Impact of molecular testing in advanced melanoma on outcomes in a tertiary cancer center and as reported in a publicly available database

Maya Dimitrova1 | Min Jae Kim2 | Iman Osman1 | George Jour1

1Langone Medical Center, New York University, New York
2School of Medicine, New York University, New York

Correspondence
Maya Dimitrova, Langone Medical Center, New York University, 550 1st Ave., New York, NY 10016, USA.
Email: maya.dimitrova@nyulangone.org

Funding information
NYU melanoma SPORE, Grant/Award Number: P50 P50CA225450

Abstract

Background: In patients with advanced melanoma (MM), genomic profiling may guide treatment decisions in the frontline setting and beyond as specific tumor mutations can be treated with targeted therapy (TT). The range of panel sizes used to identify targetable mutations (TM) can range from a few dozen to whole exome sequencing (WES).

Aim: We investigated the impact of panel size and mutation status on first-line treatment selection and outcomes in MM.

Methods and Results: We analyzed data for 1109 MM patients from three cohorts: 169 patients at NYULH and profiled with the 50 gene Ion Torrent panel (IT), 195 patients at MSKCC, profiled with the 400-gene MSK-IMPACT panel (MSK-I) and 745 patients at seven different sites profiled with WES. Data for cohorts 2 and 3 were extrapolated from the publicly available cBioPortal.

Treatment information was available for 100%, 25%, and 0% of patients in cohort 1, 2, and 3, respectively. BRAF and NRAS were among the top five most commonly mutated genes in the IT and MSK-I, whereas for WES only BRAF was a top five mutation. There was no significant difference in OS for BRAF MUT patients treated with immune checkpoint inhibitors (ICI) vs TT in cohort 1 (P = .19), nor for BRAF MUT patients from cohort 1 treated with ICI vs those from cohort 2 treated with TT (P = .762).

Conclusion: Public datasets provide population-level data; however, the heterogeneity of reported clinical information limits their value and calls for data standardization. Without evidence of clear clinical benefit of a larger panel size, there is a rationale for adopting smaller, more cost effective panels in MM.

Keywords
cancer genetics, melanoma, mutations, targeted therapy
INTRODUCTION

Activating somatic mutations in *BRAF* are present in approximately 50% of advanced melanomas. Molecular testing for this mutation has become a standard of care as recommended by the National Comprehensive Cancer Network (NCCN) and the European Society for Medical Oncology (ESMO) for stage III or stage IV disease.\(^1\) The combination of *BRAF* and MEK targeted inhibition (TT) led to the first significant improvements in progression free survival of patients with metastatic melanoma.\(^3\) Targeted therapy and checkpoint inhibitors are both preferred frontline treatments for metastatic melanoma.\(^2\) However, at this time, there are no formal recommendations on how these therapies should be sequenced in patients with *BRAF* positive tumors. In addition, depending on the clinical practice, the panels used to identify targetable alterations can range from small panels of 30 to 40 genes to whole exome sequencing (WES).\(^5\) Next generation sequencing (NGS) and WES have demonstrated utility in identifying other potentially clinically relevant mutations in melanoma but none that change frontline treatment at this time.\(^6\)\(^-\)\(^8\) To our knowledge, there are no studies investigating the size of a molecular panel and its impact on the choice of frontline treatment in cutaneous metastatic melanoma. Although WES may identify potential biomarkers for response to immunotherapy such as microsatellite instability (MS), homologous recombination scores, or tumor mutational burden (TMB), none has been validated in prospective trials in a variety of solid tumors including melanomas.\(^9\)

Herein, we examine the utilization of a targeted molecular sequence panel in a cohort of patients with advanced melanoma at NYULH and compare that with publicly available datasets from other tertiary medical centers using larger panels ranging from 400+ genes to WES to examine the effects that molecular panel sizes and mutational information have on treatment selection and patient outcomes.

PATIENTS AND METHODS

2.1 | Patient selection

We analyzed data for 1109 MM patients from three cohorts. Cohort 1 included 169 patients with advanced (stage III or V) melanoma enrolled at NYULH (Cohort 1) and profiled with the 52 gene Ion Torrent panel (IT). Cohort 2 included 195 patients enrolled at MSKCC (Cohort 2), profiled with the 400-gene MSK-IMPACT panel (MSK-I). Cohort 3 included 745 patients enrolled at seven different sites reporting information to cBioPortal and profiled with WES.

2.2 | Data collection

Data for cohorts 2 and 3 were extrapolated from publicly available data using cBioPortal (completion of data acquisition October 20, 2019). We examined sex, age, cancer stage, lactate dehydrogenase levels (LDH), Eastern Cooperative Oncology Group (ECOG) performance status, number of metastatic sites, *BRAF* status, treatment received and response to treatment as available by the reported data. We searched clinicaltrials.gov for actively recruiting clinical trials as of April 10, 2020 on patients with advanced melanoma harboring each of the top 50 mutations (excluding *BRAF*) as identified by the NYULH, MSK-IMPACT, and WES panels.

2.3 | Statistical analysis

We tested associations between molecular data, treatment choice and overall survival (OS), adjusting for baseline characteristics when available. Statistical tests including t tests, log rank test for survival analysis were carried using Graphpad Prism V.8 (\(P < .05\)).

RESULTS

3.1 | Clinicopathological characteristics of the three cohorts studied

The NYULH cohort of patients consisted of 169 patients with a mean age of diagnosis of 61.6 (Table 1). The male to female ratio was 1:1. 40% of the patients had molecular testing when presenting with stage III disease and 60% were stage IV. The majority of patients (64%) had a normal LDH and an excellent performance status of 0 (60%). In cohort 2 \((n = 195)\), all data points were missing with the exception of gender distribution (Male = 115, Female = 80). It was not possible to know the mean age of the patients in MSK-IMPACT cohort though the M:F ratio was similar. Stage, LDH, and ECOG status were unknown. Although there is more information available in aggregate for the rest of the cBioPortal cohort (Cohort 3), the absolute percentages for stage, LDH, and ECOG are low (<10% of total with concrete data).

3.2 | Comparison of targeted panels and their genomic design reveals significant heterogeneity

We looked at shared genes in the panel design across the targeted molecular panels used by different institutions (NYULH, MD Anderson, MSKCC, and Foundation Medicine) and identified 23 genes that overlapped across five panels (Figure 1). We noticed a significant heterogeneity in the structure of these “targeted panels.” Some panels included more than 300 genes. Others were more restrictive, focusing on oncogenes and tumor suppressor genes with an FDA indication, such as the NYULH Oncomine panel. When focusing on the top five most commonly mutated genes in the cohorts, we found that the five most common alterations between the three are also very heterogeneous and noted that only *BRAF* appears among all three. For instance, while *BRAF* and NRAS appear as expected among the most commonly mutated genes in cohorts 1 and 2, NRAS does not appear as a top five mutation in the WES panels. Instead, the other four most common gene alterations in this group are *LRP1B*, *PCLO*, *FAT4*, and *MGAM*. Interestingly, *BRAF* was not the most common mutation in the melanoma samples tested by the IMPACT panel but the *TERT* hot spot promoter mutation appeared with a frequency of 73%.
3.3  |  Impact of upfront molecular testing on treatment decisions and outcomes in NYULH cohort

Frontline treatment modality was only available for the NYULH cohort. Based on the data reported on cBioPortal, we were able to infer that 25% of the patients in the MSK-IMPACT cohort received TT by analyzing the referenced trial on the portal (Table 2). We observed that regardless of mutation status (BRAF mutant vs BRAF wild type vs other) immunotherapy was the first-line treatment choice in Cohort 1 (Table 3). Targeted therapy was more commonly chosen over immunotherapy as a second line treatment in BRAF mutant melanoma. At the time of data review, 67.5% of patients in Cohort I were
still alive, 30.2% were deceased and there was insufficient data on 2.4% regarding survival. This compared similarly to the numbers that were available for Cohort 2 with 61%, 39%, and 0%, respectively, however, the numbers for Cohort 3 were skewed by gaps in the reported data. In cohort 1, 36% (16/45) of BRAF MUT patients received first-line TT. There was no significant difference for BRAF MUT patients treated with ICI vs TT in cohort 1 in OS (P = .19), nor for BRAF MUT patients from cohort 1 treated with ICI vs those from cohort 2 treated with TT (OS P = .762). There was no data on sequence of treatment in the studies that were reported in cohort 3 or on the response to such treatment.

### 3.4 | Comparison of genomic events detected between cohorts 1, 2, and 3 and their impact on eligibility for clinical trials

We then compared the top 50 most frequently affected genes from the three genomic platforms (NYULH, MSK-IMPACT, and WES) and their impact on trial inclusion. Based on the NYULH panel, genomic information from these genes would have served as an inclusion criteria in 61 actively recruiting clinical trials, whereas this number decreases to 39 trials using the top 50 most frequent genes from the MSK-IMPACT panel and is only seven trials (for one candidate gene-NRAS only) using WES (Figure 2 and Table S1).

### 3.5 | Comparison of the database parameters used to source cohorts 1, 2, and 3

There was significant heterogeneity in the data reported by each of the trials that was included in the cBioPortal database and from which
the molecular data was derived. The lack of standardized reporting of clinical information made it challenging to compare across trials and datasets. For example, sequencing of treatment types was not identifiable using the cBioPortal portal. That is, if treatment was reported at all.

**FIGURE 2** Number of eligible clinical trials out of the 50 most frequently occurring mutations in cohorts 1 to 3. A, A total of 26 targets detected in Cohort 1 for which there was an enrolling clinical trial. B, A total of 17 targets detected in Cohort 2 for which there was an enrolling clinical trial. C, A total of 1 target detected in Cohort 3 for which there were enrolling clinical trials.

**DISCUSSION**

Large panels and WES can identify many more mutations than guidelines recommend testing for or for which there are available treatments. Small molecular panels that fulfill international guidelines...
and FDA recommended therapeutics may be the most cost efficient and easily applicable paradigms to use in the frontline setting. In fact, at this time, there is no data from randomized prospective trials to dictate sequence of therapy in advanced and metastatic melanoma based on molecular profiling; current recommendations are made based on retrospective reviews. As demonstrated by cohort I, there does not appear to be a significant difference in outcome whether patients received IO or TT first. One retrospective review suggests that IO followed by TT leads to superior responses compared to the inverse. Other studies have not found a difference in survival based on sequence but suggest there might be a higher rate of response to IO when it is given after TT. In a cohort of Italian patients, those who received ipilimumab prior to vemurafenib or dabrafenib had better outcomes compared to patients who were treated with TT first. In general, most clinicians are starting treatment with immunotherapy unless a patient has large volume, symptomatic disease. TT has been shown to be superior in this clinical scenario. Given this area of clinical uncertainty, there are two ongoing clinical trials that may establish the sequence of IO and TT: the SECOMBIT (NCT02631447) and DREAMseq studies (NCT02224781).

There is evidence that larger testing platforms may provide more data to guide clinical decision making. In fact, a retrospective analysis of 10,000 patients profiled using the MSK-IMPACT panel demonstrated that patients’ whose tumors had a high TMB had better outcomes with IO vs non-IO treatments. While this finding may be relevant to justify IO regimens in numerous solid tumor types, its usefulness in the particular setting of melanoma remains limited. Although melanoma has already been identified as having the highest TMB from the initial TCGA study, TMB is not used to stratify patients to IO vs other therapies. These findings further support the argument

| Platform | Number of Genes | Total Genes |
|----------|----------------|-------------|
| MSK IMPACT | 6 | PTPRT GRIN2A PTPRD BRAF NRAS ROS1 |
| WES | 94 | IL7R ERBB4 MTOR ATRX SMARCA4 ASXL2 BRD4 KDR RAC1 NTRK1 TP53 Pten EP300 MCD1 HIF1A TET2 EPHA3 SETD2 APC FLT1 TGFB2 PI3KCA CDKN2A ERG PTPRB PTPCH1 NSD1 IGF1R KMT2D SF3B1 MET TSHR BLM BCOR AXL ATR CREBBP CSF1R NTRK3 NOTCH3 NCOA1 BRC2A PLCG2 AR FGFR2 FAT1 FLT4 ARID5B BRC1A1 EPHA7 ARID2 NTRK2 NOTCH1 PDGFRA PAK5 TET1 TERT GLI1 KMT2C HGF DNMT1 ZFHX3 DOTT1 ASXL1 ANKR011 RET ARID1A CARD11 NF1 MED12 EPHB1 PIK3CG TP63 SPEN CTNB1B1 EGFR MAP2K1 KMT2A DICER1 CBL IKZF1 PRDM1 MGA PGR PBRM1 CIC PIK3C2G NOTCH2 FLT3 ALK NOTCH4 BIRC3 ATM POLE |
| WES | 94 | FLG2 ADAMTS20 RYR3 COL5A1 TPT1 TACC2 OBSCN DCC COL3A1 MGAM MXRA5 COL11A1 Dnah8 CD163 TENM3 SCN10A NF1X COL22A1 TRANK1 PRUNE2 ASXL3 ADGRQ4 CMYA5 SVEP1 ZNF804A ERICH3 RP11 CSMD1 TTN COL4A5 MUC17 Dnah11 FAT4 FLG MAGEC1 ADGRV1 RELN UNC79 XRIP2 HMCN1 PREX2 STAB2 Dnah9 FAM13B Dnah3 Dnah4 Ryr1 Sorl1 Apob Abca12 Csmd3 Hydin Myo18b Lrp2 Fam83B Spata1 Unc13c Syn1 Col7a1 Pgcbp Plcb4 Lrp1b Ryr2 Dnah5 Phkdl1 S1 Dscam Neb Fmn2 Usn2a Scn5a Myh1 Myh2 Mrghb2 Col4a4 Myh4 Dnah10 Fat3 Dnah17 C6 Ank3 Cacna1e Spkhap Scn11a Abca13 Dnah2 Thsd7b Podl2 Muc16 Npap1 Meme2 Pappa2 Pcdh15 Csmd2 |
that some of the genomic data generated by large panels is important in
addressing research question, but remains of limited use in the current
clinical setting.18

What may be more clinically relevant for patients is the identification
of molecular targets for which there are clinical trials and there-
fore further therapeutic options. Focusing on the 50 most frequent
genes between the larger molecular panels used in cohorts 2 and
3, there are only six in common between the two panels (PTPRD,
GRIN2A, PTPRD, BRAF, NRAS, ROS1) (Figure 3). ROS1 is the only gene
from this set that was not identified by our own IT panel for which
there are two clinical trials currently open (NCT02568267 and
NCT02465060). Twenty nine of the 50 genes (excluding BRAF) in the
IT panel were associated with clinical trials with 61 active trials at the
time of this publication vs 25 genes of the top 50 in MSK-IMPACT
(P < .0001) out of a total 400+ genes. This again demonstrates the
utility of a limited gene panel in identifying a significant number of eli-
gible clinical trials once a patient has progressed on standard therapy.
In fact, of the top 50 mutations identified by WES, only one was asso-
ciated with clinical trial eligibility (Figure 2 and Table S1).

There are several limitations to our study. Our sample size in cohort
I was the smallest of the three cohorts and the sample sizes across the
different cohorts varied significantly. Our cohort also included a signifi-
cantly larger proportion of stage III patients, which lowered the propor-
tion of BRAF mutated patients to less than 50%. However, our
169 patients compare favorably with the sample sizes of the individual
studies that have reported into cBioPortal and we think this population
is comparable to what is seen at other tertiary centers. There was a lack
of reported patient data in Cohorts 2 and 3, as results were pooled from
larger numbers of trials with no standardized data sets, which limited our
ability to compare across cohorts. Finally, this was a retrospective review
of one institutional experience, which makes our data susceptible to bias.
However, our results support other published studies to date which
demonstrate that sequencing immunotherapy as a frontline therapy may
confers the best survival advantage to patients, or at least be noninferior
to targeted therapy.11,13-17

In conclusion, we have demonstrated that using a small, targeted
panel of only 50 genes provides sufficient information to guide clinical
decision making in the frontline treatment of advanced or metastatic
melanoma. This is very important given the financial constraints associ-
ated with lack of reimbursements by Centers for Medicare and Medicaid
Services (CMS) and third party payers when using larger panels. Although
large panels and WES may provide actionable information in relapsed or
refractory patients, their cost does not seem justified in the initial setting.
Thus, we propose reserving these panels for progressive and/or refrac-
tory disease. Our study also highlights the challenges in using publicly
available data sets to answer clinical questions. These databases are
plagued by the heterogeneity of the different data fields collected. Thus,
the utility of the “meta-data” collected is severely hampered without a
standardized format for reporting patient level data in the public domain.
To that end, we propose the adoption of the format we use in our insti-
tutional interdisciplinary melanoma cooperative group (IMCG) database
to overcome this shortage and harmonize data fields across publicly
available databases (Table S2).

ACKNOWLEDGMENTS
We thank the patients and their families who participated in this
study. This work was supported by the NYU Melanoma SPORE grant
(P50CA225450), the Perlmutter Cancer Center Support grant
(P30CA016087), and the Melanoma Research Alliance.

CONFLICT OF INTEREST
The authors declare no conflicts of interest.

AUTHORS’ CONTRIBUTIONS
All authors had full access to the data in the study and take responsi-
bility for the integrity of the data and the accuracy of the data analy-
sis. Conceptualization, I.O. and G.J.; Methodology, I.O. and G.J.;
Validation, M.D., M.J.K., I.O., and G.J.; Investigation, M.D., M.J.K., and
G.J.; Formal Analysis, M.D. and G.J.; Data Curation, M.J.K.; Writing -
Original Draft, M.D. and G.J.; Writing - Review & Editing, M.D. and G.J.;
Visualization, M.D., M.J.K., I.O., and G.J.; Supervision, I.O. and G.J.; Pro-
ject Administration, I.O. and G.J.; Funding Acquisition, I.O.

ETHICAL STATEMENT
All patients were accrued to the IRB-approved New York University
Interdisciplinary Melanoma Cooperative Group (NYU IMCG) protocol
with patient consent.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study come from two
sources: one part of the data is openly available in cBioPortal at
cbioportal.org. The other part of the data that support the findings of
this study are available on request from the corresponding author.
The data are not publicly available due to privacy or ethical
restrictions.

ORCID
Maya Dimitrova https://orcid.org/0000-0002-7421-4074
Min Jae Kim https://orcid.org/0000-0003-3241-530X

REFERENCES
1. Garbe C, Amaral T, Peris K, et al. European consensus-based interdisci-
plinary guideline for melanoma. Part 1: diagnostics - update 2019.
Eur J Cancer. 2020;126:141-158.
2. National Cancer Center Network. Melanoma (Version 2.2020).
https://www.nccn.org/professionals/physician_gls/pdf/cutaneous_
melanoma_blocks.pdf.
3. Flaherty KT, Infante JR, Daud A, et al. Combined BRAF and MEK inhi-
bition in melanoma with BRAF V600 mutations. N Engl J Med. 2012;
367(18):1694-1703.
4. Robert C, Grob JJ, Stroyakovskiy D, et al. Five-year outcomes with
Dabrafenib plus trametinib in metastatic melanoma. N Engl J Med.
2019;381(7):626-636.
5. Cheng L, Lopez-Beltran A, Massari F, MacLennan GT, Montironi R.
Molecular testing for BRAF mutations to inform melanoma treatment
decisions: a move toward precision medicine. Mod Pathol. 2018;31(1):
24-38.
6. Garg S, Gernier S, Misoya M, et al. Assessing the diagnostic yield of
targeted next-generation sequencing for melanoma and gastrointestinal
 tumors. J Mol Diagn. 2020;22:467-475.
7. Nagahashi M, Shimada Y, Ichikawa H, et al. Next generation sequencing-based gene panel tests for the management of solid tumors. Cancer Sci. 2019;110(1):6-15.
8. Ticha I, Hojny J, Michalkova R, et al. A comprehensive evaluation of pathogenic mutations in primary cutaneous melanomas, including the identification of novel loss-of-function variants. Sci Rep. 2019;9(1):17050.
9. Samstein RM, Lee CH, Shoushtari AN, et al. Tumor mutational load predicts survival after immunotherapy across multiple cancer types. Nat Genet. 2019;51(2):202-206.
10. El-Deiry WS, Goldberg RM, Lenz HJ, et al. The current state of molecular testing in the treatment of patients with solid tumors, 2019. CA Cancer J Clin. 2019;69(4):305-343.
11. Luke JJ, Flaherty KT, Ribas A, Long GV. Targeted agents and immunotherapies: optimizing outcomes in melanoma. Nat Rev Clin Oncol. 2017;14(8):463-482.
12. Pavlick AC, Fecher L, Ascierto PA, Sullivan RJ. Frontline therapy for BRAF-mutated metastatic melanoma: how do you choose, and is there one correct answer? Am Soc Clin Oncol Educ Book. 2019;39:564-571.
13. Ackerman A, Klein O, McDermott DF, et al. Outcomes of patients with metastatic melanoma treated with immunotherapy prior to or after BRAF inhibitors. Cancer. 2014;120(11):1695-1701.
14. Aya F, Fernandez-Martinez A, Gaba L, et al. Sequential treatment with immunotherapy and BRAF inhibitors in BRAF-mutant advanced melanoma. Clin Transl Oncol. 2017;19(1):119-124.
15. Johnson DB, Pectasides E, Feld E, et al. Sequencing treatment in BRAFV600 mutant melanoma: anti-PD-1 before and after BRAF inhibition. J Immunother. 2017;40(1):31-35.
16. Ascierto PA, Simeone E, Sileni VC, et al. Sequential treatment with Ipilimumab and BRAF inhibitors in patients with metastatic melanoma: data from the Italian cohort of the Ipilimumab expanded access program. Cancer Invest. 2014;32(4):144-149.
17. Saab KR, Mooradian MJ, Wang DY, et al. Tolerance and efficacy of BRAF plus MEK inhibition in patients with melanoma who previously have received programmed cell death protein 1-based therapy. Cancer. 2019;125(6):884-891.
18. Alexandrov LB, Nik-Zainal S, Wedge DC, et al. Signatures of mutational processes in human cancer. Nature. 2013;500(7463):415-421.

SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Dimitrova M, Kim MJ, Osman I, Jour G. Impact of molecular testing in advanced melanoma on outcomes in a tertiary cancer center and as reported in a publicly available database. Cancer Reports. 2021:e1380. https://doi.org/10.1002/cnr2.1380