methods such as gene expression arrays, SNP array and microsatellite instability (MSI) are also used as diagnostics to define risk for metastasis in UM (Chapters 22 and 23). These tests also could be useful to distinguish benign lesions from a potentially malignant one or melanoma of unknown origin when traditional pathological and immunohistochemical methods do not provide definite answers (Chapter 12).

Several studies also identified BRAF and NRAS mutations as prognostic markers and association with worse outcome in patients with melanoma. BRAF mutations are more frequent in cutaneous superficial spreading melanomas (SSM) developing in areas of the body with intermittent sun exposure (50 % of tumors), while the rate of BRAF mutations is lower in acral, mucosal, and cutaneous melanomas with evidence of CSD, and they are essentially absent in uveal melanomas. Melanomas associated with CSD commonly have NRAS (15–20 %) and sometimes KIT (2 %) oncogenic mutations.

In vivo imaging of melanoma is also of great importance to improving therapy. Molecular probes targeting tumor specific biomarkers that are capable of greatly improved melanoma detection and accurate melanoma assessment are being developed, contributing to the improvement of personalized management of melanoma (Chapter 30).

Immune Response Markers

Immunotherapy with immunological drugs such as interferon alpha (IFN-α) 2b and interleukin (IL)-2 is characterized by response rates in the range 10–20 %, with approximately 5 % of the patients exhibiting long-term durable responses (Chapter 4). Alongside the recent success of molecularly based targeted drugs therapeutic blockade of immunologic checkpoints demonstrated better efficacy in inducing responses in melanoma patients than nonspecific immune activators. Anti CTLA-4 antibody ipilimumab showed improvement in clinical outcome in patients with metastatic melanoma compared with control therapy (Chapter 6). More recently inhibition of another checkpoint pathway using PD-1 specific antibody entered clinical trials in melanoma patients. As complete responses are achieved with this treatment in a subgroup of patients and it is associated with relatively high toxicity immune profiling as an approach to individualized treatment would greatly improve its clinical utility (Chapter 6).

Promising markers associated with response to ipilimumab were identified. Expression of FOXP3, $ICOS^{hi}$ T cells and indoleamine 2, 3-dioxygenase (IDO) on tumor infiltrating T cells as well as high titers of anti- melanoma associated antigen NY-ESO-1 antibodies in blood, respectively, can predict response to ipilimumab. Tumor cells and stromal cells express PD-1 ligands (PD-L1 or B7-H1 and PD-L2 or B7-DC). The expression of PD-L1 correlates with response to treatment suggesting that it could be a critical component of successful therapy with anti PD-1 and it could serve as predictive marker for individualized treatment with this agent (Chapters 1 and 2). The prognostic significance of tumor infiltrating lymphocytes (TILs) in melanoma was in detail discussed in Chapter 16. More, recently combination of targeted therapy and immunotherapy as well as sequential treatment with different immunotherapy agents are tested (Chapter 7). Combination therapies might provide synergistic effect but there is a need to define an algorithm based on serological markers and tumor profiling to optimally sequence these treatment modalities. Given that treatment with individual agents is associated with toxicity identification of patients who will respond to combination therapy is of high importance. Considering limitations of the tests for individual markers, novel multiparametric system approaches could be best suited for the assessment of the complex system such as immunological function especially in combination treatment setting (Chapter 31).

The significance of the host genetic background needs to be considered as a critical factor for determining melanoma patient survival as well. Polymorphisms in immune response genes in melanoma, such as INFγ and certain chemokines and cytokines, are established prognostic factors and are closely associated with a melanoma outcome. Specific haplotypes of human leukocyte antigen (HLA) class I and II or costimulatory molecules such as B7-H can determine melanoma prognosis and treatment response (Chapters 18 and 19). Sequence analysis also suggests that specific haplotypes associated with the CTLA-4 gene predict effectiveness of anti CTLA-4 immunotherapy.

Another approach that is showing promising results in patients with metastatic melanoma is adoptive cell therapy with use of tumor infiltrating lymphocytes (TIL). In this approach T cell receptors (TCRs) directed against tumor antigens have been genetically engineered to recognize the specific epitope (e.g., NY-ESO-1). Chimeric antigen receptors (CARs) in which antibody-combining site has been genetically linked to TCR-signaling domain have also been used to target T cells to the tumor via recognition of the specific antigen. The efficacy of these multiple immunotherapy approaches critically depends on the presence in the tumor of the antigenic target for immunotherapy. Patient inclusion criteria in the specific treatment approach include tumor that expressed melanoma-specific antigens such as MAGE proteins, MART-1/MelanA, NY-ESO-1, gp100, HMB-45 and tyrosinase or the mutated form of the antigen. Protein-based approaches or genomic tools including RT-PCR or sequencing of the tumor tissue are used to determine the antigen expression or mutational signature of the tumor and to generate or identify specific TILs.

The advances in basic understanding of the immune system and the host–tumor interactions should ultimately lead to more effective and tailor-made immunotherapy. With promising new drugs modulating immune response such as ipilimumab or anti PD-1 and PD-L1 directed therapy, it is essential to further validate the existing markers and search for new diagnostics to identify patient populations that would benefit from these treatments.

Aims and Approach

The primary goal of this volume is to provide an overview of criteria and methods currently used to determine diagnosis, prognosis and predict the response to treatment of patients' with melanoma. Protocol-based chapters focusing on well-established assays that are currently used in clinical laboratories include a sufficient amount of technical detail so that the informed reader can understand the technology to establish the assay for clinical use. We discuss mutational testing as well as promising serologic markers with a potential to impact patient care via stratification of patients for targeted therapies and immunotherapy. Companion diagnostics have shown their clinical utility (e.g., BRAF and KIT) to determine therapeutic eligibility for the melanoma patients. Other markers show their utility to improve diagnosis and prognosis (e.g., CGH, Gene expression profiling, SNPs and MSI). Several assays to detect lymphatic invasion (Chapter 15), tumor infiltrating lymphocytes (Chapter 16) or quantitative measurements of marker expression using IHC-based approaches (Chapters 13, 14 and 32) that improve standard histopathology methods are discussed. Several novel markers with a potential to be included in standard testing are also described (Chapters 28 and 29). In addition, several chapters address novel technologies with the potential to improve mutation detection (Chapter 33) or identify novel approaches for marker detection (Chapters 26, 27, 31, 33, 34 and 35). Review chapters provide an

overview of current strategies in clinical practice (Chapters 1–4) and challenges in clinical management of melanoma patients including the role of markers. The significance of these chapters is to provide background information and the context for the chapters describing specific assays for marker detection. Important issues of specimen collection and statistical design of marker study are addressed (Chapters 36 and 37).

We have tried to include examples of the established methods which represent the major technologies in the field with sufficient technical information and the extensive background information so the reader can both understand the technology and the context of the test. The target audiences are clinical laboratory-based investigators and laboratory scientists who are developers of application of the technologies but other target groups are the users including pathologists and oncologists. We hope that provided examples represent the state of the art in melanoma diagnostics and the review chapters enhance an understanding of the status and challenges in melanoma diagnostics and treatment. As such, hopefully this volume will lead to improvement of the melanoma diagnostic and overall management of patients with this disease.

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