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Liquid Biopsies for Assessing Metastatic Melanoma Progression

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Abstract

The field of genomic biomarkers in melanoma has evolved dramatically in the past few decades. Whereas much of the prior focus has been on molecular assessment of tumor tissue, circulating tumor cells (CTCs) and cell-free circulating tumor DNA (ctDNA) as sources of a “liquid biopsy” in cancer patients provide promising potential as a method to assess tumor progression, identify targets for therapy, and to evaluate clinical response to treatment. Blood biomarker assays have the advantage of being non-invasive, allow for dynamic evaluation of disease over a serial time frame, and help to address the issue of tissue sampling bias and tumor heterogeneity. However, there still remains an assortment of technologies and techniques to isolate and detect CTCs and ctDNA and a standardized method is yet to be established. Despite these challenges, multiple studies have already demonstrated the clinical prognostic utility of blood-based genomic biomarker assays. With the advent of next generation sequencing and genome-wide ctDNA analysis, this will undoubtedly lead to an improved understanding of tumor progression, help to identify new targets for treatment, and improve monitoring of treatment response and development of resistance.

Keywords

Melanoma; liquid biopsy; metastasis; blood; circulating tumor cells; cell-free DNA

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In Memoriam

As a giant in the field of surgical oncology and melanoma, Dr. Donald Morton was a revered surgeon, clinician, professor, researcher, and innovator. As an academic surgeon, he was a strong proponent of clinical and basic science translational research in melanoma and his success in the field spanned six decades, garnered numerous consecutive NIH NCI program project grants, greater than 800 peer reviewed publications, and he has the distinction of being the surgeon with the highest continual NIH grant funding. From the beginning, he had the foresight to establish and maintain the John Wayne Cancer Institute's world-renowned melanoma tumor and blood specimen repository—the largest, oldest, and most clinically well-annotated melanoma bank worldwide. His pioneering discoveries in melanoma immunotherapy and human tumor immunology paved the way for countless translational studies using this specimen repository to study the molecular genetics of tumorigenesis and metastasis. He was always driven by his desire to help improve the management and therapies available for melanoma patients with an ultimate goal of finding a cure for the disease. Through our collaborations, we have been at the forefront of melanoma biomarker assays and are now ushering in an exciting new era where next generation sequencing will provide us with even more answers and help improve melanoma patient's care. If he were alive today, he would likely be impressed by the advances that have been made in this field but already looking forward to tackling the next challenge, overcoming the next obstacle, and finding the answers that still elude this field.

I. INTRODUCTION

The incidence rate of melanoma has been increasing for at least the past 30 years and in 2015, an estimated 73,870 new cases will be diagnosed in the United States.¹ Although early stage melanoma is completely curable with surgical resection alone, 5-year survival rates with stage IV disease are <15%.¹ Historically, standard chemotherapy using cytotoxic agents such as dacarbazine and temozolamide has not achieved satisfying response rates and does not prolong survival.²⁻⁴ High dose interleukin-2 invokes a complete response in a minority of patients with metastatic melanoma, but its benefit is limited by a significant toxic side effect profile.^{5,6}

With such bleak options available for patients with advanced melanoma in the past, the need for new therapeutic approaches has been immense. Despite the slow progress, in the past decade and more recently in the past five years, there has been an exponential growth in the potential treatments for metastatic melanoma with promising results. The discovery of a missense mutation in the serine threonine kinase BRAF at codon 600 and its high frequency of occurrence in cutaneous melanoma led to the translational development of *Braf*^{V600} inhibitors for patients with metastatic disease.⁷⁻⁹ In 2011, a phase III study using vemurafenib was the first targeted therapy in melanoma to demonstrate an improvement in overall survival (OS).¹⁰ Compared to the control group who were treated with dacarbazine, the median OS was statistically significantly longer in the vemurafenib group (9.7 mo. vs. 13.6 mo.; HR 0.70, $p=0.0008$) as well as the median progression free survival (1.6 mo. vs. 6.9 mo.; HR 0.38, $p<0.0001$).¹⁰

Immune therapies such as ipilimumab, a monoclonal antibody that activates the immune system by targeting cytotoxic T-lymphocyte antigen-4 (CTLA-4), a protein receptor that down regulates the immune system, have revolutionized the field of melanoma therapy.¹¹ In 2010, Hodi et al. published their phase III randomized controlled trial in patients with unresectable stage III or IV melanoma who received ipilimumab plus gp100 vs. ipilimumab alone vs. gp100 alone.⁶ The median OS with ipilimumab alone was 10.1 mo. compared with 6.4 mo. in patients receiving gp100 alone (HR 0.66; $p=0.003$). Although mostly reversible with the appropriate treatment, the enthusiasm for ipilimumab as an agent that extends OS has been tapered by the concern for its immune-related side effects such as dermatitis, colitis, and endocrinopathies, as well as the 2.1% rate of drug-related deaths.⁶ Despite these valid concerns, the breakthrough with ipilimumab has led to further development of alternate immunotherapies for melanoma with the latest focus on a molecule called programmed cell death 1 (PD-1). PD-1 is another inhibitory cell receptor protein that negatively regulates T-cell activation and their effector mechanisms.^{12,13} It binds to PD-L1, which is expressed on cancer cells and tumor-infiltrating macrophages and PD-L2, which is preferentially expressed on antigen presenting cells.¹⁴ Nivolumab, a PD-1 immune checkpoint inhibitor, was studied initially in patients with advanced treatment-refractory melanoma, non-small cell lung cancer, and kidney cancer and showed a 20–30% objective tumor regression rate.¹⁵ When the melanoma patients were followed for survival outcomes, the median OS in nivolumab-treated patients was 16.8 mo. and 1- and 2-year survival of 62% and 43%, respectively, with a durable median response of 2 years.¹⁶ However, despite the rapid approval of multiple drugs for melanoma in the last 4 years, the OS for advanced stage

melanoma has seen only minor improvement. It is now recognized that monotherapy is not effective but combinatorial treatment strategies are important for achieving successful outcomes. However, we still face the challenges of detecting disease progression at an early stage and developing efficient methods for monitoring patients during treatment to assess therapy response.

With these breakthroughs in melanoma treatment, there has been a renewed vigor in translational research focusing on molecular assessment of melanoma metastasis. Historically, US FDA approval of blood cancer biomarkers has been tepid and lags far behind when compared to the degree of progress made in the approval of new melanoma therapies. In the last two decades, advances in the detection and characterization of circulating tumor cells (CTCs) and cell-free circulating tumor DNA (ctDNA) in plasma and serum of cancer patients, including those with advanced melanoma, has revolutionized the field of genomic biomarker assessment. As a non-invasive test, the diagnostic and prognostic potential of CTC and ctDNA analyses as a liquid biopsy provides actionable molecular information that was previously only available using tumor tissue samples. Our group has been assessing CTC and ctDNA in melanoma since the early 1990's and pioneered many of the sentinel studies in this field.^{17–22} We have demonstrated the utility of blood biopsies in melanoma patients of different clinical stages and while undergoing various treatments. In the case of melanoma, as with other solid tumors, sites of metastases such as visceral organs or the brain may represent sensitive and technically challenging areas to access for biopsies. Even when attainable, these biopsies are subject to selection bias secondary to tumor heterogeneity and represent a single timeframe of disease progression. Tissue biopsies are not always accurate and molecular tumor heterogeneity in melanoma is highly evident.

ctDNA, which has been shown to be shed into the bloodstream by cancer cells undergoing apoptosis or necrosis but also released by living cells,²³ has been studied for decades as it was viewed as a potentially more efficient, accurate, and clinically useful biomarker source. Analysis of ctDNA can provide information about the genomic alterations in the primary tumor and serial monitoring of tumor clones through repetitive blood draws and real-time detection of disease as it correlates to stage and prognosis. CTCs represent cancer cells that have dissociated from the primary or metastatic tumor and can be identified in the peripheral blood of cancer patients.²⁴ CTC analysis as another source of information from the liquid biopsy has been applied to many different cancer types, and has been shown in several studies to be predictive of subclinical metastatic disease and potentially serve as a surrogate marker of treatment outcome in melanoma patients.²⁵

II. TISSUE IS THE ISSUE...OR IS IT?

Currently, tumor biopsy remains the gold standard for primary melanoma diagnosis or confirmation of metastatic disease. The recent advances in targeted therapy in melanoma were made possible by molecular studies on tissue to identify and better understand the primary mechanism of action of these targeted drugs and also the pathways that lead to eventual treatment resistance. However, despite the wealth of information that can be obtained from a tissue sample, biopsies are not without their limitations. As an invasive procedure, biopsies can be uncomfortable, lead to complications such as bleeding,

inflammation, or organ injury, and there are reported cases of biopsies of solid tumors causing recurrence due to needle-track seeding.²⁶

Technically a biopsy can be challenging if the tumor is small (sub centimeter), difficult to image or localize even with high resolution CT scans or MRIs, or are in an anatomically challenging area to access such as the brain or visceral organs.^{27, 28} From a processing standpoint, adequate sample size as well as the method of tissue acquisition, processing, and preservation can affect the integrity of the nucleic acid sample and lead to inadequate molecular analysis.

Perhaps the biggest criticism and limitation of tissue biopsy is the issue of “tumor heterogeneity”. It has been shown that advanced cancers are heterogeneous and can often have variable genetic profiles within the same tumor specimen.^{29,30} This is also true when analyzing tissue from a primary tumor versus local metastatic site such as the lymph nodes versus distant disease. Additionally, a tissue biopsy is a static snapshot in time and does not reflect the fluidity of changes that are occurring at a molecular level in the tumor as it progresses from a primary lesion to metastasis. These issues are becoming more important in the age of molecularly targeted drugs that are dependent on specific mutations or other genomic aberrations.

Blood-based biomarker studies provide a non-invasive source of repetitive and dynamic monitoring of molecular changes in the patient’s cancer. Blood draws can easily be incorporated into a patient’s regular follow-up in the clinic, helps avoid the dangers of biopsies, provides a source of fresh DNA for analysis, and only a small amount of sample is needed to perform complex molecular studies. Blood draws can be performed in any certified clinic, which helps to simplify the logistics of establishing a consistent schedule for monitoring patients.

Nucleic acids are released via normal physiological mechanisms but as compared to healthy individuals, cancer patients have significantly higher levels of ctDNA.^{31–33} When cell death occurs via apoptosis, DNA fragments of approximately 180 bp or less in length are generated.^{34–37} With solid tumors, ctDNA can also be released via necrosis, autophagy, secretion and necroptosis but these processes generate >300bp, randomly digested DNA fragments (Figure 1).³⁸ Since the amount of ctDNA released from the tumor can vary greatly from as low as 0.01% to greater than 90% in some studies,³⁵ development of newer approaches to improve sensitivity and specificity of these assays is crucial in order to optimize detection of low disease burden. In a study we published in 2006, we utilized the serum DNA integrity, calculated as the ratio of longer fragments to total DNA, and found that the mean serum DNA integrity was significantly higher in patients with stage II-IV breast cancers as compared to healthy female volunteers.³⁹ We also *hypothesized* and found that the integrity of ctDNA had significant prognostic utility for detection of breast cancer progression and lymph node metastasis.³⁹

ctDNA blood biomarkers can be assessed by analyzing microsatellites, mutations, copy number variation (CNV), cpG gene promoter hypermethylation, and coding and noncoding regions.³⁸ Our group has been investigating these various sources of ctDNA assays in many

different cancers for over two decades and pioneered much of the work in melanoma. The results of these studies will be presented here and well as the future direction in which this field is headed in the next five years.

III. LOSS OF HETEROZYGOSITY AND MICROSATELLITE INSTABILITY IN MELANOMA ctDNA

ctDNA is composed of both genomic and mitochondrial DNA. Genetic alterations found in ctDNA that can be investigated include microsatellite instability (MSI), loss of heterozygosity (LOH), mutation, rearrangements, amplifications, CNV, and methylation.¹⁶ Our early work in the 1990's focused on discovery of tumor-specific genetic markers in the blood of cancer patients. Prior studies demonstrated that multiple DNA microsatellites with frequent LOH were found in melanoma, but it was unknown if tumor-specific free DNA could be detected in the plasma of melanoma patients, much less provide any clinically useful information.⁴⁰⁻⁴⁵

In a study by Fujiwara et al., we examined the plasma of 57 advanced and 19 early staged melanoma patients using a panel of 10 microsatellite markers representing six chromosomal regions of which 40 patients had matched tumor lesions which were also assessed.¹⁹ This novel study was the first to demonstrate that blood ctDNA analysis in melanoma correlated to clinical outcomes. Overall, we found a significant correlation ($p < 0.0001$) between LOH matched-pair plasma and tumor specimens of each individual MS marker with 21/23 (91%) patients with LOH markers in plasma also showing LOH in their respective tumors¹⁹. Interestingly, combinations of different LOH MS markers (D9S157 and D3S1293, $p=0.01$; D9S157 and D1S228, $p=0.05$; D11S925 and D3S1293, $p=0.01$) were significantly correlated with clinical progression.¹⁹

By this time, our group and others had developed mRNA-based markers using RT-PCR assays to detect melanoma cells in tumor tissue and blood with concordance of mRNA marker expression to disease progression.^{17, 18} However, these mRNA-based studies had limitations such as the potential for false positivity as well as technical challenges in mRNA degradation, processing, and integrity. On the other hand, analysis of DNA markers for somatic mutations and chromosome alterations are more tumor-specific and DNA based assays less technically challenging to perform. By applying the same concept of a multi-marker assay, we demonstrated that plasma LOH analysis could be used to dynamically monitor disease progression and potentially serve as markers for sub-clinical disease detection without requiring invasive tumor biopsies.

Our study as well as others demonstrated that as melanoma progresses, additional LOH events occur that allow the tumor to transform into a more aggressive phenotype or acquire metastatic potential.^{41, 44, 46-48} In a subsequent study, we wanted to determine whether the presence of these tumor microsatellite markers in the preoperative blood of melanoma patients with AJCC stage I-IV disease had any prognostic utility.²¹ We again showed that LOH incidence and frequency correlated with advancing AJCC stage and that in stage III patients, the presence of LOH in preoperative blood was significantly associated with an increased risk of death ($p=0.05$).²¹ By providing additional evidence that detection of

ctDNA was predictive of tumor progression and now clinical outcome, these blood based biomarker assays could potentially provide a means of serially monitoring patients for development of subclinical disease and be used to identify patients at risk for more aggressive phenotypes.

Carrying these two studies a step further, we wanted to determine if LOH in these MS markers could also be correlated with systemic treatment response. During this era, treatment options for patients with advanced melanoma were limited, and biochemotherapy (BC) regimens were shown to have increased response rates and decreased toxicities as compared with those for patients treated with chemotherapy and biologic response modifiers alone.^{49–52} In a study by Taback et al., sera from 41 patients with metastatic melanoma were drawn before the initiation of biochemotherapy and analyzed for LOH with nine microsatellite markers.⁵³ During a median follow up of 13 months, the response rate of 12 patients with LOH was 17% as compared to 72% in the 29 patients without LOH (95% CI 54–85%; $p=0.001$), and failure to respond to biochemotherapy was associated with an increased number of positive serum LOH markers ($p=0.002$).⁵³ In terms of survival data, patients with LOH in serum as compared to patients without LOH had significantly decreased median progression free survival (3 mo. vs. 9.5 mo.; $p<0.001$) and OS (6.3 mo. vs. 15 mo.; $p=0.022$) (Figure 2).⁵³ This was the first demonstration of melanoma blood biopsy utility in monitoring patients during treatment and specifically their response to combination drug therapy. Previously, the LOH assays were technically challenging. Now with parallel sequencing, new opportunities arise that may allow this type of ctDNA analysis to be of increased clinical utility since LOH is common in many cancers, particularly cutaneous melanoma.

IV. TUMOR-ASSOCIATED DNA METHYLATION IN ctDNA

With the focus shifting to ctDNA assays, studies emerged on epigenomic mechanisms regulating cancer progression. Numerous prior studies demonstrated that DNA methylation of gene promoter regions, as well as noncoding genomic sequences, were associated with tumor initiation and progression in various cancers.^{54–61} Our group first reported screening of tumor-related gene hypermethylation in primary and metastatic melanoma tumors by investigating a panel of candidate tumor suppressor genes including *RASSF1A*, *RAR-B2*, and *MGMT*.⁶² Overall, at least one gene demonstrated promoter region CpG island hypermethylation in 75% of the melanoma patients and metastatic tumors demonstrated a higher rate of tumor-related hypermethylation as compared with primaries.⁶² Using our knowledge that ctDNA in the serum of cancer patients had clinical utility, we *hypothesized* that hypermethylation of that same panel of tumor-related genes (*RASSF1A*, *RAR-B2*, and *MGMT*) in serum DNA could predict disease outcome and therapy response in metastatic melanoma patients receiving BC.⁶³ Analyzing 47 patients with stage IV melanoma who had blood drawn before administration of BC, we found that the presence of at least one serum methylated gene predicted for worse OS than in patients with no methylated genes ($p=0.010$). By multivariate analysis, *RASSF1A* methylation was the only factor that significantly correlated with OS and BC response (risk ratio 2.38; 95% CI 1.16 to 4.86, $p=0.018$; and odds ratio 0.21; 95% CI 0.05 to 0.90, $p=0.036$).⁶³

We have explored the presence of methylated ctDNA in melanoma patients as prognostic biomarkers in many studies. In another publication, we demonstrated that estrogen receptor (ER) promoter methylated ctDNA in melanoma patients is a valuable biomarker of disease progression.⁶⁴ We observed that as melanoma tumors progressed, the ER promoter became methylated. This highlights that often times, normal genes in different epigenomic states can also be used as ctDNA biomarkers during tumor progression.

Long interspersed nucleotide elements (LINEs) are noncoding repetitive sequences that are characterized as GC-poor regions with open reading frames 1 and 2 and encode a reverse transcriptase and endonuclease.^{65, 66} LINE-1 is generally methylated in normal cells whereas the high-density CpG islands found in the gene promoter region are hypomethylated in several malignancies.^{67–69} *Absent in melanoma-1 (AIM1)* is a tumor suppressor gene located on chromosome 6.^{70, 71} Hypomethylation of LINE-1 is associated with genomic instability and chromosome translocation. A higher prevalence of LINE-1 has been found on chromosome 6 and LINE-1 hypomethylation and CpG island hypermethylation of other tumor suppressor genes has been correlated in past studies.^{72, 73} We found that LINE-1 hypomethylation was elevated with increasing AJCC stage ($p < 0.0001$) whereas *AIM1* promoter hypermethylation was found at higher frequency in metastatic melanoma ($p = 0.005$).⁷⁴ The same pattern was shown when we analyzed serum ctDNA—unmethylated LINE-1 was found at higher levels in stage III and IV patients as compared with normal healthy donors ($p = 0.022$) and *AIM1* methylated DNA was correlated with OS in stage IV patients ($p = 0.009$).⁷⁴ Overall, stage IV patients with serum unmethylated LINE-1 or methylated *AIM1* DNA had the worse prognosis when compared with patients that had neither ($p = 0.0009$; Figure 3).⁷⁴ Thus the combination of both methylated and unmethylated cfDNA analysis was shown to have prognostic utility as a real-time detection method for assessing disease status and outcomes in melanoma patients. The study also demonstrated the utility of both the methylated status of a coding and non-coding genomic region as biomarkers in melanoma.

V. GENOME WIDE ANALYSIS OF CTCS AND ctDNA

In the past, ctDNA assessment has been hampered by several issues including technical limitations of the volume of serum/plasma required for analysis, adequacy of ctDNA extraction, and specificity/sensitivity problems of PCR based assays. It has also been limited by concordance and availability of data between primary tumors and metastases, the robustness and reproducibility of the assays, and the clinical utility and concordance of the data. Long before *Braf*^{V600} inhibitors became part of the standard armamentarium for treatment of patients with metastatic melanoma, our group investigated the incidence of *B-raf* oncogene (BRAF) mutations in primary cutaneous melanomas, their relation to tumor progression and effect on clinical outcomes.⁷⁵ Direct sequencing of PCR products was performed on DNA microdissected from primary and metastatic melanoma tumors; eighteen mutations (31%) were detected at exon 15 in primary melanoma with a significantly higher frequency in patients < 60 years of age ($p = 0.001$) and *BRAF* mutation frequency in metastatic lesions was significantly higher than in primary tumors (57% vs. 31%; $p = 0.0024$).⁷⁵ Our study suggested that *BRAF* mutation is not likely to be the only major key factor in early melanoma tumorigenesis.

Biomarkers in CTCs have been shown to correlate with increased tumor burden, recurrence risk, and poor survival in melanoma patients.^{25, 76} Yet, although extensive genome-wide profiling studies have been carried out in melanoma tumors, the same detailed genomic characterization of CTCs in melanoma is still lacking. In combination with LOH, single nucleotide polymorphism (SNP)-derived copy number aberrations (CNA) are important genomic aberrations in cancer progression. We therefore postulated that certain genomic signatures in CTCs of patients with locally advanced melanoma patients (Stage IIIB/C) could be correlated with aggressive disease and provide real-time genetic information about the metastatic process.⁷⁷

We performed an array-based genome-wide CNA characterization of melanoma CTC and identified 251 CNAs from 13 patients that showed >90% genomic concordance with CTC-metastasis pairs.⁷⁷ A biomarker panel of 5 CTC-associated CNA [CUB and Sushi multiple domains 2 (*CSMD2*), 1p35.1; contactin associated protein-like 5 (*CNTNAP5*), 2q14.3; necessary for RNA interference, domain containing 2 (*NRDE2*), 14q32.11; ADAM metalloproteinase domain 6, pseudogene (*ADAM6*), 14q32.33; and transient receptor potential cation channel, subfamily M, member 2 (*TRMP2*), 21q22.3] was used to categorize 35 patients with stage IIIB/C melanoma into high- and low risk groups.⁷⁷ The patients in the high-risk group had worse clinical outcomes as compared to the low-risk group with 5-year disease-free survival rates of 13% vs. 69% ($p=0.0006$) and OS of 29% vs. 94% (there were limited events in one group to report an accurate p value) (Figure 4).⁷⁷ A risk score was then generated from the 5-marker CNA panel and evaluated by a multivariable Cox model with known melanoma prognostic factors and the CNA-risk score was an independent prognostic factor for 5-year melanoma recurrence (HR 1.14; CI 1.00–1.44; $p=0.0471$) and death (HR 2.86; CI 1.23–14.42; $p=0.0014$). By providing the first detailed genome-wide SNP and CNA-based analysis of melanoma CTCs, we showed there was a close genomic concordance between CTC and tumor metastases. We also were able to identify a high-risk subgroup of locally advanced melanoma patients with increased risk of developing distant metastasis that may warrant closer follow up. These pioneer studies demonstrated that CTC genomic status is important and are valuable as genomic biomarkers.

VI. THE FUTURE FRONTIER: NEXT GENERATION SEQUENCING

Several of the criticisms and limitations of ctDNA analysis have been directed towards the low specificity and sensitivity of the assays. Low disease burden in early stage patients or those with small tumors, as well as tumor heterogeneity, continue to present challenges to this field of biomarker discovery.

Most recently, high-throughput technology and next generation sequencing (NGS, parallel sequencing) have improved the degree of information and detail available for ctDNA blood-based monitoring assays. Identifying clinically relevant and specific gene mutations and amplifications is important for understanding tumor progression, discovering new mutant drivers of therapy resistance, and diagnosing pre-clinical metastasis. Digital sequencing eliminates the false positives inherent in sequencing low concentrations of DNA and enables a comprehensive method of ctDNA analysis that includes complete exon coverage for tumor mutations and amplifications of melanoma-related genes.⁷⁸

However, so far these NGS studies have been hampered by small sample size, high cost, and inadequate consistency of the data. Needless to say, much of the development in this field is yet to come as it will require industry investment and clinical trials conducted in a multi-institutional setting to validate the clinical significance of these ctDNA biomarkers and their utility for early detection, monitoring of disease progression, assessment of treatment response, and therapy resistance.

In a pilot study we presented at the 2014 ASCO Annual Meeting, we utilized a novel single-molecule digital sequencing assay (Guardant360) for ctDNA analysis in melanoma patients that enables detection of rare genomic abnormalities with ultra-high sensitivity and specificity.⁷⁸ We assessed plasma and paired metastatic tumor tissues in 18 AJCC stage IV melanoma patients and found ctDNA with somatic mutations in 6/8 (75%) matched tumor and plasma specimens with an overall concordance of 86% for the status of *BRAF* and *TP53* in a single bleed. In another 10 patients with matched tumor and plasma samples, ctDNA mutation were found in 8/10 (80%) cases and overall concordance of ctDNA mutation with metastatic melanomas across all 54 genes and 80 kbps was 49%, with individual single gene concordance even higher. Using this unique approach, a more comprehensive method for blood tumor genetic profiling during disease progression is feasible and will help identify multiple targetable genes without the need for tissue biopsies.

VII. CONCLUSIONS

Whereas, in the past, therapeutic options available for patients with advanced stage melanoma were dismal, treatments have drastically changed and there has been an explosion of exciting data in the last 5 years showing improvement in survival.^{6,10,16} We are at a pivotal time where targeted treatments have demonstrated impressive clinical results with a paradigm shift from use of less specific cytotoxic agents to more highly selective therapies. Concordantly, genomic biomarkers have become useful not only for diagnosis but, more importantly, for drug targeting and monitoring of therapy response and development of resistance. Analysis of CTCs and ctDNA allows for tracking tumor dynamics in real-time fashion and will hopefully one day lead to development of personalized cancer care geared towards each individual patient's genomic signatures.

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ABBREVIATIONS

AIMI	Absent in melanoma-1
BC	biochemotherapy
CAN	copy number aberration
CTCs	circulating tumor cells
ctDNA	cell-free circulating tumor DNA

LINEs	long interspersed nucleotide elements
LOH	loss of heterozygosity
OS	overall survival
PD-1	programmed cell death 1

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Figure 1.
Cell-free nucleic acids in the blood.

Figure 2. Kaplan-Meier analysis of progression-free (A) and overall survival for (B) patients with distant metastatic melanoma according to serum loss of heterozygosity (LOH) status at the initiation of concurrent biochemotherapy.

Figure 3.
Kaplan-Meier survival curves for stage IV patients in serum.

Figure 4.
CTC-associated genomic aberrations associated with clinical outcome.