Current and Emerging Biomarkers in Melanoma

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Medical Director, Solid Tumor Molecular Oncology, ARUP Laboratories
Investigator, Huntsman Cancer Institute
University of Utah

1

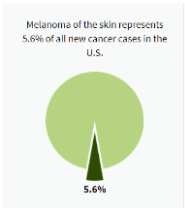
Learning Objectives

- **Understand the *major mutational drivers* in cutaneous melanoma and how somatic mutation testing *guides treatment decisions* for advanced disease.**
- Understand the **diagnostic utility** of somatic mutation testing for resolving diagnostic uncertainty in metastatic melanoma.
- Realize **unmet clinical needs** where molecular/genomic biomarkers may have utility
  - Improving relapse risk stratification of Stage II-III patients.
  - Predicting survival benefit and immune related adverse events with immune checkpoint blockade.
- Review recent clinical trial and preclinical studies that define a new paradigm for **combining immune checkpoint blockade with targeted therapy**
- Discuss **investigational biomarkers** for melanoma staging and predicting therapeutic response
  - Liquid biopsy, circulating tumor DNA (ctDNA)
  - Inflammatory gene expression profiling of the tumor
  - Tumor mutation burden

2

Melanoma is a fairly common cancer

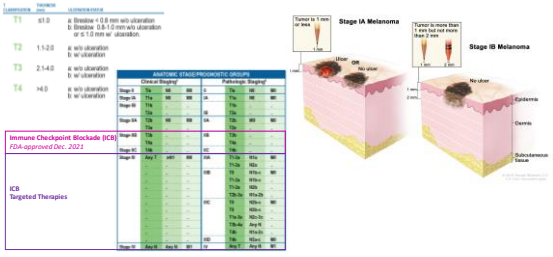
NCI SEER Cancer Database



https://seer.cancer.gov/statfacts/html/melan.html

3

Highly aggressive disease:
risk of metastasis is measured in millimeters



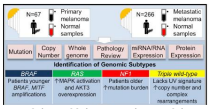
https://www.cancer.gov/types/skin/hp/melanoma-treatment-pdq

4

Framework

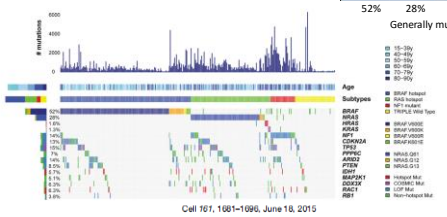
Genomic Classification of Cutaneous Melanoma

The Cancer Genome Atlas



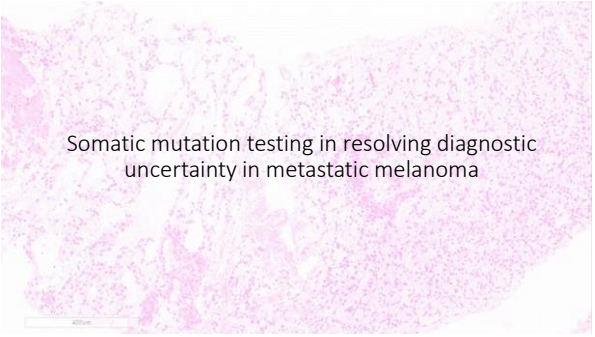
52% 28% 14% 6%

Generally mutually exclusive



Cell 161, 1681–1696, June 18, 2015

5



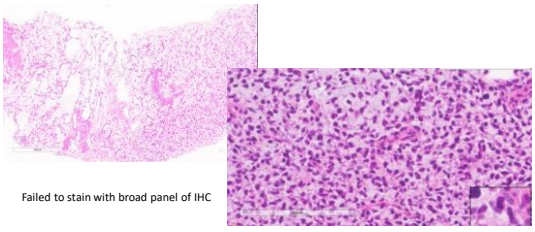
Somatic mutation testing in resolving diagnostic uncertainty in metastatic melanoma

6

62yo male referred to HCI, large axillary mass

outside dx = sarcoma (extraskeletal myxoid chondrosarcoma vs. myxoid liposarcoma vs. other)

now growing quickly through radiation (unlike sarcoma), referred to UU/HCI Sarcoma Center



Failed to stain with broad panel of IHC

7

Electronic Health Record (outside records review)

history of melanoma, ipsilateral arm, 18 mos prior

UU/HCI over-read diagnosis

High grade undifferentiated neoplasm, can not exclude metastatic melanoma,

Recommend molecular testing

↓

Surgical Resection

↓

BRAF V600E Detected

↓

Surgical Oncology question

Sarcoma vs. melanoma, which one?

8

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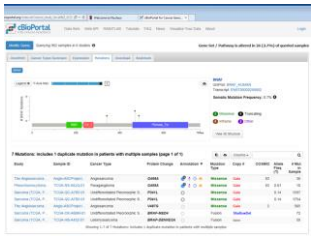

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Surgical Resection

↓

BRAF V600E Detected

↓

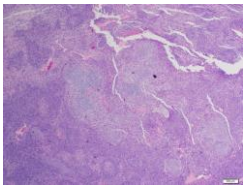
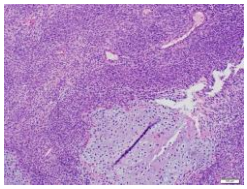
Surgical Oncology question

Sarcoma vs. melanoma, which one?

0/529 sarcomas *BRAF* V600E (absent-exceedingly rare in sarcoma)

9

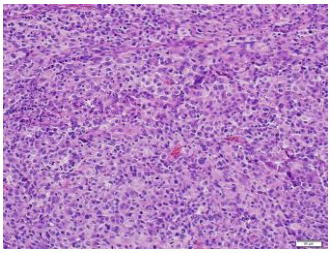
47yo male with axillary mass, and liver masses, transferred care to UU/HCI

Outside diagnosis (3 different reports) = Mesenchymal Chondrosarcoma
NO molecular confirmation with *HEY1-NCOA2* testing

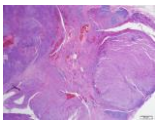
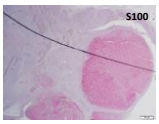
10

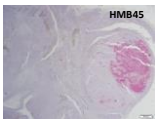
Multiple nodules with distinct epithelioid morphology + pleomorphism

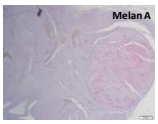


11

Immunostains → Melanoma





12

NGS testing confirms diagnosis of metastatic melanoma

NRAS c.34G>C, p.Gly12Arg (p.G12R)

- Interpretation: This *NRAS* (p.Gly12Arg) mutation activates the MAPK pathway (Rajalingam et al., 2007), and it has been reported in melanoma patients (COSMIC database, accessed December 8, 2015). Patients with *NRAS*-mutant melanoma may benefit from systemic immunotherapy (Johnson et al., 2015) as well as treatment with MEK inhibitors (Ascierto et al., 2013; Grimaldi et al., 2014; Thumar et al., 2014).

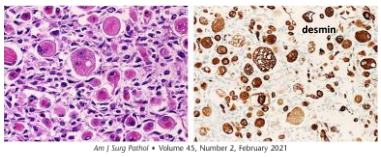
This patient received combo nivolumab/ipilimumab immunotherapy and the liver metatstases regressed!

13

Resolving diagnostic uncertainty in melanoma

melanomas frequently dedifferentiate when metastatic and/or can display a variety of misleading mesenchymal features

- Spindled, pleomorphic, small round/primitive blue cell, rhabdoid
- Myxoid, osteocartilagenous, lipoblastic metaplasia



Am J Surg Pathol • Volume 45, Number 2, February 2021

14

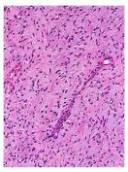
Dedifferentiated and Undifferentiated Melanomas

Report of 35 New Cases With Literature Review and Proposal of Diagnostic Criteria

Abbas Agaimy, MD, Robert Stoehr, PhD,* Arnkathrin Hornung, MD,† Judith Popp, MD,† Michael Erdmann, MD,† Lucie Heinzerling, MD,†‡ and Arndt Hartmann, MD**

Am J Surg Pathol • Volume 45, Number 2, February 2021

- n=35 unpublished cases, n=50 previously published cases, n=85 total
- negative for S100, SOX10, Melan-A, HMB45, pan-melanoma IHC
- Initial diagnoses (known in 66 cases)
  - undifferentiated/unclassified pleomorphic sarcoma (n=30)
  - unclassified epithelioid malignancy (n=7)
  - Pleomorphic rhabdomyosarcoma (n=5)
  - other specific sarcoma types (n=6)
  - poorly differentiated carcinoma (n=2)
  - collision tumor (n=2),
  - atypical fibroxanthoma (n=2)
  - reactive osteochondromatous lesion (n=1)
- 16.6% diagnosis of melanoma was considered
- **Axilla**, inguinal or other nodal basin, variety of visceral organs and body cavities, soft tissue, bone



15

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Melanoma compatible somatic mutation detected in 73% of cases

TABLE 4. Comparison of Demographic and Genetic Features of Different Categories of DM and UM

Tumor Category	Male:Female Ratio	Age, Median (Range) (y)	BRAF Mutations, (%)	NRAS Mutations, (%)	Non-V600E/All BRAF Mutations, (%)	NF1 Mutations/ Tested Case
Dedifferentiated primary melanoma	1.8:1	66 (47-74)	2/14 (14)	2/14 (14)	0/2 (0)	2/2*
Undifferentiated metastatic melanoma with known primary	1.5:1	67 (24-88)	20/48 (41.6)	15/48 (31.2)	1/20 (5); V600K	0/0*
Undifferentiated metastatic melanoma of unknown primary	6.5:1	59 (35-86)	3/15 (20)	9/15 (60)	2/3 (66); both V600K	1/1*
Total	3.3:1	64 (24-88)	25/77 (32.5)	26/77 (33.8)	3/25 (12)	3/3

*Cases were examined with an NF1-containing next-generation sequencing panel either initially or after negative *BRAF/NRAS/KIT* testing.

16

Authors proposed criteria for the diagnosis undifferentiated metastatic melanoma

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TABLE 5. Criteria Proposed to Diagnose Undifferentiated Metastatic Malignant Melanoma

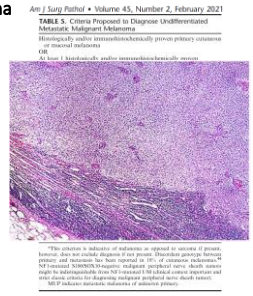
Histologically and/or immunohistochemically proven primary cutaneous or mucosal melanoma
OR
At least 1 histologically and/or immunohistochemically proven dedifferentiated melanoma MUP
Exploration of the remote clinical history (primary tumor might have been excised decades ago)
Review of previously excised melanocytic lesion/s to exclude melanoma before adopting a diagnosis of MUP
Undifferentiated histology in the metastasis (UPS) with multiple cytologic (epithelioid, rhabdoid, spindled and pleomorphic cells) patterns
Inconclusive immunophenotyping with either sarcoma-only immunophenotype or pluripotypes/patterns of limited specificity, eg, pleomorphic rhabdomyosarcoma, smooth muscle like, myoepithelial-like, osteosarcomatoid, primitive small cell or multiple patterns
Lack of histologic patterns that are known to be associated with specific or defining genetic alteration/etiology such as synovial sarcoma, EWSR1-positive Ewing sarcoma, conventional leiomyosarcoma, etc
Undifferentiated metastasis not epicentered at a site of previous irradiation (otherwise postradiation sarcoma should be considered)
Demonstration of a melanoma-compatible or melanoma-typical genotype or of a mutation known to have occurred in the primary melanoma, eg, *BRAF*, *NRAS*, *KIT*, or *NF1* mutation*
In the very exceptional cases with frankly epithelial differentiation, eg, adenocarcinoma-like, exclude another primary, eg, *BRAF* mutated colorectal carcinoma, etc, by appropriate clinical examination/imaging
In the exceptional cases with frankly possible *BRAF* mutation such as anaplastic thyroid carcinoma by appropriate immunohistochemistry and/or clinical examination/imaging

*This criterion is indicative of melanoma as opposed to sarcoma if present, however, does not exclude diagnosis if not present. Discordant genotype between primary and metastasis has been reported in 18% of cutaneous melanomas.[9] NF1-mutated S100/SOX10-negative malignant peripheral nerve sheath tumors might be indistinguishable from NF1-mutated UM (clinical context important and strict classic criteria for diagnosing malignant peripheral nerve sheath tumor).
MUP indicates melanoma, melanoma of unknown primary.

17

helpful clues to aid in the diagnosis undifferentiated metastatic melanoma

- History of melanoma
- *Remote* h/o melanoma
  - Higher risk stage?
- Axilla, groin or other LN basin!!!
- Obviously in a LN!!!
- Melanoma-compatible mutation detected



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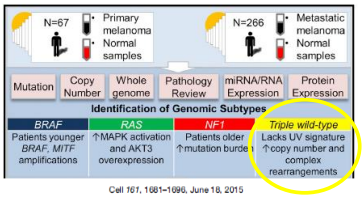
18

Resolving diagnostic uncertainty in melanoma

- Detection of ***BRAF*** and ***NRAS*** mutations (≥70% cutaneous melanoma) can help distinguish undifferentiated melanomas, or melanomas mimicking mesenchymal neoplasms, from soft tissue, bone or visceral sarcomas. Exceptionally rare in sarcoma.
  - More challenging to distinguish undifferentiated carcinoma from melanoma by mutation
- ***KIT*** mutations would not be surprising in metastatic melanoma from older patient with chronic sun damage – and/or could suggest acral, mucosal origin (assuming ruled out GIST)
- ***NF1*** mutations do occur in both melanoma and sarcoma (especially MPNST) – more limited diagnostic utility

19

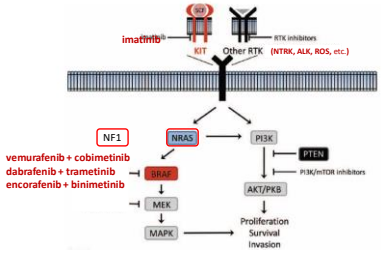
wild type result does Not exclude melanoma



Cell 161, 1681–1696, June 18, 2015

20

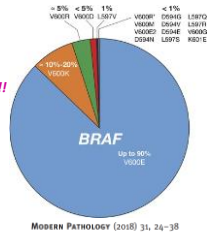
Testing for Actionable Mutations



21

Actionable/potentially actionable mutations are common:
somatic mutation testing is standard of care

- *BRAF*$^{V600E/K}$ (~50%)
  - RAF and MEK inhibitors
    - Adjuvant Stage III
    - Unresectable Stage III
    - Stage IV
    - ***Contraindicated in BRAF wild type melanoma!!!***



Modern Pathology (2018) 31, 24–38

22

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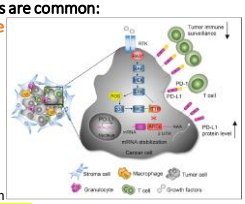
    - ***Contraindicated in BRAF wild type melanoma!!!***
- ***NRAS***$^{Q61R/K/L}$ (15-30%)
  - Major unmet clinical need, ongoing trials
  - Correlates with poor survival
  - Minority respond to targeted MEK inhibition
  - Immune Checkpoint Blockade = First line therapy


Immunity 47, December 19, 2017

23

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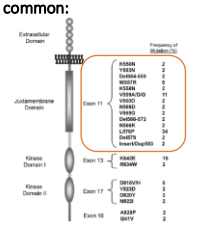
  - Minority respond to targeted MEK inhibition
  - Immune Checkpoint Blockade = First line therapy
- ***KIT* exon 11** (10-15% of acral, mucosal melanoma)
  - Also enriched in melanoma with chronic sun damage
  - Targeted therapy responses are limited and not durable
- ***NTRK, ALK, ROS*** fusions (<1%)



Biochem Pharmacol. 2010 Sep 1; 80(5): 568–574.

24

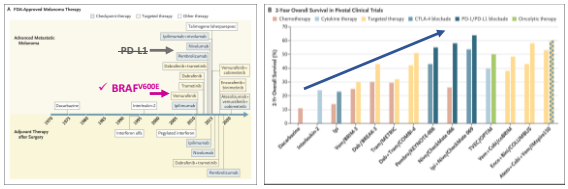
NCCN Clinical Practice Guidelines (version 2.2022)
Indications for Somatic Mutation Testing

- Stage III
  - Eligibility for RAF + MEK inhibitors as adjuvant therapy (*BRAF*^{V600}-mutant)
  - Ongoing trials for neoadjuvant RAF + MEK inhibition (*BRAF*^{V600}-mutant)
- Stage IV – newly diagnosed and relapsed, eligibility for targeted tx
  (Retesting after progression on targeted therapy is not recommended)

  Broad panel testing (such as NGS) is recommended if feasible or when initial single gene testing for *BRAF* is negative/not detected.

25

Despite major advances in the treatment of advanced-stage melanoma:
NO new standard-of-care biomarkers since 2011



Brendan D. Curti, M.D., and Mark B. Faries, M.D.
N ENGL J MED 384;23 NEJM.ORG JUNE 10, 2021

26

Unlike NSCLC and other carcinomas,
PD-L1 testing is NOT required in melanoma

- Tumor PD-L1 staining can identify patients more likely to respond
- *but* patients with PD-L1 negative tumors may still respond and benefit from anti-PD-1 immunotherapy.
- Stage IIB,IIC, III, IV melanoma are eligible for anti-PD-1 therapy

27

Important Clinical Question:
Most effective method for combination treatment?

Immune Checkpoint Blockade (ICB) + targeted therapy

DREAM-seq, NCT02224781
Two-year outcome results reported at the ASCO Annual Meeting, June 2021

SECOMBIT, NCT02631447

ImmunoCobiVem; NCT02902029

28

ASCO Plenary Series

DREAMseq (Doublet, Randomized Evaluation in Advanced Melanoma Sequencing) a Phase III Trial: ECOG-ACRIN EA6134

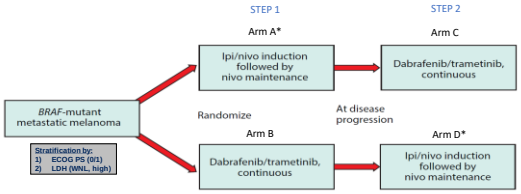
Michael B. Atkins[1], Sandra Lee[2], Bartosz Chmielowski[3], Antoni Ribas[3], Ahmad A. Tarhini[4], Thach-Giao Truong[5], Diwakar Davar[6], Mark O'Rourke[7], Brendan D. Curti[8], Joanna M. Brell[9], Kari L. Kendra[10], Alexandra P. Ikeguchi[11], Jedd D. Wolchok[12], John M. Kirkwood[6]

[1]Georgetown Lombardi Comprehensive Cancer Center, Washington DC; [2]Dana-Farber Cancer Institute, Boston MA; [3]Jonsson Comprehensive Cancer Center University of California Los Angeles, Los Angeles CA; [4]H. Lee Moffitt Cancer Center and Research Institute, Tampa FL; [5]Kaiser Permanente Northern California, Vallejo CA; [6]Pittsburgh Cancer Institute, Pittsburgh PA; [7]Greenville Health System Cancer Institute, Greenville SC; [8]Providence Cancer Institute, Portland OR; [9]MetroHealth Medical Center, Cleveland OH; [10]Ohio State University Comprehensive Cancer Center, Columbus OH; [11]University of Oklahoma Medical Center, Oklahoma City OK; [12]Memorial Sloan Kettering Cancer Center, New York NY

Slide Courtesy Michael B. Atkins, MD

29

DREAMseq Trial Treatment Schema

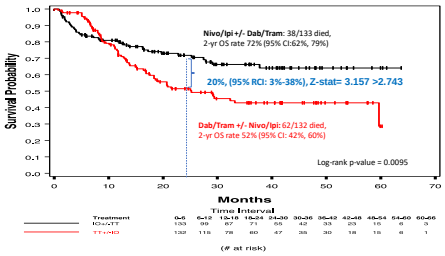


*Nivo/Ipi Induction = 12 wks; nivo maintenance = 72 wks

Slide courtesy Michael B. Atkins, MD

30

Improved Overall Survival (OS) leading with Nivo/Ipi



Slide courtesy, Michael B. Atkins, MD,

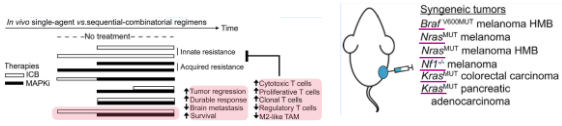
31

Recent preclinical studies suggest a promising new combo treatment paradigm for multiple cancer types

Anti-PD-1/L1 lead-in before MAPK inhibitor combination maximizes antitumor immunity and efficacy

Yujue Wang, Sixue Liu, Zhentao Yang, Alain P. Algazi, Shirley H. Lomeli, Yan Wang, Megan Othus, Aayoung Hong, Xiaoyan Wang, Chris E. Randolph, Alexis M. Jones, Marcus W. Bosenberg, Stephanie D. Byrum, Alan J. Tackett, Henry Lopez, Clayton Yates, David B. Solit, Antoni Ribas, Marco Piva, Gatien Moriceau, and Roger S. Lo

Cancer Cell 39, 1375–1387, October 11, 2021



Syngeneic tumors
Braf^{V600MUT} melanoma HMB
Nras^{MUT} melanoma
Nras^{MUT} melanoma HMB
Nf1$^{-/-}$ melanoma
Kras^{MUT} colorectal carcinoma
Kras^{MUT} pancreatic adenocarcinoma

32

Chasing with Biomarkers, charting unknown waters

Which patients are likely to receive benefit from ICB?

Which patients are *not* likely to receive benefit from ICB?

33

Management of Immune-Related Adverse Events in Patients Treated With Immune Checkpoint Inhibitor Therapy: ASCO Guideline Update

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J Clin Oncol 39:4073-4126. © 2021

- Rash or Inflammatory Dermatitis
- Bullous Dermatoses
- SCAR (Stevens-Johnson syndrome, toxic epidermal necrolysis, acute generalized exanthematous pustulosis and drug reaction with eosinophilia and systemic symptoms or drug-induced hypersensitivity syndrome
- Hemolytic Anemia, aplastic anemia
- HUS
- Acquired TTP
- Lymphompenia
- ITP
- Acquired hemophilia A
- Colitis
- Hepatitis
- Pneumonitis
- Endocrinopathies (adrenal, thyroid, pituitary, diabetes)
- Autoimmune arthritis
- Myositis, polymyositis-like syndrome
- Nephritis or acute kidney injury
- Myocarditis, Pericarditis, Arrhythmias, Impaired Ventricular Function With Heart Failure, and Vasculitis
- Venous Thromboembolism
- Uveitis or iritis, episclerits
- Myasthenia Gravis
- Guillain-Barre syndrome
- Peripheral Neuropathy
- Autonomic neuropathy
- Aseptic meningitis
- encephalitis
- Demyelinating Diseases, Including Multiple Sclerosis, Transverse Myelitis, ADEM, ON, and NMO
- Infusion reaction

34

Mounting Evidence within Tumors: immunogenicity and inflammation

- **immune cell infiltration**
  - activated T cells vs. dysfunctional T cells
  - immunosuppressive regulatory T cells and M2-like tumor associated macrophages
- tumor immunogenicity: **tumor mutation burden (TMB)**, neoantigen load, neoantigen heterogeneity
- expression of genes involved in antigen presentation
- specific gene mutations associated with resistance
- adaptive immune resistance, PD-L1 and LAG-3 expression
- **inflammatory gene expression** (particularly the IFNγ pathway)

35

Retrospective study

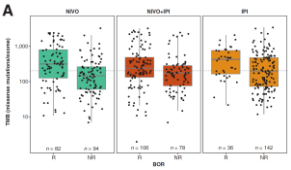
CheckMate 066 (NCT01721772)

CheckMate 067 (NCT01844505)

- whole exome sequencing
  - germline
  - Pre-treatment tumor
- *somatic missense* mutations
- calculated median for each trial cohort (**mutations/exome**)
- TMB^{HIGH} > median
- TMB^{LOW} < median

TMB and Inflammatory Gene Expression Associated with Clinical Outcomes following Immunotherapy in Advanced Melanoma

F. Stephen Hodi[1], Jedd D. Wolchok[2,3,4,5], Dirk Schadendorf[6], James Larkin[7], Georgina V. Long[8,9], Xiaozhong Qian[10], Abdel Saci[11], Tina C. Young[12], Sujaya Srinivasan[10], Han Chang[12], Hao Tang[12], Megan Wind-Rotolo[10], Jasmine I. Rizzo[13], Donald G. Jackson[10], and Paolo A. Ascierto[14]



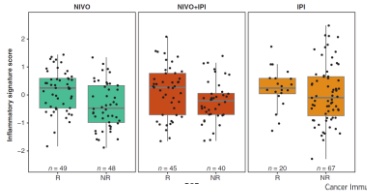
High variance TMB among both Responders vs. Non-responders

Cancer Immunol Res; 9(10) October 2021

36

High Variance in Tumor Inflammation Score (TIS) among both responders and nonresponders

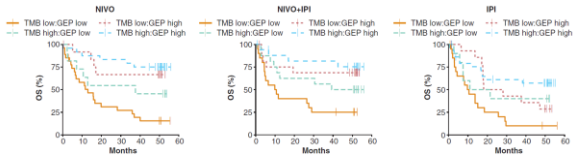
- RNA sequencing (RNA-seq), FFPE tumors, pretreatment
- *CD274* (PD-L1), *CD8A*, *LAG3*, *STAT1*



Cancer Immunol Res; 9(10) October 2021

37

Overall Survival is Stratified by TMB and TIS

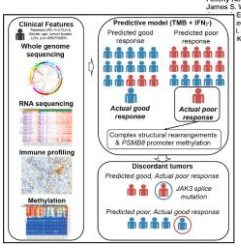


Cancer Immunol Res 2021;9:1202–13

38

Multiomic profiling of checkpoint inhibitor-treated melanoma: Identifying predictors of response and resistance, and markers of biological discordance

Felicity Newell,[1,17] Ines Pires da Silva,[2,4,5,6,17] Peter A. Johansson,[1,17] Alexander M. Menzies,[2,5,6,7,17] James S. Wilmott,[2,3,4,17] Venkateswar Addala,[1,8] Matteo S. Carlino,[2,4,9,10] Helen Rizos,[11] Katia Nones,[1] Edwards,[2,3,4] Vanessa Lakis,[1] Stephen H. Kazakoff,[1] Pamela Mukhopadhyay,[1] Peter M. Ferguson,[2,4,11] eonard,[1] Lambros T. Koufariotis,[1] Scott Wood,[1] Christian U. Blank,[12,13] John F. Thompson,[2,4,14] I. Spillane,[2,4,11] Robyn P.M. Saw,[2,4,11,14] Kerwin F. Shannon,[2,7,14] John V. Pearson,[1] Graham J. Mann,[2,3,4,15] K. Hayward,[1,17] Richard A. Scolyer,[2,3,4,11,16] Nicola Waddell,[1,4,18] and Georgina V. Long[2,3,4,6,7,10,16,19,*]



Restrospective study, Stage IV melanoma
IFNγ gene signature developed with melanoma
IDO1, CXCL10, CXCL9, HLA-DRA, STAT1, IFNγ

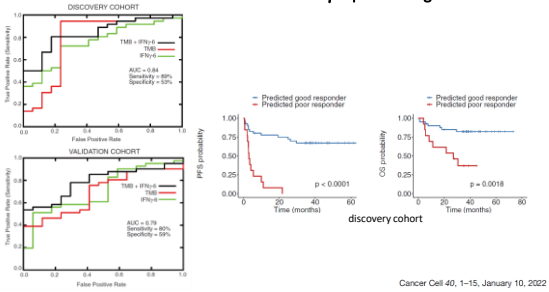
TMB and IFNγ accurately predicted response to ICB (89% sensitivity)

Failed to predict resistance (59% specificity)
no common mechanisms of resistance

Cancer Cell 40, 1–15, January 10, 2022

39

Performance of combined TMB and IFNγ expression signature



discovery cohort

Cancer Cell 40, 1–15, January 10, 2022

40

Conclusions

- TMB, neoantigen load, IFNγ expression signature, PD-L1 expression, and presence of CD8+ T cells in the tumor microenvironment are associated with *response* to ICB
- TMB and IFNγ expression signature are *independent* predictive factors
- potential predictive value of combined TMB and inflammatory gene signatures needs to be validated in prospective studies using predefined cutoffs

41

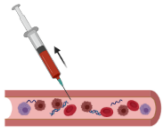
ImmunoMATCH:
next generation NCI precision medicine trials
prospective molecular profiling and biomarker stratification

S2101 BiCaZO: A Study Combining Two Immunotherapies (Cabozantinib and Nivolumab) to Treat Patients With Advanced Melanoma or HNSCC, an immunoMATCH Pilot Study

Hypothesis
- TMB and TIS will be feasible for upfront patient stratification
- Combination of Anti-PD1 and VEGFRi are effective and the response rate will be different among tumors with different TMB and TIS

Objectives
- feasibility of 14 day TAT for biomarkers
- Obtain preliminary evidence of clinical activity in pre-defined molecular subgroups (ORR, PFS, OS)

42



Liquid Biopsy
circulating tumor DNA (ctDNA)

1. Monitoring and predicting treatment efficacy in Stage IV patients
   Lancet Oncol 2021; 22: 370–80
2. Predicting relapse and survival in Stage III patients
   Annals of Oncology 30: 804–814, 2019
3. Predicting relapse and survival in Stage II/III patients
   Annals of Oncology 29: 490–496, 2018

43

Circulating tumour DNA in patients with advanced melanoma treated with dabrafenib or dabrafenib plus trametinib: a clinical validation study

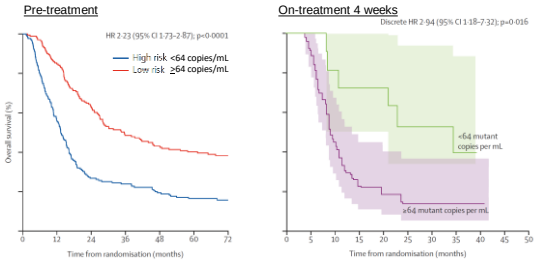
Lancet Oncol 2021; 22: 370–80

Mahrukh M Syeda, Jennifer M Wiggins, Broderick C Corless, Georgina V Long, Keith T Flaherty, Dirk Schadendorf, Paul D Nathan, Caroline Robert, Antoni Ribas, Michael A Davies, Jean Jacques Grob, Eduard Gasal, Matthew Squires, Mahtab Marker, James Garrett, Jan C Brase, David Polsky

- Retrospective study, unresectable or metastatic *BRAF* -mutant melanoma
  - Advanced stage → expect tumor shedding and detectable ctDNA pre-treatment
- phase 3 COMBI-d and phase 2 COMBI-MB trials
  - dabrafenib ± trametinib
- Measured $BRAF^{V600E/K}$ ctDNA by droplet digital PCR , n=345 patients
  - Detected in 90% of patients
- *Serially collected blood* - before treatment and on treatment week 4
- Biomarker study funded by Novartis, testing performed by NYU

44

ctDNA testing stratified high vs. low risk for progression and prognosticates overall survival in both baseline and *very early* on-treatment



45

Unmet clinical need:
Improving risk stratification for Stage III melanoma

- Stage III patients are eligible for adjuvant ICB therapy
  - Costly
  - Potential for immune related adverse events (irAEs)

- Clinical goal for biomarker development and validations
  - Ideally - avoid unnecessary treatment in patients who are cured by surgery alone
    - 40%–90% of patients with resected stage III disease treated with curative intent will relapse within 5 years
  - identify those at highest risk of relapse, where the benefits of systemic therapy may outweigh the risk of irAEs

46

Prediction and monitoring of relapse in stage III melanoma using circulating tumor DNA

Annals of Oncology 30: 804–814, 2019

L. Tan[1,2†], S. Sandhu[1,2†], R. J. Lee[3,4†], J. Li[1,2], J. Callahan[1], S. Ftouni[1], N. Dhomen[3], P. Middlehurst[3], A. Wallace[5], J. Raleigh[1], A. Hatzimihalis[1], M. A. Henderson[1,2], M. Shackleton[6], A. Haydon[7], V. Mar[6], D. E. Gyorki[1,2], D. Oudit[4,8], M. A. Dawson[1,2,9], R. J. Hicks[1,2], P. Lorigan[4,8], G. A. McArthur[1,2], R. Marais[3,4‡], S. Q. Wong[1‡] & S.-J. Dawson[1,2,9*‡]

- Tumor: mutations identified in 99/133 (74%) patients
  - *BRAF*, *NRAS*, *TERT* promoter
  - Blood: 315 prospectively collected plasma specimens
  - Pre-Op baseline
  - Post-Op
- ctDNA Assay = droplet digital PCR (ddPCR)
- ctDNA was detected in 37 of 99 (37%) individuals
- 53 of 99 (54%) had relapsed with median follow up of 18 months (range: 2–58 months) (none had received adjuvant systemic therapy)

47

ctDNA detection increases with increasing T Stage (Breslow/primary tumor thickness, ulceration, lymph node stage)

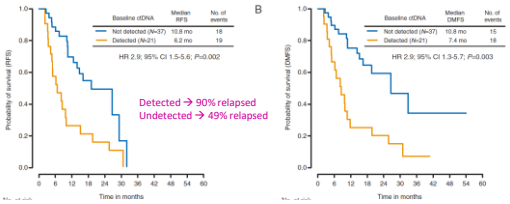
Table 1. Clinicopathological characteristics of patients in the MIST cohort according to baseline and postoperative ctDNA status

Characteristics	ctDNA baseline				ctDNA postoperative			
	Undetected *N* (%)	Detected *N* (%)	*P*	Total *N* (%)	Undetected *N* (%)	Detected *N* (%)	*P*	Total *N* (%)
Age								
<70 years	35 (67)	17 (33)	0.55	52 (76)	35 (73)	13 (27)	0.74	52 (76)
≥70 years	9 (56)	7 (44)		16 (24)	9 (75)	3 (25)		16 (24)
Sex								
Male	29 (60)	19 (40)	0.28	48 (71)	35 (78)	10 (22)	0.77	45 (66)
Female	15 (75)	5 (25)		20 (29)	17 (74)	6 (26)		23 (34)
AJCC substage								
IIIA	7 (100)	0 (0)	**0.02**	7 (10)	8 (100)	0 (0)	0.06	8 (11)
IIIB	16 (70)	7 (30)		23 (34)	21 (88)	3 (12)		24 (32)
IIIC and IIID	21 (55)	17 (45)		38 (56)	23 (82)	14 (38)		37 (49)
Breslow thickness								
≤2.0 mm	23 (79)	6 (21)	**0.046**	29 (46)	27 (87)	4 (13)	0.09	31 (53)
>2.0–4.0 mm	9 (69)	4 (31)		13 (21)	7 (88)	1 (12)		8 (14)
>4.0 mm	11 (52)	10 (48)		21 (33)	11 (58)	8 (42)		19 (33)
Ulceration								
Absent	28 (74)	10 (26)	0.39	38 (62)	31 (89)	4 (11)	**0.02**	35 (62)
Present	14 (61)	9 (39)		23 (38)	13 (62)	8 (38)		21 (38)

Annals of Oncology 30: 804–814, 2019

48

PRE-operative ctDNA detection Stage III melanoma: reduced relapse free and distant metastasis free survival

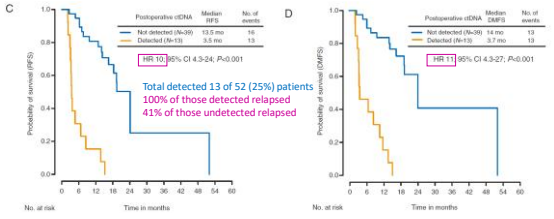


Patients treated with surgery alone - NO systemic therapy

Annals of Oncology 30: 804–814, 2019

49

POST-operative ctDNA detection Stage III melanoma reduced relapse free and distant metastasis free survival



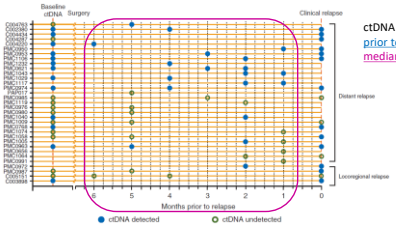
Patients treated with surgery alone - NO systemic therapy

Annals of Oncology 30: 804–814, 2019

50

Serial postoperative liquid biopsies

ctDNA was detected *prior* to relapse in half the patients

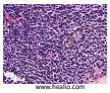


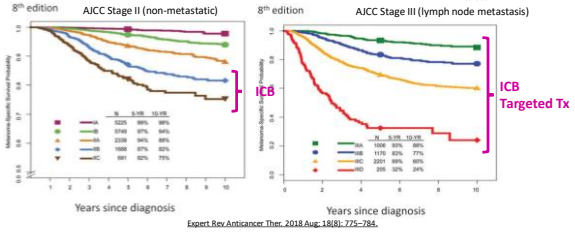
ctDNA detected 16/33 (48%) patients prior to clinical relapse median lead time of 2 months.

Annals of Oncology 30: 804–814, 2019

51

High Risk Stage II-III Melanoma:
improve risk stratification Stage IIB-C, IIIA-B?





Expert Rev Anticancer Ther. 2018 Aug; 18(8): 775–784.

52

Can ctDNA distinguish relapsers from nonrelapsers within high risk, resected, Stage II/III melanoma patients?

- Retrospective study
- Stage IIB, IIC, III melanoma
- Single plasma collection within 12 weeks after surgery (trial setting)
- ddPCR $BRAF^{V600E}$ and $NRAS^{Q61K/L}$
- detectable $\geq$1 copy of mutant DNA/2mL plasma

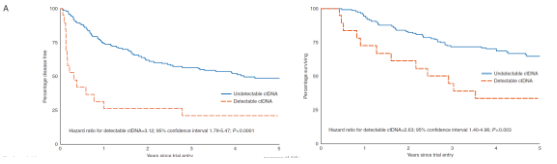
Table 1. Demographics of patients with detectable or undetectable ctDNA

Characteristic	Total *N* (%)	Undetectable ctDNA *N* (%)	Detectable ctDNA *N* (%)
Disease stage			
II	36 (22)	33 (23)	3 (16)
IIA	29 (18)	27 (19)	2 (11)
IIB	59 (37)	51 (36)	8 (42)
IIC	37 (23)	31 (22)	6 (32)
Mutation status			
BRAF V600E	132 (82)	117 (82)	15 (79)
NRAS Q61K/L	29 (18)	25 (18)	4 (21)
Total	161 (100)	142 (88)	19 (12)

Annals of Oncology 29: 490–496, 2018

53

Detection of ctDNA: reduced disease-free and overall survival (5yr) Stage II/III melanoma



Annals of Oncology 29: 490–496, 2018

54

Detection of ctDNA improves prognostication of Stage II/III melanoma

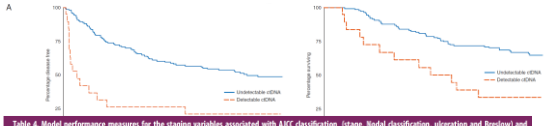


Table 4. Model performance measures for the staging variables associated with AJCC classification (stage, Nodal classification, ulceration and Breslow) and the model adjusted for ctDNA

Model Outcome Measure	AJCC staging variables			Adjusted for ctDNA		
	DFI	OS	DMFI	DFI	OS	DMFI
Prognostic separation measure D statistic	0.63 (SE = 0.17)	0.70 (SE = 0.21)	0.53 (SE = 0.18)	0.96 (SE = 0.20)	0.98 (SE = 0.23)	1.01 (SE = 0.22)
Predictive ability measure Nagelkerke's R^2	0.093	0.085	0.077	0.17	0.13	0.15
Calibration shrinkage measure	0.43	0.36	0.29	0.65	0.53	0.63

Annals of Oncology 29: 490–496, 2018

55

Summary

- Somatic mutation testing, particularly for *BRAF*, remains essential for, and will continue to guide, SOC therapy for cutaneous melanoma
- Panel testing is recommended, if feasible, to cover actionable mutations
  - *BRAF > NRAS > KIT > NF1> NTRK/ROS/ALK*
- Molecular testing may help resolve diagnostic uncertainty with metastatic melanoma
- Recent clinical trial data demonstrates improved efficacy of combo therapy → **ICB lead followed by targeted therapy**
  - Emerging data – may be relevant to other cancers
- Emerging evidence suggest genomic markers of tumor immunogenicity (TMB) and inflammation (CD8 infiltration, IFNγ gene expression signatures) identifies patients who are most likely to benefit from ICB, prospective clinical trials pending
- Liquid biopsy/ctDNA testing may improve disease monitoring and risk stratification, prospective clinical trials needed

56

Acknowledgements

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Sapna Patel, MD, Chair SWOG Melanoma Committee, Medical Oncology, MDACC

Kenneth Grossmann, MD PhD, Senior Principle Scientist, Clinical Director, Merck

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57