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Using genetics and genomics strategies to personalize therapy for cancer: focus on melanoma

Katherine L. Nathanson, MD
351 BRB 2/3, University of Pennsylvania School of Medicine, 421 Curie Blvd, Philadelphia, PA 19104

Abstract

Individualizing therapeutic selection for patients is a major goal in cancer treatment today. This goal is best facilitated by understanding both an individual's inherited genetic variation and the somatic genetic changes arising during cancer development. Clinical decision making based on inherited genetic variation is done for those patients with cancer susceptibility syndromes and more generally to personalize drug dosing. Personalized medicine based on genetic and genomic changes within tumors is being applied more widely, with increased use of therapies targeted to somatic mutations and amplifications. Somatic mutations associated with resistance also are being used to select against therapies. Somatic point mutation testing being used clinically includes direct sequencing, short sequencing and single nucleotide interrogation. Single amplifications are commonly assessed using FISH or CISH; high through-put assessment of amplifications and deletions is done mainly on a research basis. Melanomas contain complex mutational profiles that allow them to be sub-grouped by their genetic and genomic profile, each of which then can be evaluated pre-clinically to determine their response to targeted therapies. *BRAF* V600E mutations are the most commonly found in melanoma; specific inhibitors of mutant BRAF have been developed and are currently in clinical trials. In addition, other melanoma sub-groups have been identified genetically, which respond to other inhibitors. These studies focus on somatic genetic changes in cancer, which can be targeted directly by therapies. However in the future, personalized medicine will use a combination of inherited and somatic genetics to select the optimal tailored therapy for each patient.

Keywords

personalized medicine; melanoma; targeted therapy; mutation detection; somatic genetics

1.1 *Introduction*

The goal of personalizing therapy based on genetics and genomics has become a guiding force in treatment for cancer. Individualizing treatment can be done in various different ways which are at different stages of application clinically. Both the genetics of the inherited and somatic genome can be used to guide clinical decision making for the patient. The somatic genome is comprised of the inherited genome and any genetic or genomic changes that develop during tumorigenesis. Knowledge of both inherited genetic variation and deleterious mutations (e.g.

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knathans@mail.med.upenn.edu, tel: 215-573-9840, fax: 215-573-7945.

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as for *BRCA1*) can be used to delineate cancer prevention and treatment strategies for the patient, as well provide prognostic information. The identification of specific somatic genetic changes in the tumor can be used to guide therapeutic selection, both positively and negatively, and can provide prognostic information. This review will focus on the application of somatic genetics to personalizing cancer therapy, including some of the molecular techniques used clinically and research combining genetic analysis and pre-clinical studies to select optimal therapies for melanoma sub-groups. A short review of how inherited variation is used to determine specific cancer therapy also is included, as our understanding of the pathways disrupted in cancer susceptibility syndromes has been extraordinarily useful in the development of targeted therapies. The future of personalized medicine will be to utilize a combination of inherited and somatic genetics to select the optimal tailored therapy for each patient.

1.2 Inherited genetics and personalized therapy for cancer

Inherited genetics and personalized cancer therapy can be thought of in two contexts. The first is through our understanding of cancer susceptibility syndromes, associated with deleterious mutations, and the second is genetic variation, which most commonly is associated with differences in drug metabolism. Inherited mutations are identified through commercial genetic testing, which for most genes includes both point mutations and large genomic rearrangements (deletions/duplications). For most cancer susceptibility syndromes, such as Hereditary Breast and Ovarian Cancer, Hereditary Non-Polyposis Colorectal Cancer and Von-Hippel Lindau disease, standard of care screening protocols for the known manifestations of disease are available [1]. In addition, prophylactic surgery is considered standard of care for some syndromes, such as thyroidectomy for Multiple Endocrine Neoplasia type 2 [2]. Interestingly, identification of specific hereditary mutations also has been associated with differences in likelihood of metastatic disease and thus patient prognosis. As an example, approximately, one-quarter of pheochromocytomas and paragangliomas have an underlying hereditary cause [3,4]. Mutations in three of the genes of the succinate dehydrogenase complex, *SDHB*, *SDHC* and *SDHD*, are associated with susceptibility to pheochromocytomas and paragangliomas. Among those, only mutations in *SDHB* are associated with a high rate of metastatic disease, up to 98% in one series [5,6]. Genetic testing is therefore recommended for all patients with pheochromocytomas and paragangliomas, as the identification of an *SDHB* mutation will influence the initial decision about work-up for metastatic disease as well intensity of follow-up for patients. Thus, we can use our knowledge about the natural history of cancer susceptibility syndromes to guide screening and evaluation of patients with these inherited mutations.

Familial melanoma is among the few examples in which both the indication for testing and subsequent management of mutation-positive patients are not well defined. One of the reasons that genetic testing for familial melanoma is not universally recommended is the low rate of identifiable causative mutations; only two percent of melanoma families have germline mutations [7]. *CDKN2A* and *CDK4* when mutated in the germline lead to melanoma susceptibility and also are common somatic mutations in sporadic melanomas [8]. As skin tone and sun exposure both are shared among family members, it can be difficult to distinguish clustering of melanoma cases in family due to these causes from inherited mutations, particularly in areas of high sun exposure such as Australia [9]. Another reason that genetic testing familial melanoma has been controversial is because it is not entirely clear how a positive test will influence medical management. Regular skin examinations to look for melanoma and its precursors are recommended for patients with a family history of melanoma whether or not they carry a germline susceptibility mutation. However, the identification of a germline mutation may improve screening and precautionary behaviors [10]. Mutations in *CDKN2A* also predispose to pancreatic cancer, and the presence of a pancreatic cancer in the family increases the probability of mutation identification [9,11]. Thus, the question of whether

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patients with mutations should be screened for pancreatic cancer, which can include endoscopic ultrasound, abdominal imaging (CT or MRI) and endoscopic retrograde cholangiopancreatography, has arisen [12,13]. As pancreatic screening would represent a change in medical management, whether or not it is recommended for patients with *CDKN2A* mutations would be considered a rationale for offering genetic testing. Currently screening for pancreatic cancer is more likely to be recommended in mutation-positive families with a history of pancreatic cancer as there is a genotype-phenotype correlation with specific mutations conferring a higher risk of disease [11]. Recent recommendations suggest that patients with three or more melanomas and one invasive melanoma with two or more first or second degree relatives with melanoma or pancreatic cancer should be referred for genetic testing, in a moderate or high incidence population [7]. Thus, genetic testing is emerging as medical management tool, particularly for families with both melanoma and pancreatic cancers.

In patients with cancer susceptibility syndromes due to germline mutations, we know which signaling pathways are disrupted that lead to tumorigenesis. As the same pathways also are disrupted somatically in sporadic cancer, the implications of this knowledge are far-reaching and can be utilized in cancer drug development in multiple ways. The initial identification of Poly (ADP-ribose) polymerase (PARP) inhibitors as potential cancer therapies came from our understanding of the functional effects of *BRCA1* and *BRCA2* mutations in disrupting homologous recombination, a component of Double Strand Break Repair (DSBR) [14,15]. PARP plays an important role in repairing single strand breaks through base excision repair; inhibition of PARP leads to an accumulation of single strand DNA breaks and then double stranded breaks at replication forks [16]. As the cancer cell, which has lost both copies of either *BRCA1* or *BRCA2* cannot repair these double strand breaks, there is synthetic lethality with application of PARP inhibitors, killing the cancer cells specifically. PARP inhibitors have been now been successfully used to treat cancers in *BRCA1/2* mutation carriers, and are being explored in many other tumor types including glioma and pancreatic cancer, as well as myocardial infarction (www.clinicaltrials.gov [17]. PARP inhibitors are not the only example of drugs that target the disrupted pathway associated with a mutation causative of a cancer susceptibility syndrome. Patients with Tuberous Sclerosis Complex (TSC), due to mutations in *TSC1* and *TSC2*, develop angiomyolipomas of the kidneys and lymphangiomyomatosis of the lungs [18]. Loss of function of TSC1/2 leads to constitutive activation of mTOR (mammalian target of rapamycin). Sirolimus (rapamycin), an mTOR inhibitor, has been used to successfully treat angiomyolipomas and lymphangiomyomatosis in TSC patients [19-21]. However, when therapy is halted, there is regrowth of the angiomyolipomas. In melanoma, clinical trials using CDK4 inhibitors are either just beginning or on the horizon (www.clinicaltrials.gov). These examples illustrate that the same pathways are disrupted through inherited mutations in cancer susceptibility syndromes as in somatic mutations in sporadic cancers. Thus, targeted therapies can potentially be used as chemopreventative agents for patients with cancer susceptibility syndromes, but there are multiple questions about their optimal implementation that will need to be addressed in clinical trials. The dosing amount and schedule will have to be determined, as the treatment would be given for a chronic disease over many years, rather than acutely for cancer, as well as the long term side effects. In addition, as the patients with these diseases are rare, pharmaceutical companies may not support clinical trials. Nonetheless, it is important to note that targeted therapies can be used to treat inherited disease, both as those patients develop cancer and potentially as chemoprevention, as well as sporadic cancer.

Personalized therapy for cancer, as for other diseases, can be done through optimizing drug dosing based on inherited variation in metabolism genes. While variation in several genes has been found to be associated with differences in therapeutic outcome, in practice the information is little used. A recent example is variation in *CYP2D6* in relationship to tamoxifen efficacy.

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Patients have both acquired and *de novo* resistance to tamoxifen; its anti-proliferative effects are mediated by its 4-hydroxytamoxifen and endoxifen produced through metabolism by *CYP2D6* [22]. Association studies, focusing on polymorphisms in *CYP2D6*, to determine whether they affect outcome in women treated with tamoxifen have been done in retrospective cohorts of varying size [23-26]. While not all studies have supported an association between *CYP2D6* polymorphisms and clinical outcome related to tamoxifen, currently the preponderance of evidence suggests that poor metabolizers of tamoxifen have a worse outcome, as measured by recurrence rate and overall survival [22,27]. However, testing is not yet done clinically because of various concerns including the retrospective study design, use of convenience sample sets and inconsistent associations with outcomes. In addition, aromatase inhibitors are replacing tamoxifen as standard of care for breast cancer treatment. Currently, the only FDA recommended pharmacogenetic testing is for polymorphisms in *UGT1A1*, which determine the clearance of Irinotecan, used to treat colon cancer [28]. However, despite the recommendation, not all centers use the genetic information to determine dosing regimens. While inherited variation is known to impact cancer drug metabolism, and thus potentially could guide therapeutic decisions, translation into the clinical arena has been limited.

1.3 Somatic genetics and personalized therapy for cancer

Somatic genetic and genomic changes and the pathways that they activate are currently being exploited as targets for cancer therapy. In addition to therapeutic targets, somatic genetic studies have identified biomarkers for clinical outcome; usually they are directly related to the target (e.g. *BRAF* mutation and BRAF inhibitors with response or conversely *KRAS* mutations and EGFR inhibitors with resistance) [29]. While most current studies have focused on single genetic changes, many groups are looking beyond single mutations to build a profile of genetic and genomic changes that can be used to sub-classify tumors for optimal personalized therapy. The ultimate goal is the incorporation of genetic variation within the inherited genome with data on somatic genetic changes in the tumor is so that all aspects of personalized therapy can be addressed.

1.3.1 *Assessing somatic genetic changes in tumors*

Identifying somatic genetic changes in tumors has long been done on a research basis. While some types of somatic genetic testing have been used clinically for some time (e.g. translocation studies in leukemia [30]), the variety of somatic genetic testing currently being translated into the clinical arena is being greatly expanded. The methodology used to identify somatic genetic changes determines the type of results that are available, and it is very important for clinicians and researchers to understand what type of test has been done on a specific sample. In general, there are three types of mutation detection that are commonly used in clinical realm, outside of translocation studies in liquid tumors and sarcomas [31], resulting in distinct data types: direct sequencing of stretches of DNA, short sequencing (15-20 base pairs) and specific mutation interrogation. While amplifications and deletions are assessed on a large scale using SNP-based arrays or array-based comparative genomic hybridization (aCGH) and at a lower throughput using Multiplex Probe Ligation Amplification (MLPA) for research purposes, usually clinically only solitary amplifications are evaluated using techniques such as FISH. In addition, large scale sequencing of cancer genomes is underway in research laboratories, which will provide us with ever expanding amount of genetic data pertinent to cancer development [32,33].

Direct sequencing will identify all point mutations within the stretch of DNA amplified. For genes in which mutations are distributed throughout, including most tumor suppressor genes (e.g. *TP53, VHL*), direct sequencing is the optimal method for mutation detection. However, direct sequencing is the most insensitive method for mutation detection, with accuracy decreasing when the mutation accounts for less than 20% of the total DNA [34]. In addition,

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it is prone to failure when poor quality DNA, such as that extracted from tissues that have been formalin fixed paraffin embedded (FFPE), is used as the input [35]. It also is important to note that it will not identify large scale deletions or duplications that involve the entire region undergoing PCR. It is used commonly on both a research and clinical basis.

Several methods are being used for somatic point mutation detection. All of those methods solely interrogate the specific base of interest and do not provide information about any other genetic changes present in the same gene. It is important this limitation of testing be understood by all researchers and clinicians. Many of the point mutation detection methods also allow for multiplex reactions, thus interrogation of several mutations simultaneously. However, for the multiplex test to be used clinically (Clinical Laboratory Improvement Amendments [CLIA] approved) each mutation has be validated independently, so many institutions have been slow to adapt them for clinical purposes. Three commonly used single nucleotide extension techniques, Taqman, iPlex and SNaPshot, which are being adapted for clinical use, are discussed below.

Taqman® assays (Applied Biosystems, Inc., Foster City CA) are commonly used to detect single nucleotide polymorphisms (SNPs) and similarly can be used to identify somatic single base changes. Probes matched to the wild type and mutant sequence are labeled with different fluorescent tags, and visualized when there is a correct match to the sequence. Many companies, such as Roche Diagnostics, are developing and planning to market molecular diagnostic approaches for somatic mutations based on Taqman. Taqman can detect a variant present at 10% of the total sample, so improves sensitivity over sequencing [36]. Taqman assays are limited as only one nucleotide variant is detected per assay, so if there is more than one possible nucleotide leading to the same amino acid change, multiple assays will be needed.

The iPlex™ (Sequenom, Inc., San Diego CA) platform is a single nucleotide extension technique, which has been used in several studies to simultaneously interrogate multiple somatic single base genetic changes [37,38]. Base calling is based on matrix-assisted laser desorption/ionisation, time-of-flight mass spectrometry (MALDI-TOF) analysis to detect different nucleotides [39]. PCR primers are designed around the single nucleotide base change of interest, with common 10-mer tags on both the forward and reverse primers to allow multiplexing. A primer that ends at the base immediately adjacent to the base of interest used to allow for a single nucleotide extension reaction. If needed, all four nucleotides can be evaluated, but usually there are fewer possible genetic changes. The level of sensitivity is higher than sequencing, down to 10% [40]. This method allows for higher throughput assessment, as up to 10 to 12 somatic mutations can be multiplexed together. Of note, the number optimally included in a multiplex for tumor is fewer for somatic genetic changes than SNPs.

Several groups are using SNaPshot™ (Applied Biosystems, Inc., Foster City CA) for multiplexing and assessing somatic genetic changes [41]. SNaPshot also is a single nucleotide extension technique in which the region of interest is amplified using PCR. Probes of different lengths are designed so that they can be multiplexed and differentiated in fragment analysis on a sequencer. The annealing probe is generally approximately 20 base pairs in length and then different nucleotide sequences are used to extend the length of the probe, such as poly(dT) [42]. The design of these probes can be problematic. All four differentially labeled fluorescent ddNTPs are added to the reaction so that all nucleotides are interrogated. The detection sensitivity is reported to be 5% for the mutant allele [42].

An alternative to single nucleotide extension techniques, Pyrosequencing™ (Qiagen, Inc., Alameda, CA) provides short sequence data [43]. Within the sequence, all mutations are detected, so if several somatic mutations cluster within a short stretch of nucleotides, they all can be detected which confers an advantage over single nucleotide extension techniques. With

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pyrosequencing, mutation detection is possible when the mutant allele only comprises 5% of the total sample, even in DNA extracted from FFPE tissues [35]. In addition, methods have been proposed to optimize mutation detection so that very low percentage mutations can be detected, such as coamplification at lower denaturation temperatures – PCR (COLD-PCR) [44]. Each of the described techniques has been used for somatic mutation detection. The selection at any single institution depends upon the interest in through-put, availability of the necessary equipment and experience with the particular technique.

Solitary amplifications and deletions in tumor cells can be assessed using FISH (fluorescence in situ hybridization) or chromogenic in situ hybridization (CISH), which are commonly used clinically. For research purposes, array-based comparative genomic hybridization (aCGH) or SNP arrays are used to define chromosomal amplifications and deletions. The methods differ slightly. With aCGH, DNA from the tumor is mixed with control diploid DNA; they are then co-hybridized to the array [45]. With SNP arrays, the DNA from the tumor is directly hybridized to the array, and amplifications and deletions are determined as compared to either matched normal DNAs, hybridized to a different array or a pooled set of normal DNAs [46]. The use of large scale copy number assessment with aCGH or SNP arrays in cancer is, in general, confined to the research arena, although such arrays are being routinely used in clinical cytogenetics laboratories to identify pathogenic DNA changes associated with pediatric syndromes. Multiplex probe ligation amplification (MLPA, MRC-Holland, Amsterdam, Netherlands) is a lower throughput method that can be used to identify deletions and amplifications in the genome [47,48]. In MLPA, the probes anneal adjacent to each other and are ligated together. With a single reaction, up to 40 primer pairs can be multiplexed, which are normalized to control probes, so that copy number of the test probes can be quantified. This technique is being used commonly clinically to detect deletions and duplications within cancer predisposition genes (e.g. *TSC2*, *BRCA1*) and on a research basis to detect copy number changes within genes (e.g. *PTEN*, *CDKN2A*) or across a large number of genes [49-52]. MLPA has the advantages of using less DNA than aCGH or SNP arrays and there are multiplex kits available to look at copy number across single genes, exon by exon, unlike high through-put arrays, however it is limited to the probes under investigation. It may be more amenable to clinical use, based on its lower cost, familiarity to many genetics laboratories and the ability to independently validate each amplification or deletion.

Multiple studies are now taking advantage of large scale deep resequencing to better understand the cancer genome. While these studies are currently all done in research laboratories, it is very likely that deep resequencing will be used clinically in the future, if not for whole genome studies, for resequencing of multiple cancer genes simultaneously. For melanoma, whole genome resequencing has been done in COLO-829 derived from a metastasis of a malignant melanoma and compared to a matched lymphoblastoid cell line [53]. 33,345 somatic base substitutions, 680 small deletions, 303 small insertions, and 51 somatic rearrangements were initially identified. The point mutations were confirmed at a higher rate than the other types of genetic changes. A mutational signature consistent with ultraviolet exposure, with predominantly C>T/G>A transitions, was noted. Interestingly, the authors also identified a lower rate of mutations in expressed genes, consistent with preferential translational coupled repair. In addition to the known mutations in *BRAF* (V600E), *PTEN* (large genomic deletion) and *CDKN2A* (two base pair deletion), novel mutations were identified in *SPDEF*, *MMP28* and *UVRAG*. The resequencing of this cancer genome represents only one of the published large scale studies of cancer genomes, with much more data available in the near future.

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1.3.2 Combining genetic analyses and pre-clinical studies in melanoma defines multiple sub-populations with differential responses to targeted therapies

In order to optimize the approach to identify targeted therapies, a combination of genetics and genomics studies, using the techniques described above, are first done so that tumors can be grouped into homogeneous sub-sets. Once the tumors are classified, therapeutic targets can be identified using pre-clinical functional studies specific to the set of point mutations and genomic changes within the tumor sub-set. Currently there are multiple examples of research involving those types of studies; discussed below are examples from melanoma research.

The incidence of melanoma in North America is increasing. For 2009, it is estimated that there will be 69,000 new cases and 8,700 deaths from melanoma [54]. Patients with stage IV disease have less than a 5% expected long-term survival rate. Currently available systemic therapies in the metastatic setting are woefully inadequate. However, there has been significant progress in identifying critical signaling pathways that define sub-sets of melanoma and conducting clinical trials of novel targeted therapies.

Constitutive signaling through the RAS signaling pathway has been described in nearly all melanomas [55]. The best understood pathways downstream of RAS are the MAP kinase (MAPK) and PI3 kinase (PI3K) pathways. RAS itself, specifically *NRAS*, is mutated in ~12% of melanomas, and is capable, in theory, of activating both the MAPK and PI3K/Akt pathways [56]. *BRAF* mutations are present in ~45% of melanomas, representing the most commonly mutated oncogene, and constitutively activate the MAPK pathway [57,58]. The V600E mutation increases the activity of BRAF 480-fold over the wildtype form [59]. Introduction of *BRAF* V600E into melanocytes is sufficient for increased proliferation and clonogenic growth [60]. Based on this and other data, specific inhibitors of mutant BRAF were developed, and are in clinical trials to treat melanoma patients.

The first inhibitor, specific to mutant BRAF, tested in clinical trials is PLX4032 (now known as RO5185426) is a highly selective and potent, as is its structural analog PLX4720. PLX4720 has an IC_{50} of 13 nM against BRAF V600E mutated cell lines and only inhibits one other kinase from a panel of 65 that represent all the families of the human kinome [61]. It is up to 100-fold more selective for mutated BRAF than for wild type BRAF in cell lines. Treatment with PLX4032 in a phase I clinical trial resulted in an unprecedented 60% response rate in patients with *BRAF* mutant melanoma [62]. For the phase II and III trials, all patients had to have demonstrated *BRAF* V600E mutations. For the clinical trial, mutation screening was done through the pharmacology sponsor, but as the drug is approved, the testing will have to be widely available. FDA approval of drugs targeted to specific mutations, which require identification in advance, is driving the development of genetic testing, as described above. The development of therapies targeted to somatic mutations and genetic tests to identify those mutations is being done synergistically.

The vast majority of patients whose melanoma contain *BRAF* mutations appear to respond to its targeted inhibition. However even prior to the phase I clinical trial, it was thought that single agent BRAF inhibitor therapy was unlikely to adequately treat the disease over time. Multiple pathways are known to be activated in melanoma. In particular, activation of the PI3K/Akt pathway occurs in 30-40% of cases [63]. The most common mechanism of PI3K activation is loss of PTEN function, which is a late event in the progression of primary melanoma, confers a higher risk of metastatic disease and has been suggested to be the essential step that allows *BRAF* mutant melanocytes to progress to primary invasive melanoma [56,63-68]. In addition, based on experience in other cancers with targeted therapies, it is very likely that additional mutations will arise in tumors leading to acquired resistance, such as in EGFR targeted therapies and *MET* amplifications [69]. Thus, not surprisingly, preliminary data from the phase I trial suggest that essentially all patients treated with BRAF inhibitors develop progressive

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disease, after variable periods of time on therapy. Thus, there is great interest in understanding the mechanisms of therapeutic resistance to BRAF inhibitors.

We studied determinants of therapeutic resistance to BRAF-inhibitors in *BRAF* V600E mutated metastatic melanomas and melanoma cell lines. They were characterized using direct sequencing and aCGH, to identify potential genetic and genomic changes associated with resistance [70]. We identified a set of melanomas that harbored either concurrent *BRAF* V600E and *CDK4* mutations or concurrent *BRAF* V600E mutation and cyclin D1 (*CCND1*) amplification (17% of all *BRAF* mutated). It is important to note that while it has been shown in primary melanomas that mutations in the same pathway (e.g. the MAPK signaling pathway) are mutually exclusive [71], we observed multiple mutations in the same pathway in both metastatic tumors and cell lines derived from metastatic tumors. Increased resistance to a BRAF-inhibitor was not seen in cell lines with a *CDK4* mutation alone, but was in a cell line with a *BRAF*, *CDK* mutation and *CCND1* amplification. We overexpressed CCND1 alone, and in the presence of CDK4, in a drug sensitive melanoma line. CCND1 overexpression increased resistance, which was enhanced with concurrent overexpression of CDK4. Thus, increased levels of CCND1, resulting in some cases from genomic amplification, may contribute to the BRAF-inhibitor resistance of *BRAF*-V600E mutated melanomas. These data support the importance of building a genetic and genomic profile of the tumor, rather than just determining the presence of a single mutation, so that potential mechanisms of resistance to therapy can be identified.

Several recent studies have examined the effect of BRAF inhibition on cell lines with wild-type BRAF and either mutant or wild-type RAS [72,73]. In the absence of *BRAF* mutations, RAF inhibitors activate the MAPK signaling pathway in a RAS-dependant fashion. Binding of the RAF inhibitors and induction of conformational changes in BRAF kinase domain activates wild-type BRAF binding to CRAF, localization to the plasma membrane and increases CRAF homodimerization. In cell lines with mutant RAS, tumor growth is enhanced by the use of BRAF inhibitors, potentially accounting for the increased rate of and quickly growing squamous cell cancers that have been observed in patients treated with BRAF inhibitors [62]. These data reinforce the importance of accurate genotyping for patients treated on clinical trials with targeted inhibitors.

The preponderance of mutations in *BRAF* are the kinase activating V600E, however there also are other rarer mutations, both in the glycine loop and in the activation segment (within the third cysteine-rich domain) [57]. We examined whether cell lines with mutations in the amino terminal portion of the activation segment (termed non-canonical mutations) responded differently to therapies than V600E mutant melanoma cell lines. Using direct sequencing and one of the single nucleotide extension techniques, we were able to identify a panel of melanoma lines with the non-canonical *BRAF* mutations, G469E and D549V. A prior study had suggested that low-activity mutants of *BRAF* signal via CRAF [59]. Although the cell lines were highly resistant to MEK inhibition, they were sensitive to the CRAF inhibitor sorafenib, whereas melanoma cell lines carrying the *BRAF* V600E mutation were not. Sorafenib was originally developed as a CRAF inhibitor and has a four-fold higher selectivity for CRAF over BRAF, as well as inhibitory effects against a number of other kinases [74]. CRAF is known to suppress apoptosis through a direct association with the mitochondria. In agreement with the idea of the sorafenib effects being CRAF mediated, we found that there was a preferential loss of mitochondrial membrane potential only in the melanoma cells with low activity *BRAF* mutations. Both treatment of the non-canonical mutant cell lines with sorafenib and shRNA targeted to CRAF had the effect of down-regulating its targets, with a MEK-independent decrease in Bcl-2 expression and apoptosis. Therefore, consideration should be given to treating patients with melanomas that containing low activity or non-canonical *BRAF* mutations with sorafenib.

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Approximately 40% of cutaneous melanomas do not contain activating mutations in*BRAF* or *NRAS*; identifying the underlying genetics and genomics of those tumors will be crucial to develop treatments for them. *KIT* mutations also have been demonstrated in cutaneous melanomas, but are associated much more commonly with acral and mucosal melanomas [75,76]. In order to identify additional sub-types of melanoma, without mutations in *BRAF*/*NRAS*/*KIT*, we used an expression profiling approach and identified a group of melanoma cell lines with co-overexpression of *CDK4* and *KIT*. Quantitative PCR confirmed the existence of a similar *KIT*/*CDK4* amplified sub-group in human melanoma samples. Pharmacological studies showed they were resistant to BRAF inhibitors but sensitive to imatinib both *in vitro* and *in vivo*. Pharmacological studies showed the *KIT*/*CDK4* amplified sub-group to be resistant to BRAF inhibitors but sensitive to imatinib in both *in vitro* and *in vivo* melanoma models. Mechanistically, imatinib treatment led to increased apoptosis and G1-phase cell cycle arrest associated with the inhibition of phospho-ERK and increased expression of $p27^{KIP}$. Other melanoma cell lines, which retained some KIT expression but lacked phospho-KIT, were not sensitive to imatinib, suggesting that KIT expression alone is not predictive of response. Co-overexpression of *KIT*/*CDK4* may be a potential mechanism of oncogenic transformation in some *BRAF*/*NRAS* wild-type melanomas. Again, these data support the importance of building a somatic genetic profile for each patient that includes multiple mutations, as patients whose tumors contain unusual mutations, such as the non-canonical *BRAF* mutations or those in *KIT*, may need to be treated with alternative therapies.

1.4 *Conclusion*

These studies serve as an example of how genetics and genomics studies of tumors can inform the selection of targeted therapies and be validated using pre-clinical studies. Many tumor types are being and will be characterized somatically so that patients can be selected for clinical trials of the therapeutic option best suited to their tumor profile. High-throughput characterization of somatic mutations is currently being offered at several academic institutions, with many more in the process of setting it up [41]. Optimally, rather than a single mutation being examined in each tumors, multiple mutations, amplification and deletions will be assayed simultaneously so that the genomic profile of each tumor can be used to provide prognostic information and assist with treatment selection. Inherited variation can provide information about drug dosing and for patients with cancer susceptibility syndromes contribute to treatment choice. Ideally going forward, genetic mutations in somatic genomes and variation in the inherited genome will be used together to select the best possible therapy for the individual patient.

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