Laboratory Investigation (2013) 93, 858–867

PATHOBIOLOGY IN FOCUS

Melanoma genotypes and phenotypes get personal

Jose M Pimiento[1,2,3], Eileen M Larkin[3], Keiran SM Smalley[4], Ginger L Wiersma[3], Noel R Monks[2], Inna V Fedorenko[4], Chelsea A Peterson[3] and Brian J Nickoloff[2,3,5]

Traditionally, the diagnosis of metastatic melanoma was terminal to most patients. However, the advancements towards understanding the fundamental etiology, pathophysiology, and treatment have raised melanoma to the forefront of contemporary medicine. Indeed, the evidence of durable remissions are being heard ever more frequently in clinics around the globe. Despite having more gene mutations per cell than any other type of cancer, investigators are overcoming complex genomic landscapes, signaling pathways, and immune checkpoints by generating novel technological methods and clinical protocols with breath-taking speed. Significant progress in deciphering molecular genetics, epigenetics, kinase-driven networks, metabolomics, and immune-enhancing pathways to achieve personalized and positive outcomes has truly provided new hope for melanoma patients. However, obstacles requiring breakthroughs include understanding the influence of sunlight exposure on melanoma etiology, and overcoming all too frequently acquired drug resistance, complicating targeted therapy. Pathologists continue to have critically important roles in advancing the field, particularly in the area of transitioning from microscope-based diagnostic reports to pharmacogenomics through molecularly informed tumor boards. Although melanoma is no longer considered just 'one disease', pathologists will continue this rapidly progressing and exciting journey to identify tumor subtypes, to utilize tumorgraft or so-called patient-derived xenograft (PDX) models, and to develop companion diagnostics to keep pace with the bewildering breakthroughs occurring on a regular basis. Exactly which combination of drugs will ultimately be required to eradicate melanoma cells remains to be determined. However, it is clear that pathologists who are as dedicated to melanoma as the pioneering pathologist Dr Sidney Farber was committed to childhood cancers, will be required as the battle against melanoma continues. In this review, we describe what sets melanoma apart from other tumors, and demonstrate how lessons learned in the melanoma clinic are being transferred to many other types of aggressive neoplasms.

Laboratory Investigation (2013) **93,** 858–867; doi:10.1038/labinvest.2013.84; published online 1 July 2013

KEYWORDS: *BRAF*; genomics; immunotherapy; MAPK signaling; patient-derived tumorgrafts; personalized medicine; vemurafenib

Remarkable and rapid progress has been made in basic and clinical research, including new translational approaches of relevance to malignant melanoma (MM). MM remains one of the most virulent neoplasms capable of evading the body's defense mechanisms. Whether it be strengthening the molecular links between sunlight and MM etiology, broadening the genomic landscape, employing more precise diagnostic tools, probing more deeply into MM pathobiology, or developing more effective agents for advanced disease, it is clear that lessons learned from MM studies are permeating throughout the medical community. MM was previously renowned for its resistance to 'killing' by conventional therapies. Now, a more complete delineation of driver mutations, a better understanding of the aberrant signaling pathways, and tumor microenvironmental influences (including exosomes) have converged together with the application of genomically informed tumor board principles and harnessing of the immune system to benefit MM patients. This review integrates major scientific and clinical advances pertaining to MM and discusses implications for future progress. Owing to space constraints, the primary focus among genomic and signaling events will highlight a core

[1]Division of Surgical Oncology, Lacks Cancer Center at Mercy Health-Saint Mary's, CHE/Trinity Health, Inc, Grand Rapids, MI, USA; [2]Center for Translational Medicine, Van Andel Institute, Grand Rapids, MI, USA; [3]College of Human Medicine, Michigan State University, Grand Rapids, MI, USA; [4]Department of Oncological Sciences, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA and [5]Dermatopathology Unit, Lacks Cancer Center at Mercy Health-Saint Mary's, CHE/Trinity health, Inc, Grand Rapids, MI, USA

Correspondence: Dr BJ Nickoloff, MD, PhD, at the Nicholas V. Perricone, MD, Division of Dermatology and Cutaneous Sciences, Michigan State University College of Human Medicine, 15 Michigan Street NE, Suite 419, Grand Rapids, MI 49503, USA or Center for Translational Medicine, Van Andel Institute, 333 Bostwick Ave. NE, Room 5008, Grand Rapids, MI 49503, USA.

E-mail: nickolo4@msu.edu or brian.nickoloff@vai.org

Received 8 May 2013; revised 28 May 2013; accepted 1 June 2013

MM-relevant pathway involving Ras/Raf/MEK/ERK. The ultimate goals remain: improved methods for prevention and early detection; more accurate diagnostic tools and predictive preclinical models; and combination therapies that overcome survival pathways; prevention of drug resistance using on–off scheduling; and enhanced long-term survival for MM patients and their families in a personalized medicine era. As is abundantly clear, MM is not one disease. The MM moniker covers dozens of heterogeneous disease states with clinically distinct subsets, thereby presenting sufficient challenges requiring many additional years of intense study and rigorous cooperation by pathologists and non-pathologists to effectively deal with this common, complicated, and deadly tumor that kills one patient every hour. Just as every individual is unique, each MM tumor has a uniquely altered genome. Despite a great deal of genetic heterogeneity, grouping of this information into common pathways facilitates recognition of common therapeutic targets useful in molecularly defined groups of MM patients. As new data emerge from the ENCODE project, and other studies of the functioning elements of the genome, discoveries of molecular events in the 90%+ of the so-called 'junk' DNA (noncoding), or dark matter of DNA are likely to shed new light on tumors derived from brown/red/black pigment-producing cells located throughout the body.

Birth of Nevi and Nevi at Birth: Timing of Mutations

From an embryological and developmental perspective, the neural crest complements traditional ectoderm, mesoderm, and endoderm germ layers. Neural crest-derived cells, by virtue of their relationship to an incredibly diverse assortment of human diseases, attract interest from many investigative perspectives beyond nevi and melanoma.[1] Indeed, it is now possible to direct cells into a neural crest stem cell fate to guide better understanding of diseases involving the craniofacial skeleton, cornea, teeth, and nervous systems.[1] Melanocytes are neural crest-derived and are responsible for producing melanin by virtue of uniquely possessing tyrosinase, the enzyme used for creating the distinguishing pigment. The majority of nevi occurring after birth possess a mutation in the *BRAF* gene that encodes a serine/threonine kinase central to the mitogen-activated protein kinase (MAPK)-signaling pathway designated BRAFV600E.[2] Mutations in *BRAF* are common in numerous malignancies and occur in about 50% of all MM.[3] As the vast majority of acquired nevi do not progress to MM, the BRAFV600E mutation is necessary, but alone not sufficient for MM formation, hence requiring additional mutagenic events. Approximately 1% of newborns are affected by congenital melanocytic nevi (CMN), which may be small or may cover large portions of the body. Mutations in *NRAS* are associated not only with CMN,[4] but also with neurological lesions, indicating that a single post-zygotic event is responsible for these spatially distinct disorders.[5] Congenital expression of *NRAS* mutations is also implicated in young children with MM in the brain.[6] In two families, germline mutations in the BRCA1-associated protein-1 (BAP1) gave rise to melanocytic lesions with unusual histological appearances, including Spitz tumor-like regions.[7] In sporadic cases, loss of BAP1 is accompanied by BRAFV600E mutations, but the distinctive histological appearance of large epithelioid cells is a shared trait between familial and sporadic cases.[8] With the availability of antibodies for immunohistochemical (IHC) detection of the presence of BRAFV600E and/or loss of BAP1, rapid progress should be made in combination with whole-exome sequencing to better define these molecular events in many different subsets of nevi beyond CMN, atypical Spitz tumors, blue nevi, and so on. This will likely require new recommendations for categorization and treatment approaches.[9]

Approximately 10% of all MM have an inherited predisposition, and melanoma-prone families involve the 9p21 locus that encodes at least two distinct proteins known as p16INK4a and p19Arf.[10] The first protein regulates cell cycle activity, whereas the second protein regulates p53 stability. Epigenetic silencing of the p16INK4a locus via promoter methylation may contribute to *NRAS*-mutated MM.[11] The p16INK4a locus, besides regulating cell cycle activity, may also regulate production of reactive oxygen species (ROS) that may occur in the absence of exogenously induced oxidative stress.[12] The role for ROS in generating DNA mutations in MM, regardless of the exposure to sunlight, has gained momentum due to recent findings with red-hair-bearing mice (so-called ginger mice), in which pheomelanin predominates over eumelanin.[13] Using genomically distinct strains of mice, it was demonstrated that there might be an ultraviolet radiation-independent pathway to MM carcinogenesis. The pigmentation phenotype resulting from the ratio of pheomelanin to eumelanin reflects polymorphisms in the melanocortin 1 receptor (*MC1R*) gene and in tyrosinase gene activity that can lead to a UV-radiation-independent carcinogenesis in mice. This demonstrates complexion matters, as the oxidative-damage-repair pathways may have a role in the pathogenesis of MM.[13] In the next section, new studies related to the role of sunlight and MM are reviewed.

Shedding New Light on Links Between Sun Exposure and MM

Nevi arising *in utero* develop in the absence of sunlight exposure, and hence CMN must develop independently of UV light. Thus, whereas it is expected that CMN are likely to have a different genomic spectrum of changes compared with MM developing on sun-exposed skin, the presence of *NRAS* mutations in both CMN and MM provide evidence that linking sunlight exposure to specific DNA mutations (so-called UV signatures) may require probing deeper into the skin biology. Although there is a mountain of evidence providing strong epidemiological links between sunlight exposure (and indoor tanning) and incidence of MM, only

recently have more precise cause and molecular effectors emerged, and with some surprises. Before delving into how UV light affects melanocytes and MM cells, it must be acknowledged that UV-light exposure has a multitude of effects on the skin[14] and beyond, particularly serving as a potent immunosuppressive event. Such immunosuppressive effects are likely to have a significant role in the pathogenesis of MM, but space constraints preclude a more extensive review of this topic.[15] However, when direct mutagenic events and MM are considered, a significant conundrum was apparent following the genomic identification of the BRAFV600E mutation in MM. Although BRAFV600E is not a UV-signature mutation, such MMs do occur on sun-exposed skin, pointing to a link between DNA damage and *BRAF* that we do not yet fully understand. Traditionally, the UV-light DNA signature was focused on cytidine–to–thymidine transition at a dipyrimidine motif (for example, C to T transitions; note that the ROS generated by sunlight can mediate many other types of DNA damage), therefore investigators had to dig deeper because over 50% of all MMs are related to the driver mutation without UV-light signature (that is, BRAFV600E). This lack of sunlight signatures in coding DNA sequences, together with occurrence of MM on non-sun-exposed sites, revealed a critical gap in our knowledge. Fortunately, two new studies provide fresh and much welcomed genomic data to fill this void. Rather than focusing on coding regions of genes, it was discovered that in ~70% of sporadic and familial MMs, a UV-light signature was identified in the promoter region of the telomerase reverse transcriptase or *TERT* gene.[16,17] Moreover, these findings highlight those mutations in the regulatory regions that complement the search for mutations in coding regions of relevant genes.[17,18] These seminal discoveries are important because TERT regulates telomere length, and hence contributes to cellular immortality. In the next section, we continue this theme related to MM genomics.

MM Genetics: the Landscape Expands With More Mountains and Hills

Just over a decade ago, the entire field of MM biology and treatment underwent dramatic transformation upon recognition of frequent *BRAF* mutations from a genetic perspective.[3] Currently, it appears that a MM cell may contain more mutations per cell than any other type of malignancy.[19] Indeed, the first comprehensive somatic screen for mutations in a metastatic MM cell line (eg COLO-829) revealed more than 30 000 somatic base substitutions, with almost 300 of them in protein-coding sequences![20] In this study, a high rate of C to T transitions among various MM tumors was also identified, supporting not only a causative link between sunlight and MM, but also the genomic information presented using Circos, a circular ideogram to portray genomic information.[21] The daunting fact of hundreds, if not thousands, of nonsynonymous somatic mutations in a given MM patient becomes even more of a stark reality as molecularly informed tumor boards are convened to deliver personalized treatment options based on whole-genome sequencing.[22] Where we once considered only a small number of so-called driver mutations of relevance to MM, the genomic landscape has rapidly expanded to include numerous driver mutations, passenger mutations, and even so-called back-seat mutations.[23,24] It will also be necessary to determine the relationship between numerous oncogenes associated with the melanoma cells (for example, Notch, RAC1, GRIN2a, ERBB4, loss of INK4a, and so on.) and the most common driver mutations such as BRAFV600E, *NRAS*, PTEN loss, NF1 nonsense mutations, c-KIT mutations, and GNAQ or GNA11 mutations (Figure 1). Clearly, as in other malignancies, the MM landscape has many more mountains and hills than were detectable just a few short years ago.[25] Before exploring how to move from mutations to medicine,[26] it is vital to identify the common signal transduction pathways that will assist in designing targeted agents and combinations required to confront this genomic diversity.[27]

MM Pathophysiology: Sharing of Signal Transduction Pathways Saves the Day

To 'live to fight another day' is a not an uncommon goal for patients with advanced MM. As we attempt to confront the unbelievable diversity of MM from a genomic perspective, it becomes a more realistic fight if we view the MM cell as having certain dominant signaling pathways that can be rationally targeted. The pathophysiology of melanoma is a complex and ongoing subject of research. One of the most important pathways in oncology involves the MAPK-signaling cascade that involves several kinases acting in a coordinated fashion including RAS/RAF/MEK/ERK.[28] Although molecular pathways are simplified in an unidirectional, linear fashion, one must not overlook the great complexity between the pathways,[29] and keep in mind the intricacy of communicating networks, cross-talk, and up- and downstream effectors.[30] Furthermore, a pathophysiologic harmonization between tumor cells and their environment must exist during each of the phases of MM growth, including radial and vertical growth phases and metastases.[31] It is also important to recognize that MM has traditionally been viewed as an immune-responsive tumor (perhaps related to the large number of mutations) because of documented spontaneous remissions and previous reports demonstrating occasional responses to interferons, interleukins (IL-2), and cell-based immunotherapies.[32] Not only is immunity important, but also inflammatory responses in the tumor microenvironment contribute to the plasticity of both tumor cells and immunocytes, which may have a role in tumor heterogeneity as well as therapeutic resistance.[33] More details updating the value of immune-based therapies based on overcoming immune checkpoints in MM will be covered in a following section.

One important reason for deciphering signaling networks is to identify an 'Achilles heel', by which the numerous

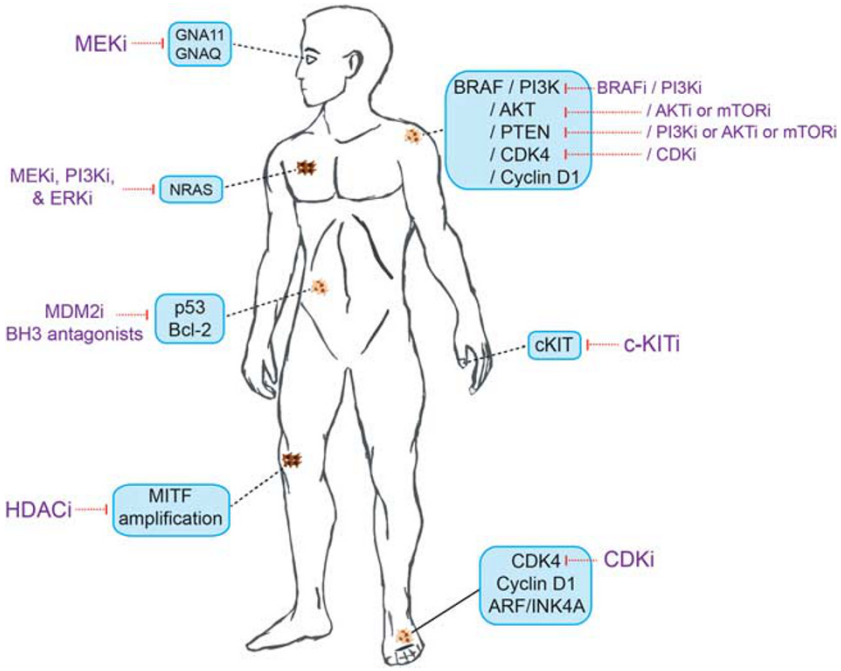

Figure 1 Different molecular subtypes of melanoma require 'personalized' therapeutic strategies. Although all melanomas arise from melanocytes, the driver oncogenes can be quite different depending upon site of origin (the eye *vs* the skin) and the level of sun exposure (chronic: more often *NRAS*, acute: most often *BRAF*, and sun-protected or mucosal: enriched for *KIT* mutations). Among the genetic groups defined so far, great complexity exists with concurrent mutations in multiple driver oncogenes reported (for example, *BRAF* mutant/PTEN loss, *BRAF* mutant/CDK4 mutant, *BRAF* mutant/MITF amplification). The complex mutational profiles will likely require multidrug combinations that will have to be personalized to individual patients.

survival pathways present in a MM cell can be overcome. Indeed, optimal therapies are those that kill MM cells rather than produce cytostatic effects. MM cells constitutively possess a plethora of prosurvival mechanisms and also inactivate proapoptotic pathways. An exhaustive description of all these proteins and processes is beyond the scope of this review, but some of the key prosurvival proteins include Bcl2, Mcl-1, and survivin, whereas proapoptotic members include Bad, Bak, Bim, Bmf, Noxa, and so on.[34]

BRAF, the most commonly mutated gene in MM, encodes a serine/threonine-protein kinase.[3] *BRAF* mutations of the V600E/K type cause a markedly increased constitutive kinase activity, as opposed to activation by *NRAS* and AKT in wild-type *BRAF*-bearing cells.[35] As *BRAF* belongs to the MAPK pathway, its increased activation ultimately stimulates MM cellular proliferation.[36–38] *BRAF* mutations have been associated with activation of AKT or loss of PTEN.[39–41] Furthermore, aberrations in the G proteins, GNAQ and GNA11, stimulate *BRAF* and its downstream effector, MEK. Mutations in these proteins further stimulate the MAPK pathway and have been associated with MM, particularly of the uvea.[42,43] Inactivation of NF1/neurofibromin cooperates with *BRAF* mutations in MM.[44] Finally, *BRAF* overactivation in association with CCND1/cyclin D1 overexpression may contribute to melanoma metastasis.[45]

Mutations of *NRAS*, a small GTPase directly upstream of *BRAF*, are observed in ~20% of melanomas[35,36,40,46] and are mutually exclusive from *BRAF* mutations.[39] Abnormalities in KIT, the receptor tyrosine kinase immediately upstream of RAS and PI3K, are found in roughly 27% of mucosal, 24% of acral, and 21% of melanomas on chronically sun-damaged skin, but are seldom found in melanomas on skin without chronic sun damage.[47–51] KIT stimulates both the MAPK and PI3K-AKT pathways; thus, mutations in KIT lead to deregulated growth and survival.[52] As KIT stimulates MITF, c-KIT aberrations can overactivate MITF, and cause proliferation and reduced apoptosis. One method by which mutant *BRAF* can influence the survival of MM cells is by reducing the levels of proapoptotic proteins, such as Bim and Bmf to name a few.[53] Before ending this section, it should be noted that the downstream component of MAPK is ERK, and MMs bearing the BRAFV600E mutation are considered by some investigators to be driven by deregulated ERK signaling.[54]

MM Stem Cells, Microenvironment, and Exosomes

It is clear from the pioneering work by Dr Mihm and fellow dermatopathologists in Boston that MM becomes exceptionally virulent when it progresses into the so-called vertical growth phase.[55] It is also important to note that MMs possess differentiation plasticity, a feature shared with stem cells during development.[56,57] As delineated in this section, the general scientific community is divided as to the presence, frequency, and overall contribution of MM stem

cells to tumorigenesis, treatment, and drug resistance. However, MM is probably not entirely exempted from virulence-conferring, stem-like cells.

Whether MM stem cells are frequent or infrequent remain a controversial point, and conclusions unfortunately depend on the selection of animal strains, markers utilized, and contribution of phenotypic switching.[58,59] Currently, there are reports supporting both a stochastic, as well as hierarchical, stem cell model involving MM propagation.[60] Regarding attempts to identify MM stem cells, a remarkably diverse set of cell markers have been used for the identification of MM stem cells and/or tumor-initiating cells. Perhaps as perplexing as the frequency and phenotypic identity of MM stem cells and plasticity of MM cells[33] is the complex role for the microenvironment of both primary and metastatic lesions as important contributors to the biology and drug responses involving MM.[61] As MM cells do not reside in a vacuum, the surrounding cells and microenvironment contain a confederacy of cell types that are being dissected in primary and metastatic lesions as an active area of research. Indeed, discussions governing the relative role for the seed and soil in cancer biology dates back a generation,[62] with studies of MM having an early and central role to better understand this complex set of interactions.[63] Not only is there cellular heterogeneity among various normal and reactive cell types, but also defining MM cell heterogeneity using genomics and other techniques, including IHC,[64] remains to be more extensively pursued to define the number and evolution of clones as identified in other neoplasms.[65] Tumor cell heterogeneity includes different clones within a single primary lesion or differences between the primary MM and a metastatic MM lesion, as well as among different metastatic lesions. Such genomic heterogeneity presents diagnostic as well as therapeutic challenges (not only for selection of frontline therapy, but also for contributing to the development of drug resistance) as described in the following sections.[66] Not only do MM cells absorb and internalize ultraviolet-light-derived signals, but they also produce various extracellular small membrane vesicles known as exosomes.[67,68] Interestingly, MM-derived exosomes (containing TYRP2, VLA4, HSP70, MET, and Rab27a proteins) can have an impact on cells and organs both locally and at distant sites (lymph nodes, bone marrow), with a net effect of enhancing invasion and priming a metastatic niche. Thus, it is clear that much remains to be learned about MM microenvironments, stem cell behavior and their secretory capability to influence cellular and molecular events both locally and throughout the body.

Diagnostic Challenges: Microscope *vs* Machines

The current gold standard in evaluating pigmented lesions remains histopathological evaluation using a microscope, although alternate approaches are emerging.[69,70] Criteria for histopathological diagnosis of MM by dermatopathologists, and our understanding of melanoma biology, have evolved over decades and was based initially on correlating clinical and histological features of MM.[55] Despite the historical importance of light microscopy and refinement in diagnostic criteria, inter-observer discordance of pigmented lesion diagnosis does occur,[71] necessitating additional tools in reaching the life-changing diagnosis of MM. More recent diagnostic approaches include the use of IHC or fluorescence *in situ* hybridization (FISH).[72] IHC, an antibody-based staining procedure identifies specific protein epitopes in biopsies, but unfortunately no marker has been identified that unequivocally distinguishes a benign *vs* malignant nevomelanocytic lesion. FISH uses DNA sequence-specific fluorescent probes to detect the presence or absence of chromosome sequences associated with MM.[73]

Given the aforementioned limitations in using the microscope, physicians are now turning to the use of more sophisticated instruments to aid in identifying, and even classifying, MM on the basis of molecular abnormalities at the RNA and DNA levels. Molecular subtypes of MM, rather than traditional histological subtypes, are now taking center stage from both diagnostic and therapeutic perspectives.[35] Gene expression profiling and genome-wide sequence analysis have yielded information regarding MM progression and contributed towards biomarker detection, diagnostic tool development, prognostic markers, and molecular therapies.[74] Epigenetic modifications also have a significant role in MM and may be useful prognostic biomarkers,[75] including the example of loss of 5-hydroxymethylcytosine in MM epigenome.[76] Regarding epigenetics and MM treatment, histone deacetylases (HDAC) that modify chromatin structure are emerging as therapeutic targets because HDAC inhibitors may be useful in overcoming drug resistance in MM patients with mutant *BRAF*, as further discussed in the following section.[77,78] Epigenetic events including methylation of CpG island promoter sites (in both coding and noncoding regions),[79,80] histone modification,[81] and microRNA expression also contribute to MM biology.[82]

It is likely that a new gold standard for diagnosis, prognosis, and therapy of MM will emerge beyond use of the microscope for either primary lesions or evaluation of sentinel lymph nodes to include making daily use of DNA sequencers. Even now, formalin-fixed paraffin-embedded (FFPE) samples of MM (or any other tumor) are sent to various laboratories, and whole-exome sequencing accompanied by mutational status can be delivered back to the physician within 2 weeks. Additionally, there is a promising antibody that may be employed to localize and quantify BRAFV600E+ MM cells in FFPE samples.[83] Thus, sufficient progress has been made so that machine/technology-driven molecularly informed tumor boards, in conjunction with accurate interpretation by a highly trained dermatopathologist, might become the rule rather than the exception for MM patients in the not-so-distant future.[22]

Cracking Signaling Codes for Therapeutic Benefits and Understanding Drug Resistance

There are many therapeutic strategies emerging from the panoply of genetic alterations being detected by high-throughput sequencing technologies, facilitating development of precision medicine for MM patients.[27] One of the best therapeutic examples of exploiting the discovery of a BRAFV600E mutation identified in the majority of MM patients has been targeting signaling components linked to the Ras/Raf/MEK/ERK pathway.[84] Whereas most BRAFV600E+ MM patients initially respond to targeted therapy (vemurafenib and dabrafenib), almost all patients develop acquired drug resistance (Figure 2) and die from disease progression.[85] In 2011, the FDA approved vemurafenib for BRAFV600E/K+ patients earning MM, a mention as the 'poster child' for personalized medicine.[84] This remarkable clinical achievement reflected a new approach to drug development, in which the 'one size fits all' perspective was changed to focusing on patient subsets, in this case BRAFV600E+ MM patients, to test their responsiveness to an agent designed to hit a specific target.[86]

Experience with vemurafenib therapy in MM patients generated several notable clinical observations: first, the disappearance and reappearance of metastatic lesions appeared in such a synchronous fashions so as to indicate a 'biological clock' that was timing the emergence of drug resistance (the precise mechanism remains unclear).[87] Second, unlike gatekeeper mutations underlying drug resistance using other targeted therapies,[88,89] the number and diversity of the mechanisms suggested to mediate vemurafenib resistance are bewildering, leaning towards a conclusion that resistance is more likely and frequently linked to rewiring of intracellular signaling, rather than emergence of genomically distinct clones.[90] Third, vemurafenib has a paradoxical effect in that, while inhibiting mutant *BRAF*, it activates wild-type *BRAF*, and in cells with activated RAS it can lead to new tumors such as keratoacanthomas and cutaneous squamous cell carcinomas due to enhanced ERK signaling.[91] The next generations of agents targeting BRAFV600E are likely to be the so-called paradox-breakers, and hence are more tailored to exclude unwanted activation of the wild-type *BRAF* kinase. Another rapidly emerging MAPK pathway target is immediately downstream of *BRAF*, namely MEK.[92] Numerous clinical trials include the use of a MEK inhibitor alone, in combination with vemurafenib, or in MM patients with vemurafenib resistance.[93] Early clinical evidence indicates that a combination of a *BRAF* inhibitor with a MEK inhibitor increases progression-free survival compared with *BRAF* inhibitor alone.[94] Targeting MEK may also be useful in MM patients with RAS-mutant tumors as well.

In addition to kinase inhibitors, the FDA approved ipilimumab for MM as a monoclonal antibody that blocks

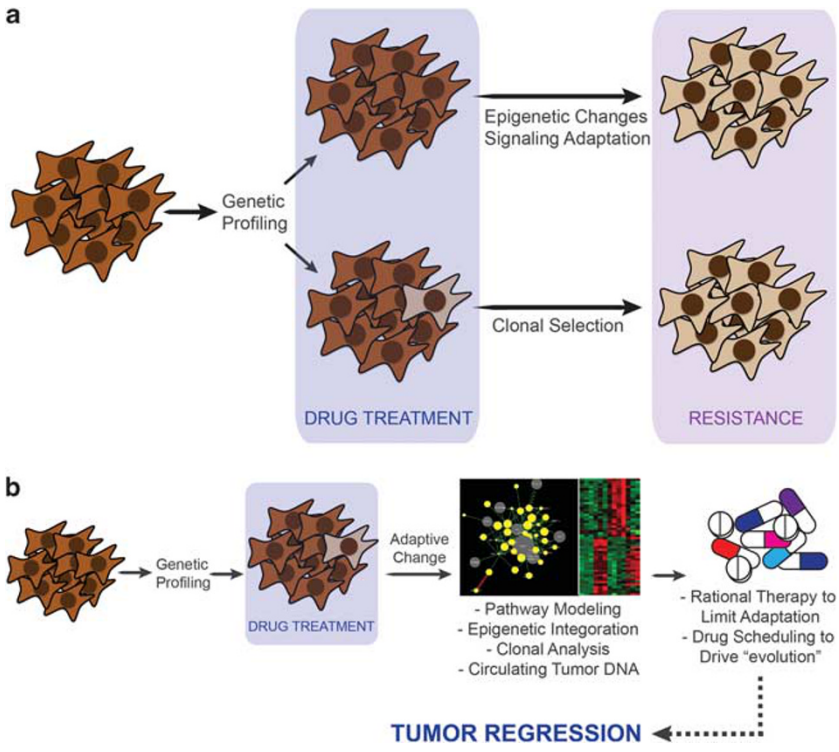

Figure 2 The current and future states of personalized therapy in melanoma. (**a**) Currently, patients are profiled for *BRAF* and *NRAS* status, and an appropriate targeted therapy selected. Following treatment, escape inevitably occurs as the likely result of selection for pre-existing resistant clones or through adaptive changes in signaling or epigenetics. (**b**) In the future, short-term drug treatment may be paired with advanced molecular techniques that allow adaptations to be defined through analysis of global signaling, pathway rewiring, clonal modeling, and circulating DNA. Rational combination therapies will then be designed that allow escape to be limited and long-term tumor regression to be achieved.

CTLA-4, one of the several immune checkpoint regulators.[95] Essentially, the rationale for the latest immunotherapeutic agents is to reverse the inhibition of tumor cell-mediated T-cell responses, thereby providing a boost to the immunocytes as they confront MM cells containing innumerable mutations, generating epitopes ripe for recognition by the patient's innate and adaptive immunocytes. Another approach is to target a different immune checkpoint protein known as PD-1, as well as to explore combining targeted therapy with immunotherapy in MM patients.[90] Given the multitude of drugs and emerging targets, it is clear that a gap is rapidly expanding, in which genomics and personalized medicine require relevant and validated preclinical models to fill this enlarging void. In the next section, various MM models are reviewed with particular emphasis on the patient-derived tumorgraft model, as cell lines and xenografts (cell line injected into immunodeficient rodents) have been recognized as not necessarily yielding results that translate in human cancer patients.[96]

MM Modeling

There are several different types of MM models, and given the progress in using animal models, some experts suggest that no human clinical trial should be initiated until a preclinically relevant and validated animal model of MM is conducted with positive outcomes.[97] Among MM models, only a few will be highlighted because of space constraints. One *in vitro* technique is to employ three-dimensional human skin reconstructs.[98] Moving to *in vivo* platforms, many groups employ genetically engineered mouse models of MM.[99] With this approach, tissue-specific expression of various oncogenes can be introduced, and phenotypes can be enhanced by selection of appropriate backcrossing using susceptible genetic backgrounds. In the third type of MM model, cells grown *in vitro* are injected into the subcutaneous tissue of immunodeficient mice to create xenografts. However, xenograft models do not exactly mimic human MM, as the cell lines are often immortalized and are of high passage number, which may contain many other genomic or epigenetic alterations than present in the original tumor. The significance of tumor environment must not be forgotten, and the fourth model system uses patient-derived tumorgrafts to optimize preservation of appropriate three-dimensional anatomical hallmarks and surrounding stroma. Before discussing tumorgraft models, it should be noted that another approach for investigating MM behavior *in vivo* is to inject cell lines into the dermis of normal human skin that has been engrafted to immunodeficient mice.[100]

Patient-derived tumorgraft models, or so-called patient-derived xenograft (PDX) models, are gaining more attention as a practical method for screening drugs, singly and in combination, to serve as 'avatars' for patients with MM and other malignancies.[101,102] Indeed, as better therapeutic outcomes are allowing longer progression-free survival intervals, investigators have an extended window of opportunity in which the unique features of a tumor for a given patient can be examined in real time, thereby assisting in selection of additional therapies either before or after resistance develops. One group has recently described a model of acquired vemurafenib resistance using BRAFV600E+ tumorgrafts.[103]

Human MM modeled in an immune-deprived environment may behave differently in an immunodeficient mouse model (although Nude mice do retain an intact innate immune system), and hence investigators have created mice with a human immune system. Notably, NOD-SCID (non-obese diabetic/severe combined immunodeficient) IL-2Rγnull mice (abbreviated as NSG), created by backcross of severe combined immunodeficient mice, engraft human stem cells readily and facilitate the reconstitution of human immune systems in murine models.[104] Thus, humanized mice can potentially reveal human MM cell/human immune cell interactions in model form.

In addition to mice, other species have been used to model MM, although to a much lesser extent. Dogs, having the advantage over laboratory animals as being an immune-competent large mammal living in the same environment as humans, spontaneously develop, with breed-specific proclivity, highly aggressive forms of MM in the oral cavity, digit/footpad, and mucocutaneous junctions with prognosis correlating directly with tumor stage, as in human MM.[105,106] Gray horses have genetic susceptibility and develop MMs with similar histological and immunohistological features as in humans, undergo spontaneous distant metastasis to similar organs as in human disease, and can suffer aggressive disease in which oncologic therapies have only limited effects.[107,108] The main limitations to a larger use of these latter animal models, however, remains the expense of housing, owner compliance, and the difficulty of genetic modification and incomplete annotation of their respective genomes.

Major Unresolved Questions to be Answered

Perhaps the single most challenging aspect of MM is defining exactly how many different diseases are actually represented by this moniker.[35] Is there any other tumor system that can present with such panoply of clinical presentations from cradle to grave? Does it make sense to even use the same diagnostic label for tumors that arise on sun-exposed skin, glabrous skin, retina, mucosa, nail plates, non-sun-exposed skin, acral regions, and so on? Indeed many MMs actually are amelanotic and thus, perhaps we should consider a label such as 'neurocristoma' followed by genomically informed subtype! Next, as regards melanocyte-based skin lesions, it is not always easy for pathologists to distinguish between an atypical nevus *vs* an early-stage MM lesion. Using routine light microscopic-based criteria, several studies demonstrated that difficulty in reproducibility among experts, and identification of IHC-based reagents to serve as unequivocal diagnostic markers is an unrealized dream at

this time. Perhaps the greatest promise for more diagnostic certainty will go beyond use of FISH markers to incorporate whole-genome DNA sequencing.

The second major challenge centers on effective, intelligently designed combination therapy for patients with advanced MM that significantly extends the length and quality of life. To accomplish this goal, more effective killing of MM cells is required, most likely requiring drug combinations. Will adding further agents that target the MAPK pathway become a more commonly used intelligent drug combination cocktail component?[109] Until the frontline therapy is improved, the other significant challenge is to better understand and prevent or prolong onset of acquired drug resistance.

As each MM cell may harbor more mutations per cell than any other neoplasm (particularly involving MMs derived from chronically sun-exposed skin sites), how do we determine a treatment recommendation when there are thousands of nonsynonymous somatic mutations? There is no question that as data continue to pour out of global DNA-sequencing efforts, like the ENCODE project revealing that 99% of noncoding DNA (situated in the so-called dark matter of the genome) actually contributes to how genetics and cancer intersect, many more surprises and new drug targets of relevance to MM will emerge, but are tumorgraft models the best MM model to rapidly exploit the genomic data?

If patient-derived tumorgrafts serve to relieve the impending bottleneck that is likely to emerge owing to the rapid and relatively affordable whole-genome sequencing, what role will pathologists have in setting standards for these avatar mice as regards model validation from a pathophysiologic viewpoint, and characterization of tissue responses to new agents or combinations of agents? Will treatment results from a fragment of one portion of a primary or metastatic lesion be applicable to a patient's overall clinical response? Can we eliminate the need for performing invasive procedures for diagnosis or can we rely on detection of circulating MM cells derived from needle pricks?[110] How do we change the behavior of individuals—both young and old to avoid indoor tanning facilities?[111] For many other unanswered questions, another group has compiled an additional list of MM-related challenging questions.[26]

Future Directions

With the rising frequency of patients and/or physicians seeking high-throughput sequencing (either whole exome from FFPE or whole genome from fresh/cryo-matched with normal cells), it is clear that pathologists will continue their historical leadership role in the cancer fight (dating back to famed pathologist Dr Sidney Farber, father of chemotherapy).[112] Pathologists must develop validation testing (perhaps using synthetic agents) to ensure that sequencing data from one hospital or test site is identical to results from other sites, as is being done for lung cancer patients and molecular testing guidelines.[113] Pathologists will also be critical to the natural evolution from morphology and site-based tumor classifications to a diagnostic approach that is more molecularly informative, reproducible, objective, and shared-pathway defined. Moving from diagnostic directions to future therapeutic opportunities, it is envisioned that MM treatment strategies are likely to include combinatorial approaches for cures (or more durable remissions).[85] Hence, initial targeted therapies aimed at driver mutations/pathways and/or virulence-conferring cells are used to shrink down the bulk of tumors, coupled with immune enhancement in which both antigenic epitopes are created/exposed and more favorable microenvironments created that are conducive to immunocytic-mediated MM cell eradication.[114–116]

The searches for better understanding of cancer genome landscapes and signaling pathway alterations, in concert with drug development efforts, present great opportunities and challenges for patient suffering from MM and many other malignant tumors.[117]

ACKNOWLEDGEMENTS

We thank our financial supporters, including institutional support from our respective organizations and the Stand Up to Cancer Melanoma Dream Team, and Melanoma Research Alliance (BJN). We also thank Dr George Vande Woude for his helpful suggestions. We apologize to those colleagues whose reports were not cited due to space constraints, and acknowledge reviewer comments that improved this presentation.

DISCLOSURE/CONFLICT OF INTEREST

The authors declare no conflict of interest.

1. Menendez L, Kulik MJ, Page AT, *et al.* Directed differentiation of human pluripotent cells to neural crest stem cells. Nat Protoc 2013;8:203–212.
2. Pollock PM, Harper UL, Hansen KS, *et al.* High frequency of BRAF mutations in nevi. Nat Genet 2003;33:19–20.
3. Davies H, Bignell GR, Cox C, *et al.* Mutations of the BRAF gene in human cancer. Nature 2002;417:949–954.
4. Bauer J, Curtin JA, Pinkel D, *et al.* Congenital melanocytic nevi frequently harbor NRAS mutations but no BRAF mutations. J Invest Dermatol 2007;127:179–182.
5. Kinsler VA, Thomas AC, Ishida M, *et al.* Multiple congenital melanocytic nevi and neurocutaneous melanosis are caused by postzygotic mutations in codon 61 of NRAS. J Invest Dermatol; advance online publication, 7 February 2013; doi:10.1038/jid.2013.70 (e-pub ahead of print).
6. Pedersen M, Kusters-Vandevelde HV, Viros A, *et al.* Primary melanoma of the CNS in children is driven by congenital expression of oncogenic NRAS in melanocytes. Cancer Discov 2013;3:458–469.
7. Wiesner T, Obenauf AC, Murali R, *et al.* Germline mutations in BAP1 predispose to melanocytic tumors. Nat Genet 2011;43:1018–1021.
8. Busam KJ, Sung J, Wiesner T, *et al.* Combined BRAF(V600E)-positive melanocytic lesions with large epithelioid cells lacking BAP1 expression and conventional nevomelanocytes. Am J Surg Pathol 2013;37:193–199.
9. Krengel S, Scope A, Dusza SW, *et al.* New recommendations for the categorization of cutaneous features of congenital melanocytic nevi. J Am Acad Dermatol 2013;68:441–451.
10. Berwick M, Orlow I, Hummer AJ, *et al.* The prevalence of CDKN2A germ-line mutations and relative risk for cutaneous malignant melanoma: an international population-based study. Cancer Epidemiol Biomarkers Prev 2006;15:1520–1525.
11. Jonsson A, Tuominen R, Grafstrom E, *et al.* High frequency of p16 (INK4A) promoter methylation in NRAS-mutated cutaneous melanoma. J Invest Dermatol 2010;130:2809–2817.

12. Jenkins NC, Liu T, Cassidy P, *et al.* The p16(INK4A) tumor suppressor regulates cellular oxidative stress. Oncogene 2011;30:265–274.
13. Mitra D, Luo X, Morgan A, *et al.* An ultraviolet-radiation-independent pathway to melanoma carcinogenesis in the red hair/fair skin background. Nature 2012;491:449–453.
14. Zaidi MR, De Fabo EC, Noonan FP, *et al.* Shedding light on melanocyte pathobiology *in vivo*. Cancer Res 2012;72:1591–1595.
15. Chu CC, Ali N, Karagiannis P, *et al.* Resident CD141 (BDCA3)+ dendritic cells in human skin produce IL-10 and induce regulatory T cells that suppress skin inflammation. J Exp Med 2012; 209:935–945.
16. Huang FW, Hodis E, Xu MJ, *et al.* Highly recurrent TERT promoter mutations in human melanoma. Science 2013;339:957–959.
17. Horn S, Figl A, Rachakonda PS, *et al.* TERT promoter mutations in familial and sporadic melanoma. Science 2013;339:959–961.
18. Rass K, Reichrath J. UV damage and DNA repair in malignant melanoma and nonmelanoma skin cancer. In: Reichrath J (ed) Sunlight, Vitamin D and Skin Cancer, New York, NY, USA, 2008 162–178.
19. Berger MF, Hodis E, Heffernan TP, *et al.* Melanoma genome sequencing reveals frequent PREX2 mutations. Nature 2012;485: 502–506.
20. Pleasance ED, Cheetham RK, Stephens PJ, *et al.* A comprehensive catalogue of somatic mutations from a human cancer genome. Nature 2010;463:191–196.
21. Krzywinski M, Schein J, Birol I, *et al.* Circos: an information aesthetic for comparative genomics. Genome Res 2009;19:1639–1645.
22. Roychowdhury S, Iyer MK, Robinson DR, *et al.* Personalized oncology through integrative high-throughput sequencing: a pilot study. Sci Transl Med 2011;3:111ra21.
23. Mehnert JM, Kluger HM. Driver mutations in melanoma: lessons learned from bench-to-bedside studies. Curr Oncol Rep 2012;14: 449–457.
24. Hodis E, Watson IR, Kryukov GV, *et al.* A landscape of driver mutations in melanoma. Cell 2012;150:251–263.
25. Wood LD, Parsons DW, Jones S, *et al.* The genomic landscapes of human breast and colorectal cancers. Science 2007;318:1108–1113.
26. Tsao H, Chin L, Garraway LA, *et al.* Melanoma: from mutations to medicine. Genes Dev 2012;26:1131–1155.
27. Flaherty KT, Hodi FS, Fisher DE. From genes to drugs: targeted strategies for melanoma. Nat Rev Cancer 2012;12:349–361.
28. McArthur GA. The coming of age of MEK. Lancet Oncol 2012;13: 744–745.
29. Hearing VJ, Leong SPL. From melanocytes to melanoma: the progression to malignancy. In: Human Press Incorporated, 2006.
30. Chang L, Karin M. Mammalian MAP kinase signalling cascades. Nature 2001;410:37–40.
31. Leong SP, Mihm Jr MC, Murphy GF, *et al.* Progression of cutaneous melanoma: implications for treatment. Clin Exp Metastasis 2012; 29:775–796.
32. McArthur GA, Ribas A. Targeting oncogenic drivers and the immune system in melanoma. J Clin Oncol 2013;31:499–506.
33. Holzel M, Bovier A, Tuting T. Plasticity of tumour and immune cells: a source of heterogeneity and a cause for therapy resistance? Nat Rev Cancer 2013;13:365–376.
34. Anvekar RA, Asciolla JJ, Missert DJ, *et al.* Born to be alive: a role for the BCL-2 family in melanoma tumor cell survival, apoptosis, and treatment. Front Oncol 2011;1:34; advance online publication, 13 October 2011; doi:10.3389/fonc.2011.00034.
35. Vidwans SJ, Flaherty KT, Fisher DE, *et al.* A melanoma molecular disease model. PLoS One 2011;6:e18257.
36. Swick JM, Maize Sr JC. Molecular biology of melanoma. J Am Acad Dermatol 2012;67:1049–1054.
37. Takata M, Saida T. Genetic alterations in melanocytic tumors. J Dermatol Sci 2006;43:1–10.
38. Sullivan RJ, Flaherty K. MAP kinase signaling and inhibition in melanoma. Oncogene 2013;32:2373–2379.
39. Tsao H, Goel V, Wu H, *et al.* Genetic interaction between NRAS and BRAF mutations and PTEN/MMAC1 inactivation in melanoma. J Invest Dermatol 2004;122:337–341.
40. Goel VK, Lazar AJ, Warneke CL, *et al.* Examination of mutations in BRAF, NRAS, and PTEN in primary cutaneous melanoma. J Invest Dermatol 2006;126:154–160.
41. Govindarajan B, Sligh JE, Vincent BJ, *et al.* Overexpression of Akt converts radial growth melanoma to vertical growth melanoma. J Clin Invest 2007;117:719–729.
42. Van Raamsdonk CD, Bezrookove V, Green G, *et al.* Frequent somatic mutations of GNAQ in uveal melanoma and blue naevi. Nature 2009;457:599–602.
43. Fisher DE, Barnhill R, Hodi FS, *et al.* Melanoma from bench to bedside: meeting report from the 6th international melanoma congress. Pigment Cell Melanoma Res 2010;23:14–26.
44. Maertens O, Johnson B, Hollstein P, *et al.* Elucidating distinct roles for NF1 in melanomagenesis. Cancer Discov 2013;3:338–349.
45. Curtin JA, Fridlyand J, Kageshita T, *et al.* Distinct sets of genetic alterations in melanoma. N Engl J Med 2005;353:2135–2147.
46. Edlundh-Rose E, Egyhazi S, Omholt K, *et al.* NRAS and BRAF mutations in melanoma tumours in relation to clinical characteristics: a study based on mutation screening by pyrosequencing. Melanoma Res 2006;16:471–478.
47. Curtin JA, Busam K, Pinkel D, *et al.* Somatic activation of KIT in distinct subtypes of melanoma. J Clin Oncol 2006;24:4340–4346.
48. Woodman SE, Davies MA. Targeting KIT in melanoma: a paradigm of molecular medicine and targeted therapeutics. Biochem Pharmacol 2010;80:568–574.
49. Ashida A, Takata M, Murata H, *et al.* Pathological activation of KIT in metastatic tumors of acral and mucosal melanomas. Int J Cancer 2009;124:862–868.
50. Beadling C, Jacobson-Dunlop E, Hodi FS, *et al.* KIT gene mutations and copy number in melanoma subtypes. Clin Cancer Res 2008;14: 6821–6828.
51. Garrido MC, Bastian BC. KIT as a therapeutic target in melanoma. J Invest Dermatol 2010;130:20–27.
52. Flaherty KT, Hodi FS, Fisher DE. From genes to drugs: targeted strategies for melanoma. Nat Revi Cancer 2012;12:349–361.
53. Cartlidge RA, Thomas GR, Cagnol S, *et al.* Oncogenic BRAF(V600E) inhibits BIM expression to promote melanoma cell survival. Pigment Cell Melanoma Res 2008;21:534–544.
54. Lito P, Pratilas CA, Joseph EW, *et al.* Relief of profound feedback inhibition of mitogenic signaling by RAF inhibitors attenuates their activity in BRAFV600E melanomas. Cancer Cell 2012;22:668–682.
55. Miller AJ, Mihm Jr MC. Melanoma. N Engl J Med 2006;355:51–65.
56. Topczewska JM, Postovit LM, Margaryan NV, *et al.* Embryonic and tumorigenic pathways converge via Nodal signaling: role in melanoma aggressiveness. Nat Med 2006;12:925–932.
57. McAllister JC, Zhan Q, Weishaupt C, *et al.* The embryonic morphogen, Nodal, is associated with channel-like structures in human malignant melanoma xenografts. J Cut Pathol 2010;37(Suppl 1):19–25.
58. Girouard SD, Murphy GF. Melanoma stem cells: not rare, but well done. Lab Invest 2011;91:647–664.
59. Hoek KS, Goding CR. Cancer stem cells versus phenotype-switching in melanoma. Pigment Cell Melanoma Res 2010;23:746–759.
60. Lang D, Mascarenhas JB, Shea CR. Melanocytes melanocyte stem cells, and melanoma stem cells. Clin Dermatol 2013;31:166–178.
61. Lee JT, Herlyn M. Microenvironmental influences in melanoma progression. J Cell Biochem 2007;101:862–872.
62. Fidler IJ, Poste G. The "seed and soil" hypothesis revisited. Lancet Oncol 2008;9:808.
63. Raz A, Ben-Ze'ev A. Modulation of the metastatic capability in B16 melanoma by cell shape. Science 1983;221:1307–1310.
64. Houben R, Vetter-Kauczok CS, Ortmann S, *et al.* Phospho-ERK staining is a poor indicator of the mutational status of BRAF and NRAS in human melanoma. J Invest Dermatol 2008;128:2003–2012.
65. Navin N, Kendall J, Troge J, *et al.* Tumour evolution inferred by single-cell sequencing. Nature 2011;472:90–94.
66. Somasundaram R, Villanueva J, Herlyn M. Intratumoral heterogeneity as a therapy resistance mechanism: role of melanoma subpopulations. Adv Pharmacol 2012;65:335–359.
67. Hood JL, San RS, Wickline SA. Exosomes released by melanoma cells prepare sentinel lymph nodes for tumor metastasis. Cancer Res 2011;71:3792–3801.
68. Peinado H, Aleckovic M, Lavotshkin S, *et al.* Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET. Nat Med 2012;18:883–891.
69. Ferris LK, Harris RJ. New diagnostic aids for melanoma. Dermatol Clin 2012;30:535–545.

70. Wachsman W, Morhenn V, Palmer T, *et al.* Noninvasive genomic detection of melanoma. Br J Dermatol 2011;164:797–806.
71. Shoo BA, Sagebiel RW, Kashani-Sabet M. Discordance in the histopathologic diagnosis of melanoma at a melanoma referral center. J Am Acad Dermatol 2010;62:751–756.
72. North JP, Vetto JT, Murali R, *et al.* Assessment of copy number status of chromosomes 6 and 11 by FISH provides independent prognostic information in primary melanoma. Am J Surg Pathol 2011;35:1146–1150.
73. Wolman SR. Applications of fluorescence *in situ* hybridization techniques in cytopathology. Cancer 1997;81:193–197.
74. Leong SP, Mihm Jr MC, Murphy GF, *et al.* Progression of cutaneous melanoma: implications for treatment. Clin Exp Metastasis 2012;29:775–796.
75. Greenberg ES, Chong KK, Huynh KT, *et al.* Epigenetic biomarkers in skin cancer. Cancer Lett 2012 (in press).
76. Lian CG, Xu Y, Ceol C, *et al.* Loss of 5-hydroxymethylcytosine is an epigenetic hallmark of melanoma. Cell 2012;150:1135–1146.
77. Shao Y, Aplin AE. BH3-only protein silencing contributes to acquired resistance to PLX4720 in human melanoma. Cell Death Differ 2012;19:2029–2039.
78. Lai F, Jin L, Gallagher S, *et al.* Histone deacetylases (HDACs) as mediators of resistance to apoptosis in melanoma and as targets for combination therapy with selective BRAF inhibitors. Adv Pharmacol 2012;65:27–43.
79. Hoshimoto S, Kuo CT, Chong KK, *et al.* AIM1 and LINE-1 epigenetic aberrations in tumor and serum relate to melanoma progression and disease outcome. J Invest Dermatol 2012;132:1689–1697.
80. Tanemura A, Terando AM, Sim MS, *et al.* CpG island methylator phenotype predicts progression of malignant melanoma. Clin Cancer Res 2009;15:1801–1807.
81. Bachmann IM, Halvorsen OJ, Collett K, *et al.* EZH2 expression is associated with high proliferation rate and aggressive tumor subgroups in cutaneous melanoma and cancers of the endometrium, prostate, and breast. J Clin Oncol 2006;24:268–273.
82. Nguyen T, Kuo C, Nicholl MB, *et al.* Downregulation of microRNA-29c is associated with hypermethylation of tumor-related genes and disease outcome in cutaneous melanoma. Epigenetics 2011;6:388–394.
83. Long GV, Wilmott JS, Capper D, *et al.* Immunohistochemistry is highly sensitive and specific for the detection of V600E BRAF mutation in melanoma. Am J Surg Pathol 2013;37:61–65.
84. Smalley KS, Sondak VK. Melanoma—an unlikely poster child for personalized cancer therapy. N Engl J Med 2010;363:876–878.
85. Kwong LN, Davies MA. Targeted therapy for melanoma: rational combinatorial approaches. Oncogene; advance online publication, 18 February 2013; doi:10.1038/onc.2013.34 (e-pub ahead of print).
86. Stewart DJ, Kurzrock R. Cancer: the road to Amiens. J Clin Oncol 2009;27:328–333.
87. Kaiser J. Profile: Bert Vogelstein. Cancer genetics with an edge. Science 2012;337:282–284.
88. Gorre ME, Mohammed M, Ellwood K, *et al.* Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. Science 2001;293:876–880.
89. Pao W, Miller VA, Politi KA, *et al.* Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. PLoS Med 2005;2:e73.
90. Jang S, Atkins MB. Which drug, and when, for patients with BRAF-mutant melanoma? Lancet Oncol 2013;14:e60–e69.
91. Su F, Viros A, Milagre C, *et al.* RAS mutations in cutaneous squamous-cell carcinomas in patients treated with BRAF inhibitors. N Engl J Med 2012;366:207–215.
92. Akinleye A, Furqan M, Mukhi N, *et al.* MEK and the inhibitors: from bench to bedside. J Hematol Oncol 2013;6:27.
93. McArthur GA. The coming of age of MEK. Lancet Oncol 2012;13:744–745.
94. Flaherty KT, Infante JR, Daud A, *et al.* Combined BRAF and MEK inhibition in melanoma with BRAF V600 mutations. N Eng J Med 2012;367:1694–1703.
95. Hodi FS, O'Day SJ, McDermott DF, *et al.* Improved survival with ipilimumab in patients with metastatic melanoma. N Eng J Med 2010;363:711–723.
96. Ellis LM, Fidler IJ. Finding the tumor copycat. Therapy fails, patients don't. Nat Med 2010;16:974–975.
97. Merlino G, Flaherty K, Acquavella N, *et al.* Meeting report: the future of preclinical mouse models in melanoma treatment is now. Pigment Cell Melanoma Res; advance online publication, 27 March 2013; doi:10.1111/pcmr.12099 (e-pub ahead of print).
98. Li L, Fukunaga-Kalabis M, Herlyn M. The three-dimensional human skin reconstruct model: a tool to study normal skin and melanoma progression. J Vis Exp 2011;54:e2937.
99. Walker GJ, Soyer HP, Terzian T, *et al.* Modelling melanoma in mice. Pigment Cell Melanoma Res 2011;24:1158–1176.
100. Juhasz I, Albelda SM, Elder DE, *et al.* Growth and invasion of human melanomas in human skin grafted to immunodeficient mice. Am J Pathol 1993;143:528–537.
101. Hidalgo M, Bruckheimer E, Rajeshkumar NV, *et al.* A pilot clinical study of treatment guided by personalized tumorgrafts in patients with advanced cancer. Mol Cancer Ther 2011;10:1311–1316.
102. Monsma DJ, Monks NR, Cherba DM, *et al.* Genomic characterization of explant tumorgraft models derived from fresh patient tumor tissue. J Transl Med 2012;10:125.
103. Das Thakur M, Salangsang F, Landman AS, *et al.* Modelling vemurafenib resistance in melanoma reveals a strategy to forestall drug resistance. Nature 2013;494:251–255.
104. Shultz LD, Ishikawa F, Greiner DL. Humanized mice in translational biomedical research. Nat Rev Immunol 2007;7:118–130.
105. Pavlin D, Cemazar M, Sersa G, *et al.* IL-12 based gene therapy in veterinary medicine. J Transl Med 2012;10:234.
106. Ramos-Vara JA, Beissenherz ME, Miller MA, *et al.* Retrospective study of 338 canine oral melanomas with clinical, histologic, and immunohistochemical review of 129 cases. Vet Pathol 2000;37:597–608.
107. MacGillivray KC, Sweeney RW, Del Piero F. Metastatic melanoma in horses. J Vet Intern Med 2002;16:452–456.
108. Seltenhammer MH, Heere-Ress E, Brandt S, *et al.* Comparative histopathology of grey-horse-melanoma and human malignant melanoma. Pigment Cell Res 2004;17:674–681.
109. Morris EJ, Jha S, Restino CR, *et al.* Discovery of a novel ERK inhibitor with activity in models of acquired resistance to BRAF and MEK inhibitors. Cancer Discov; advance online publication, 24 April 2013; CD-13-0070.
110. Leary RJ, Sausen M, Kinde I, *et al.* Detection of chromosomal alterations in the circulation of cancer patients with whole-genome sequencing. Sci Transl Med 2012;4162ra54.
111. Balk SJ, Fisher DE, Geller AC. Teens and indoor tanning: a cancer prevention opportunity for pediatricians. Pediatrics 2013;131:772–785.
112. Berman DM, Bosenberg MW, Orwant RL, *et al.* Investigative pathology: leading the post-genomic revolution. Lab Invest 2012;92:4–8.
113. Lindeman NI, Cagle PT, Beasley MB, *et al.* Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: Guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology. Arch Pathol Lab Med 2013;137:828–860.
114. Frederick DT, Piris A, Cogdill AP, *et al.* BRAF inhibition is associated with enhanced melanoma antigen expression and a more favorable tumor microenvironment in patients with metastatic melanoma. Clin Cancer Res 2013;19:1225–1231.
115. Menaa F. Latest approved therapies for metastatic melanoma: what comes next? J Skin Cancer 2013;2013:735282.
116. Kudchadkar RR, Smalley KS, Glass LF, *et al.* Targeted therapy in melanoma. Clin Dermatol 2013;31:200–208.
117. Vogelstein B, Papadopoulos N, Velculescu VE, *et al.* Cancer genome landscapes. Science 2013;339:1546–1558.