Current and Emerging Biomarkers in Melanoma

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ARUP LABORATORIES

HUNTSMAN CANCER INSTITUTE UNIVERSITY OF UTAH

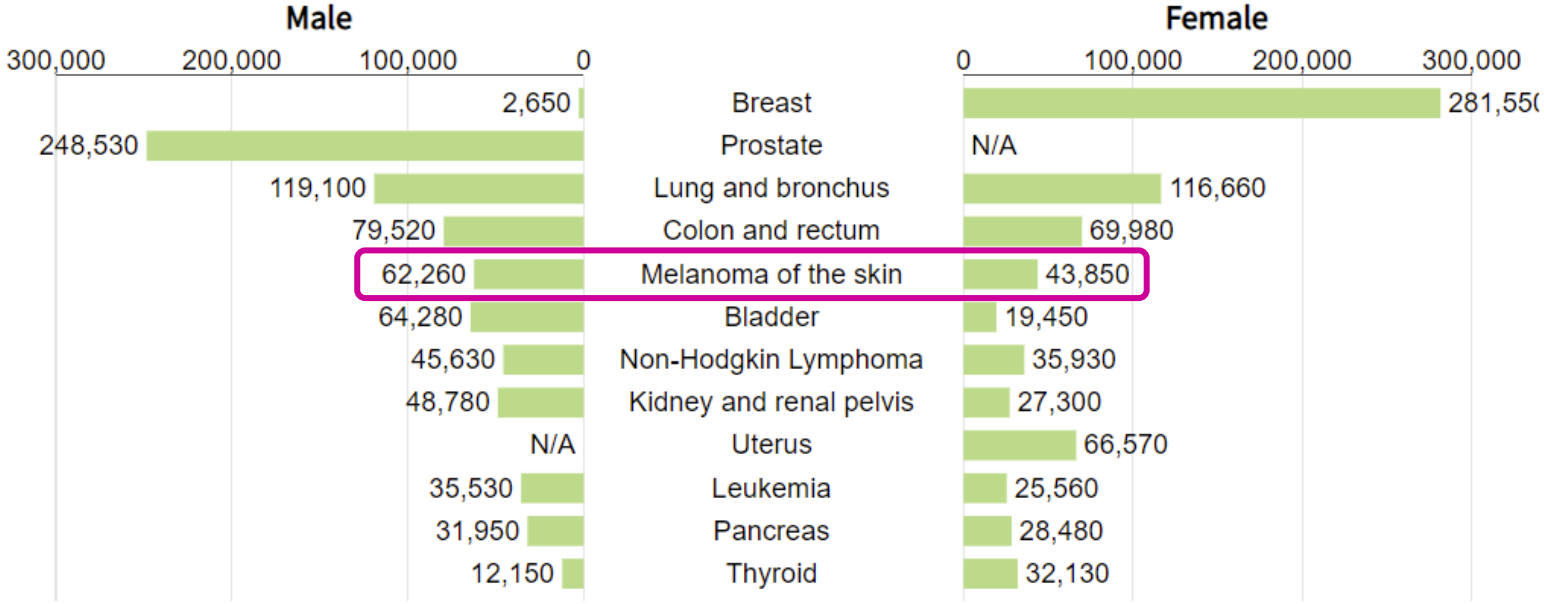
HEALTH UNIVERSITY OF UTAH

Learning Objectives

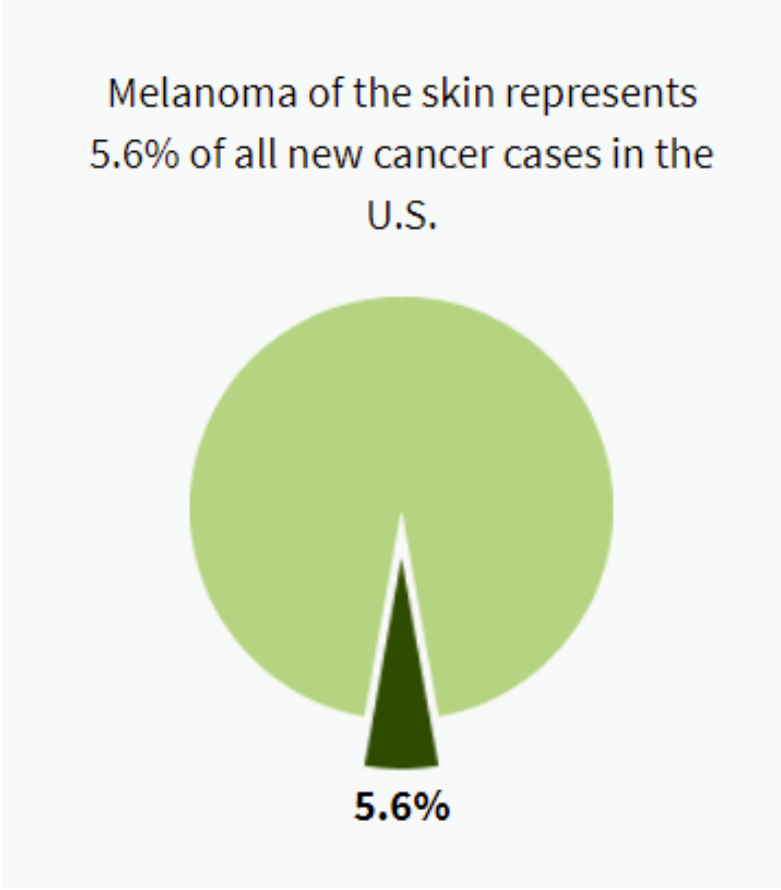
- **Understand the *major mutational drivers* in cutaneous melanoma and how somatic mutation testing *guides treatment decisions* for advanced disease.**
- Understand the **diagnostic utility** of somatic mutation testing for resolving diagnostic uncertainty in metastatic melanoma.
- Realize **unmet 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

Melanoma is a fairly common cancer

NCI SEER Cancer Database



Source: Cancer Facts & Figures 2021, American Cancer Society (ACS), Atlanta, Georgia, 2021.



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

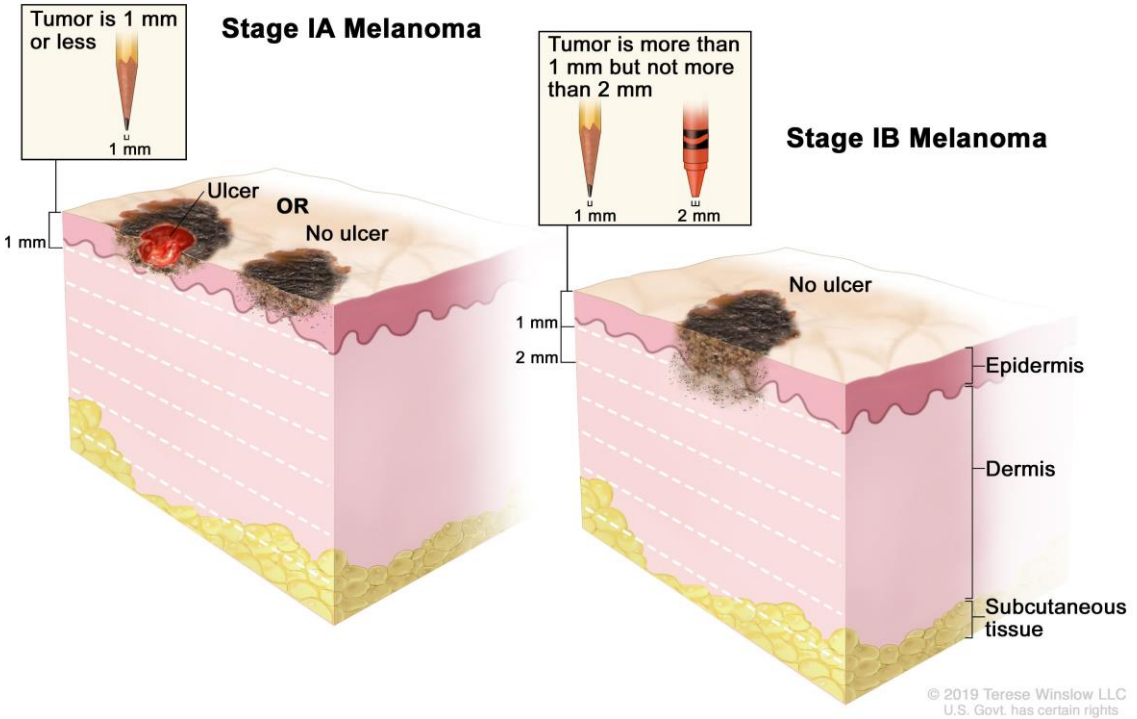
Highly aggressive disease:
risk of metastasis is measured in millimeters

T CLASSIFICATION	THICKNESS (mm)	ULCERATION STATUS
T1	≤1.0	a: Breslow < 0.8 mm w/o ulceration b: Breslow 0.8-1.0 mm w/o ulceration or ≤ 1.0 mm w/ ulceration.
T2	1.1-2.0	a: w/o ulceration b: w/ ulceration
T3	2.1-4.0	a: w/o ulceration b: w/ ulceration
T4	>4.0	a: w/o ulceration b: w/ ulceration

Immune Checkpoint Blockade (ICB)
FDA-approved Dec. 2021

ICB
Targeted Therapies

ANATOMIC STAGE/PROGNOSTIC GROUPS							
	Clinical Staging[3]				Pathologic Staging[4]		
Stage 0	Tis	N0	M0	0	Tis	N0	M0
Stage IA	T1a	N0	M0	IA	T1a	N0	M0
Stage IB	T1b	..	..		T1b	..	..
	T2a	..	..	IB	T2a	..	..
Stage IIA	T2b	N0	M0	IIA	T2b	M0	M0
	T3a	..	..		T2a	..	..
Stage IIB	T3b	..	..	IIB	T3b	..	..
	T4a	..	..		T4a	..	..
Stage IIC	T4b	..	..	IIC	T4b	..	..
Stage III	Any T	≥N1	M0	IIIA	T1-2a	N1a	M0
	..	..	..		T1-2a	N2a	..
	..	..	..	IIIB	T0	N1b-c	M0
	..	..	..		T1-2a	N1b-c	..
	..	..	..		T1-2a	N2b	..
	..	..	..		T2b-3a	N1a-2b	..
	..	..	..	IIIC	T0	N2b-c	M0
	..	..	..		T0	N3b-c	..
	..	..	..		T1a-3a	N2c-3c	..
	..	..	..		T3b-4a	Any N	..
	..	..	..		T4b	N1a-2c	..
	..	..	..	IIID	T4b	N3a-c	M0
Stage IV	Any N	Any N	M1	IV	Any T	Any N	M1

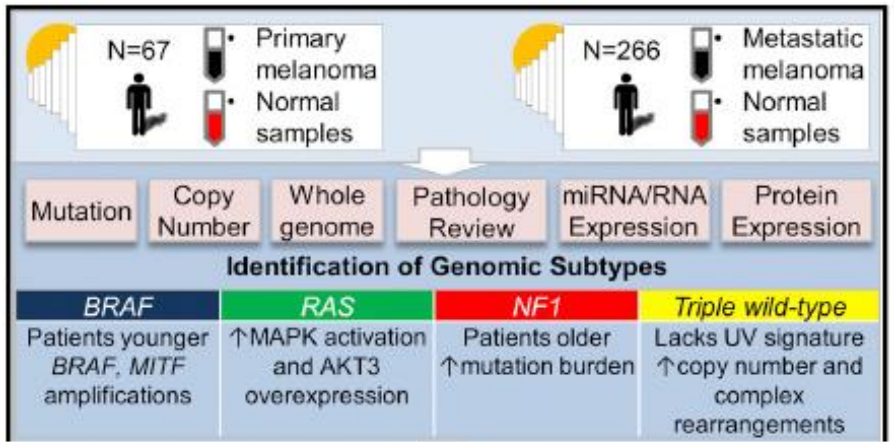


https://www.cancer.gov/types/skin/hp/melanoma-treatment-pdq

Framework

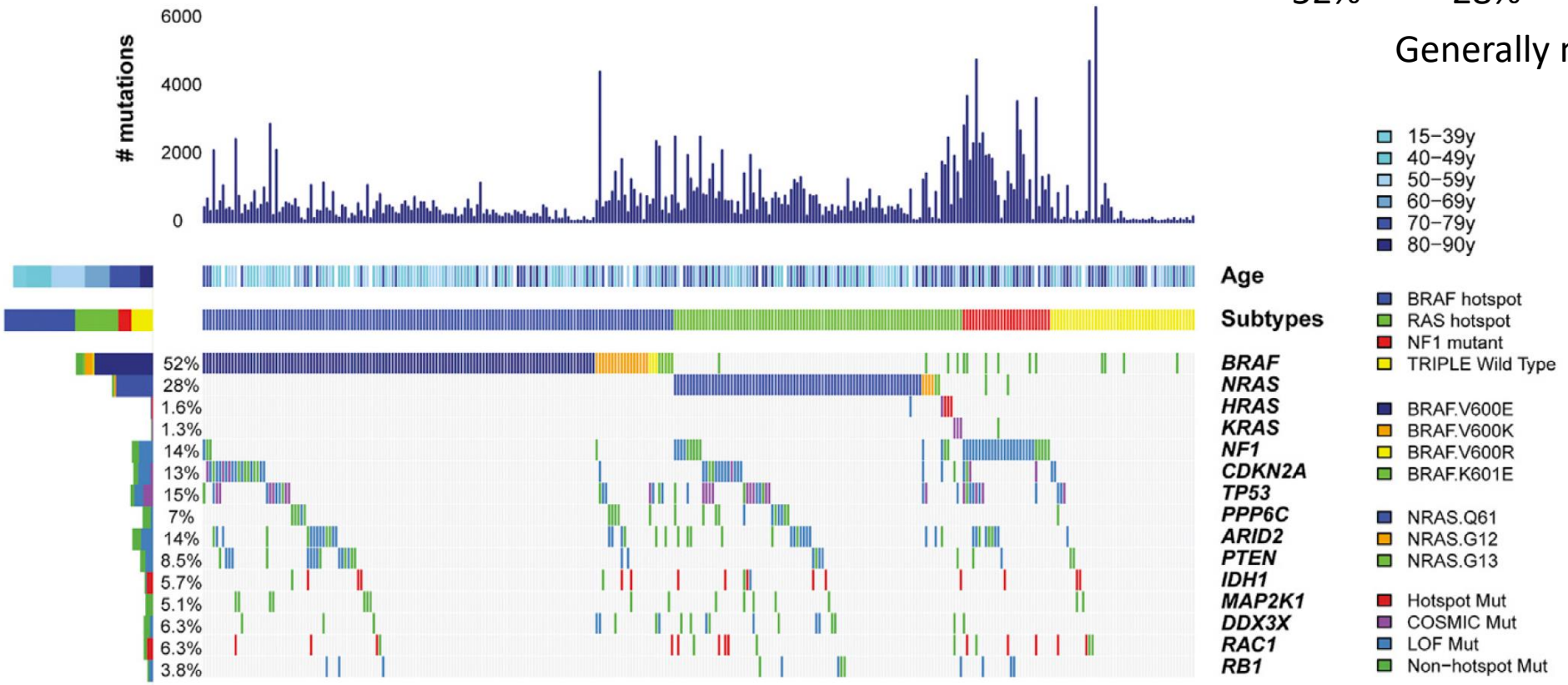
Genomic Classification of Cutaneous Melanoma

The Cancer Genome Atlas



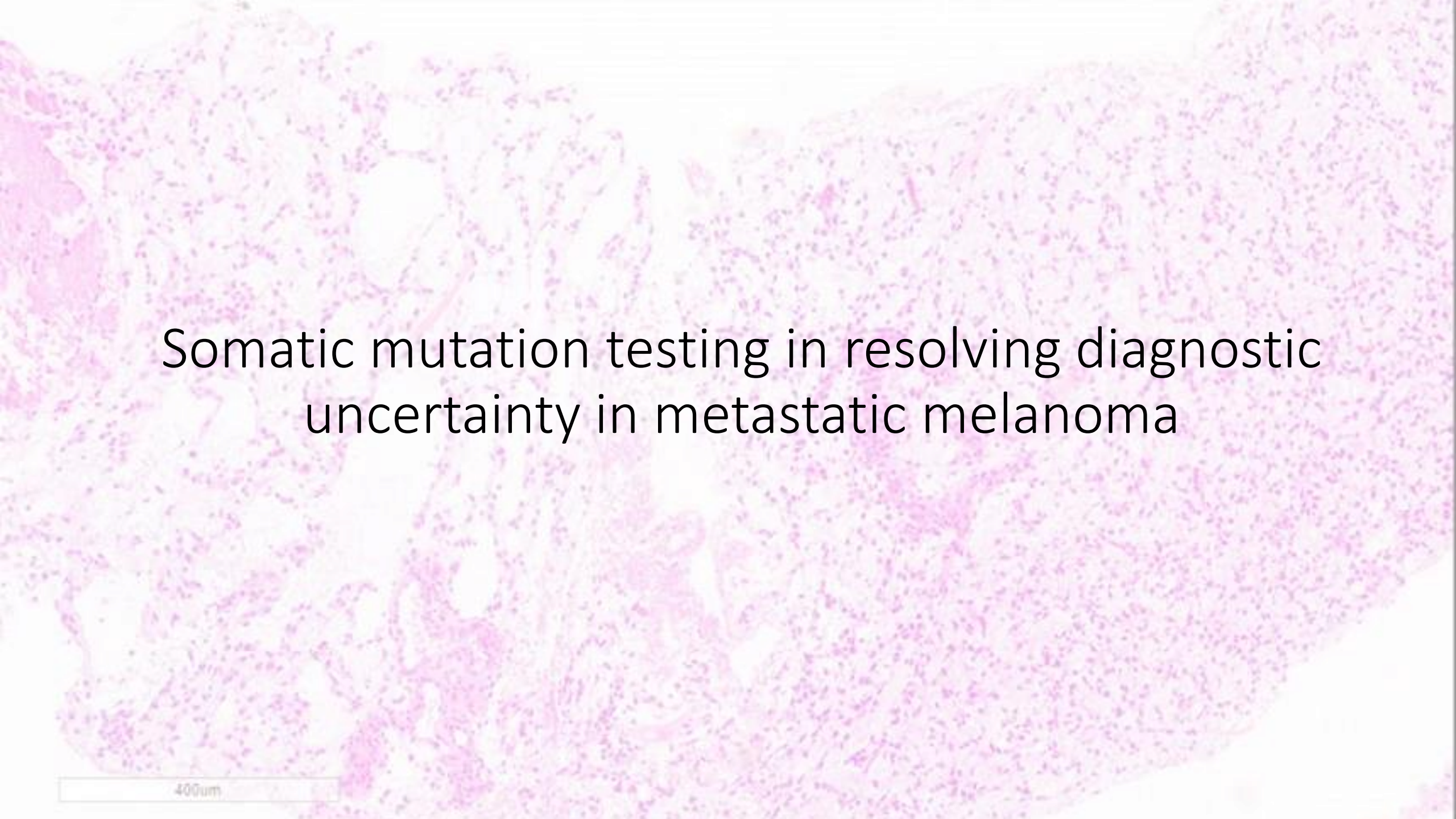
52% 28% 14% 6%

Generally mutually exclusive



Cell *161*, 1681–1696, June 18, 2015

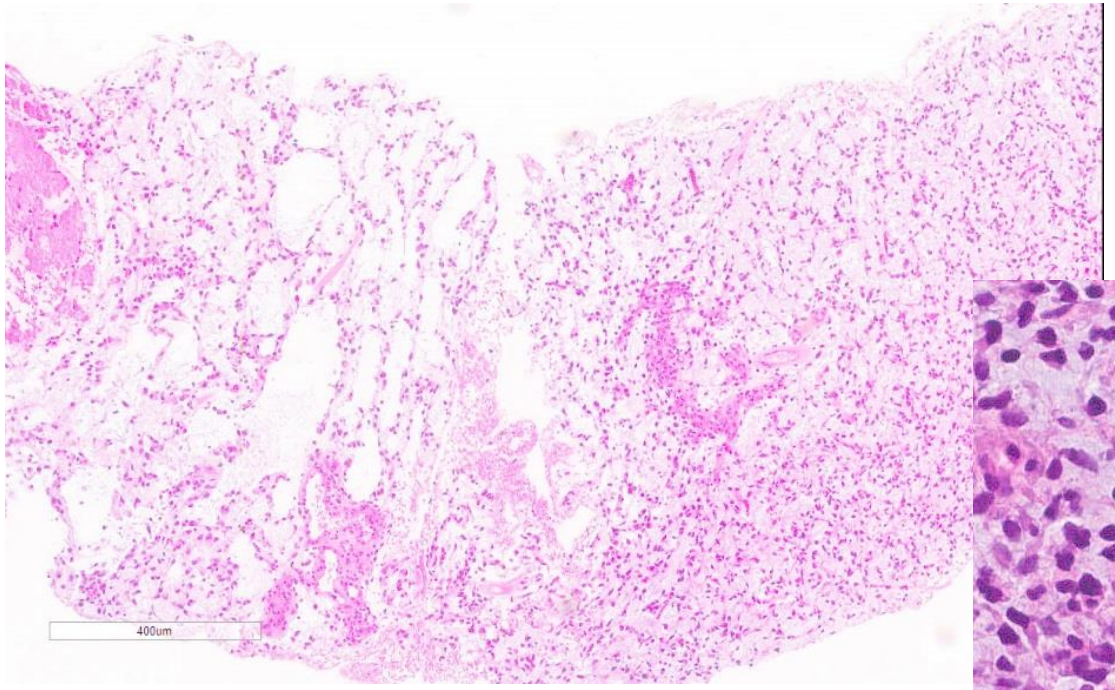
Somatic mutation testing in resolving diagnostic uncertainty in metastatic melanoma

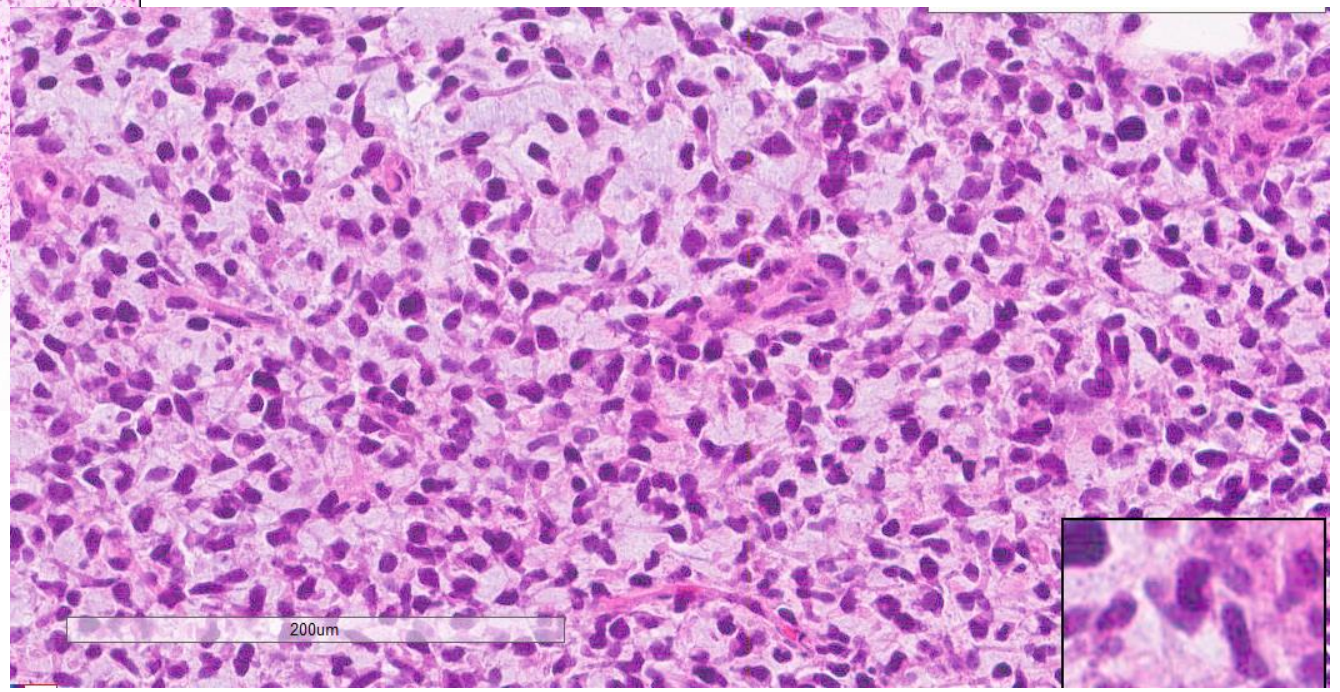


62yo male referred to HCI, large axillary mass

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

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

↓

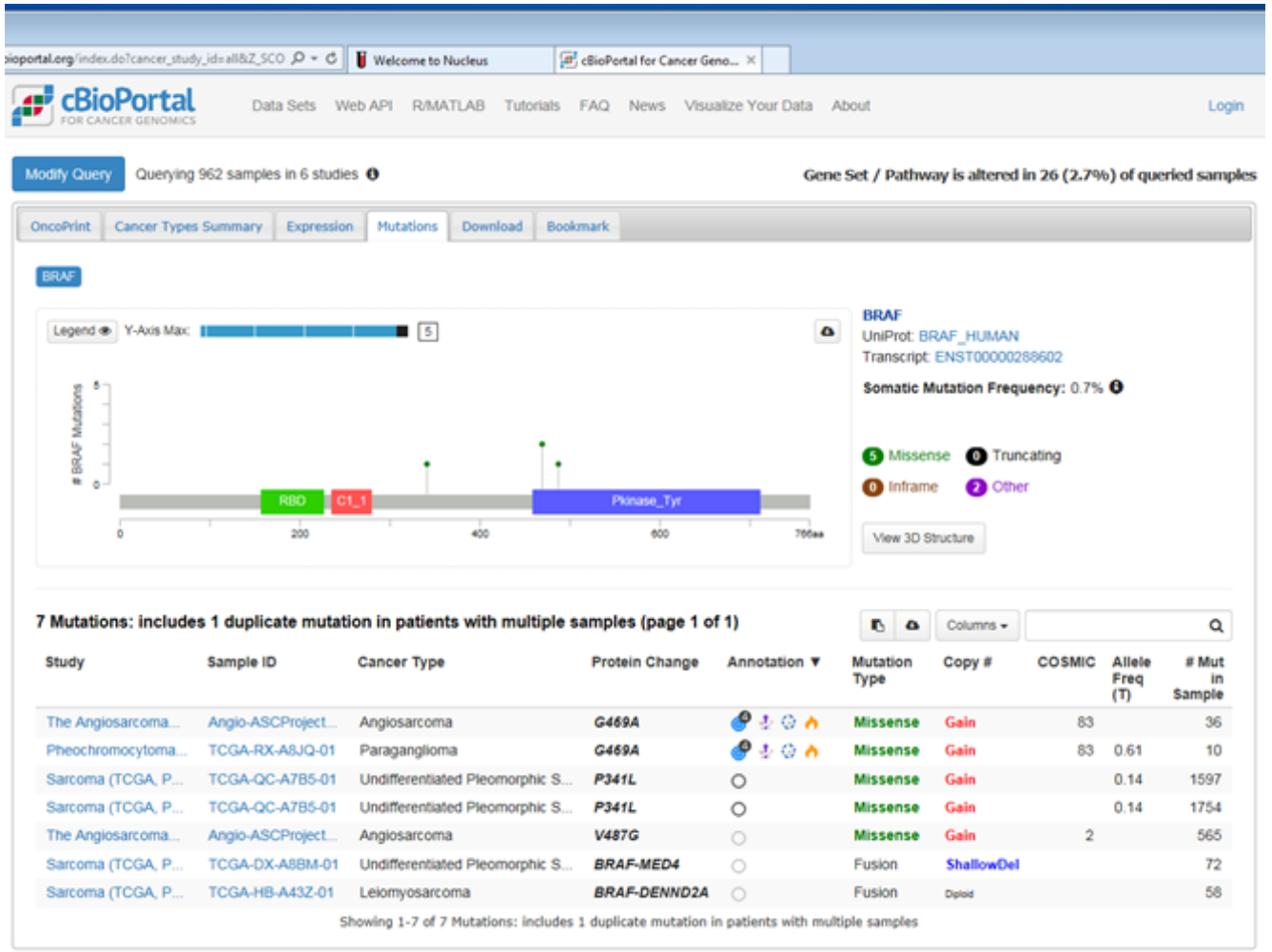
BRAF V600E Detected

↓

Surgical Oncology question

Sarcoma vs. melanoma, which one?

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

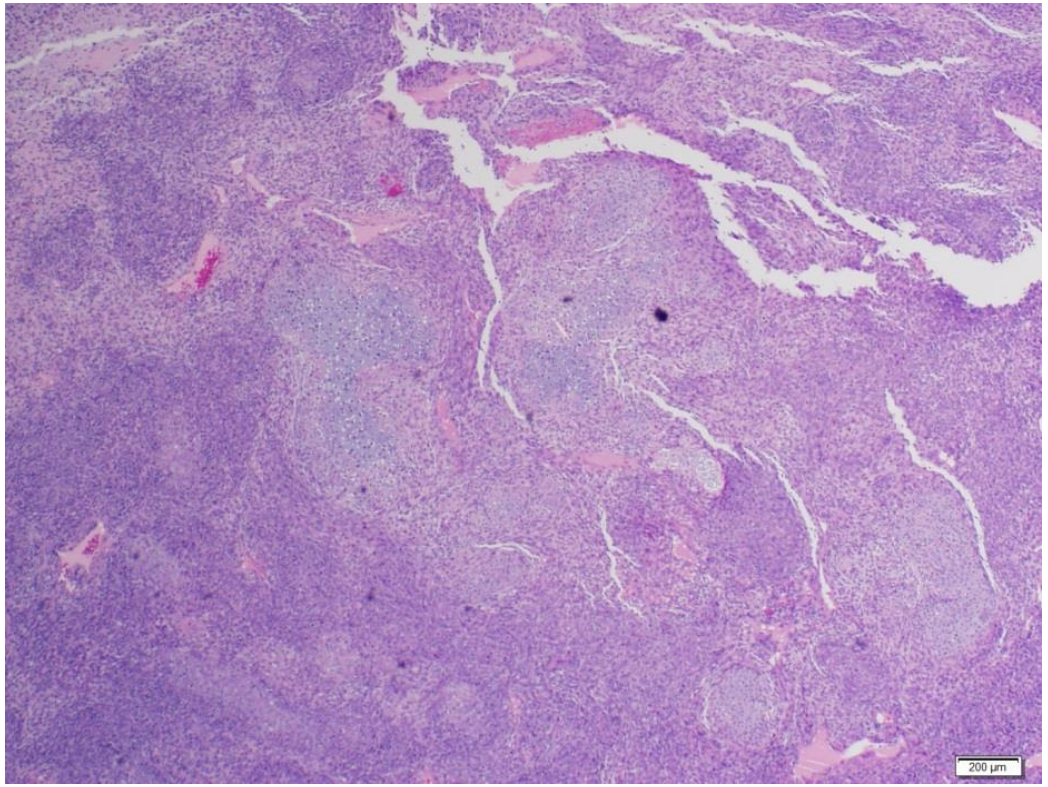
↓

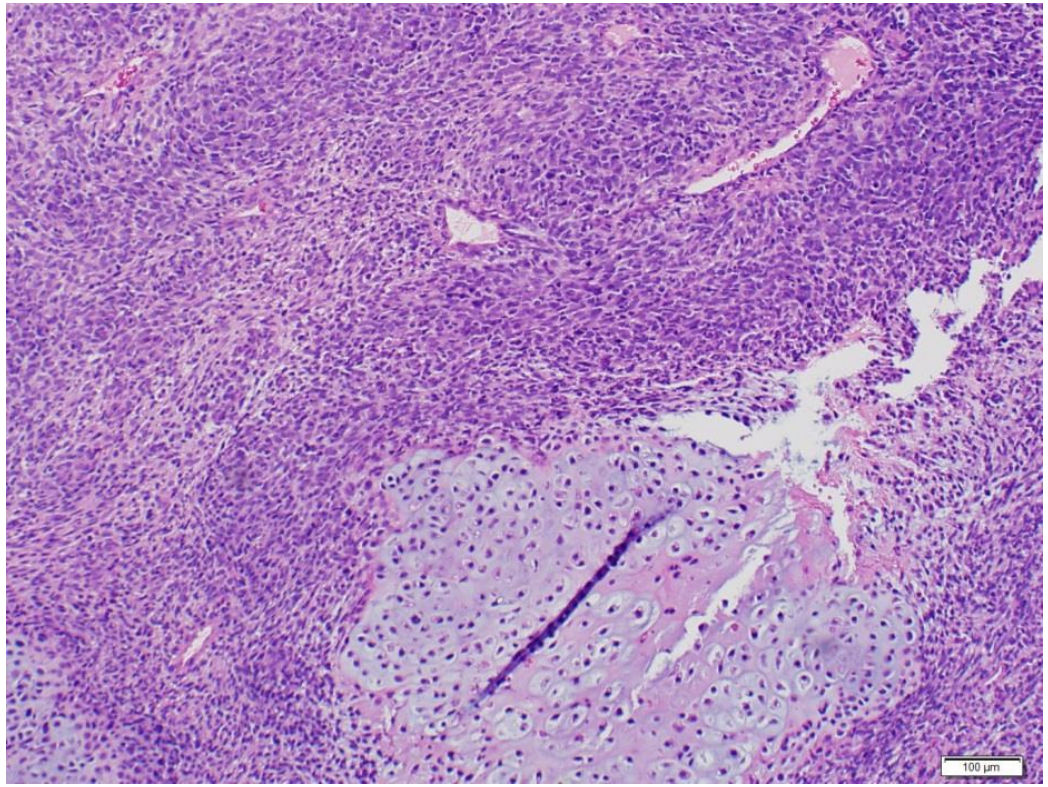
Surgical Oncology question

Sarcoma vs. melanoma, which one?

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

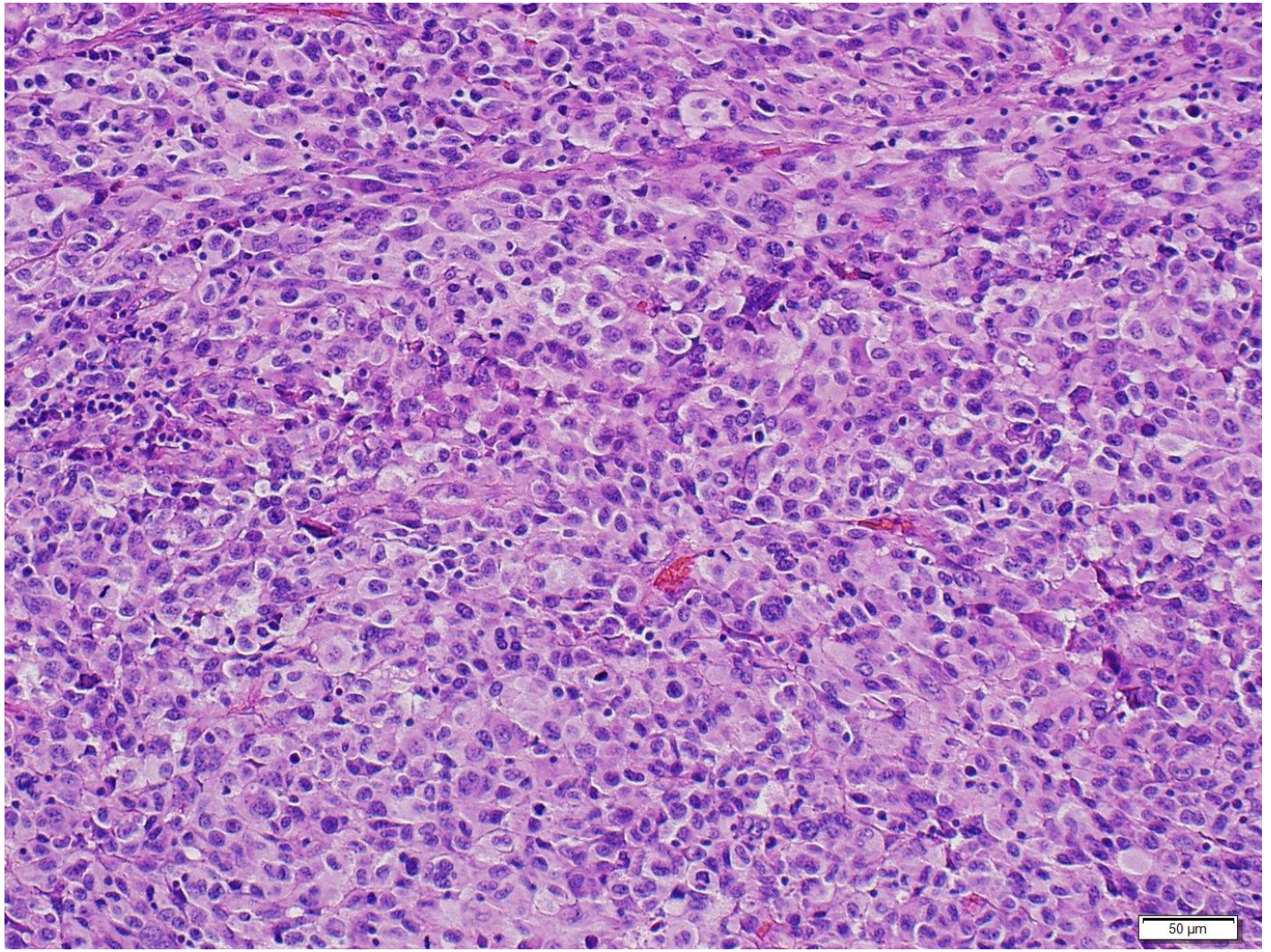
47yo male with axillary mass, and liver masses, transferred care to UU/HCI



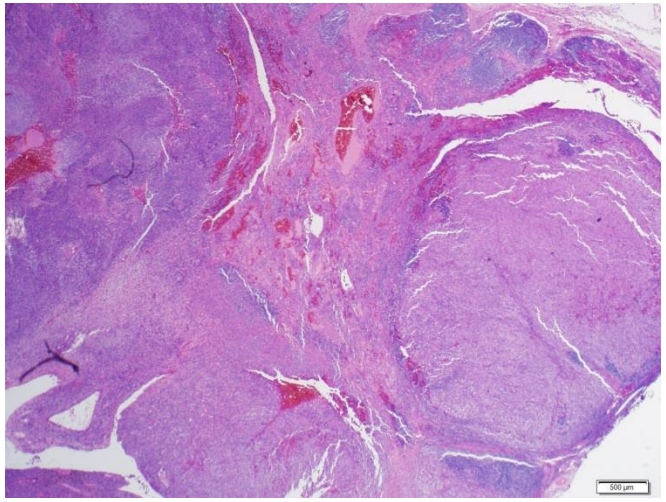


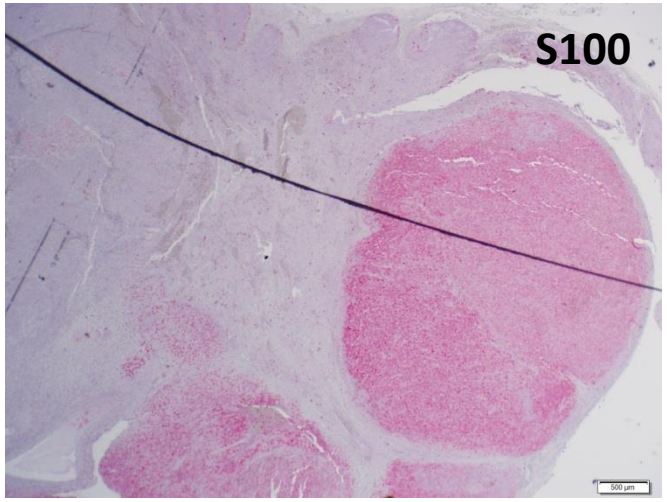
Outside diagnosis (3 different reports) = Mesenchymal Chondrosarcoma
NO molecular confirmation with *HEY1-NCOA2* testing

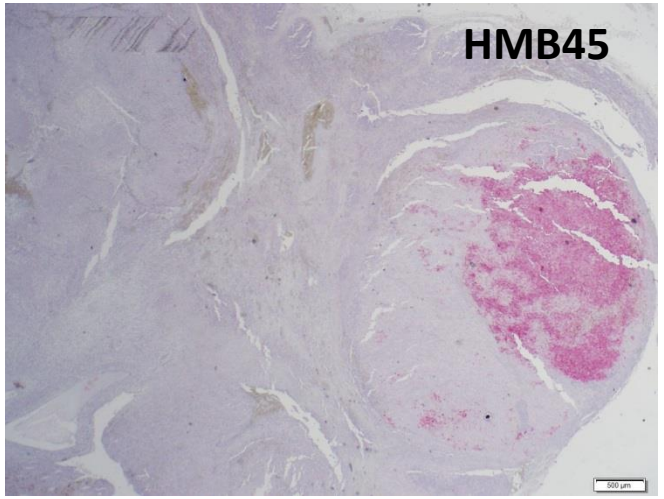
Multiple nodules with distinct epithelioid morphology + pleomorphism

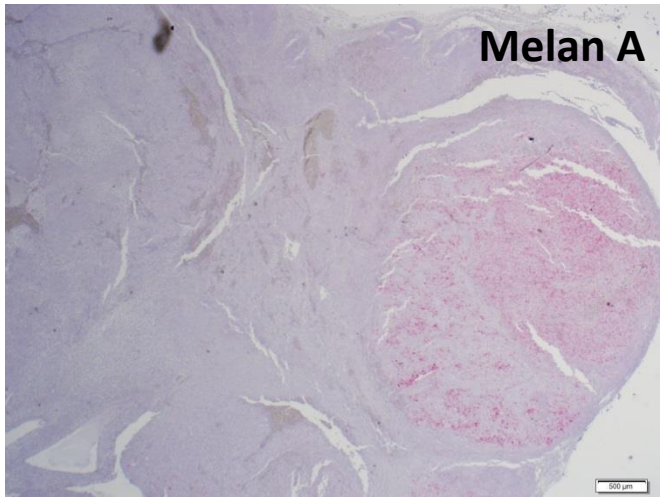


Immunostains → Melanoma









NGS testing confirms diagnosis of metastatic melanoma

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

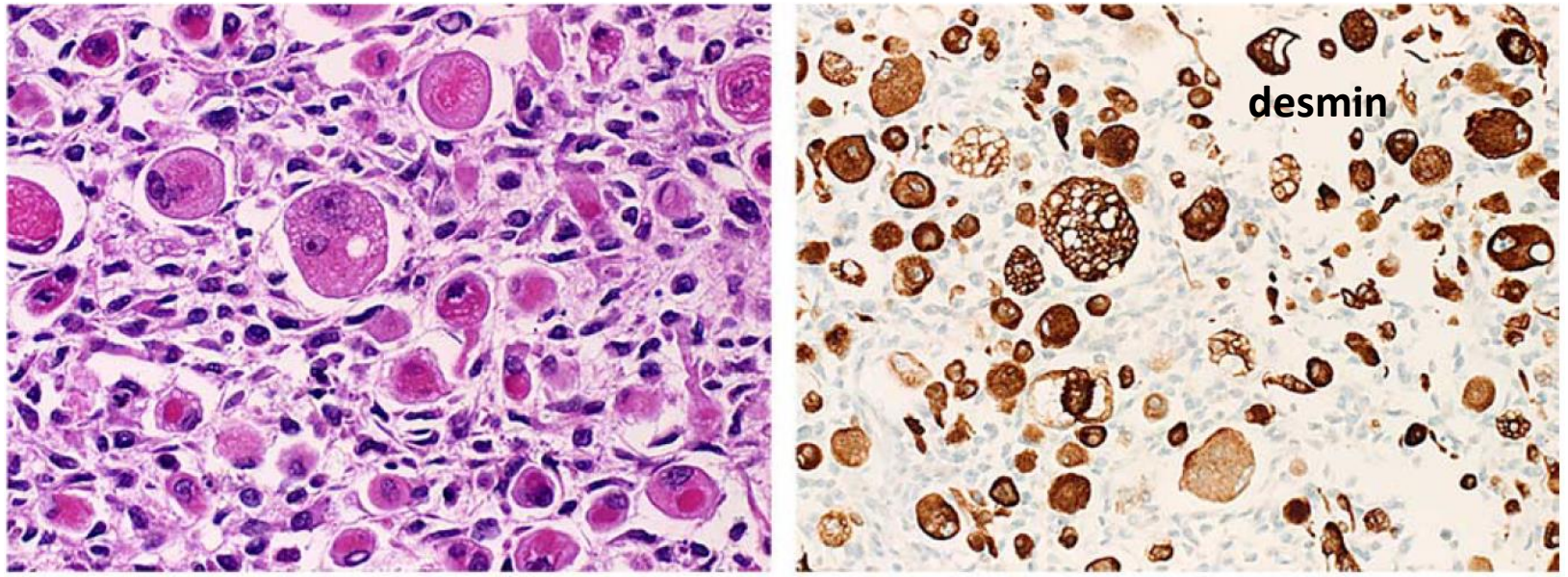
- Interpretation: This *NRAS* (p.Gly12Arg) mutation activates the MAPK pathway (Rajalingam et al., 2007), and it has been reported in melanoma patients (COSMIC database, accessed December 8, 2015). Patients with *NRAS*-mutant melanoma may benefit from systemic immunotherapy (Johnson et al., 2015) as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

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



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Dedifferentiated and Undifferentiated Melanomas

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

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

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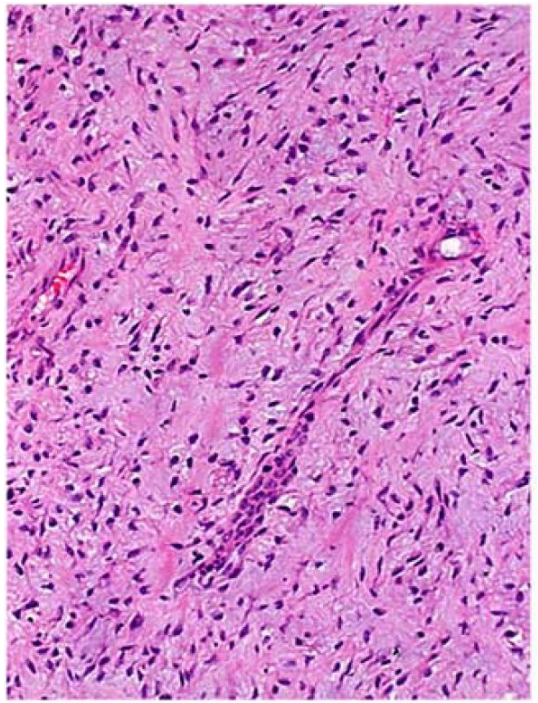

- n=35 unpublished cases, n=50 previously published cases, n=85 total
- negative for S100, SOX10, Melan-A, HMB45, pan-melanoma IHC
- Initial diagnoses (known in 66 cases)
  - undifferentiated/unclassified pleomorphic sarcoma (n=30)
  - unclassified epithelioid malignancy (n=7)
  - Pleomorphic rhabdomyosarcoma (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

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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.

Authors proposed criteria for the diagnosis undifferentiated metastatic melanoma

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

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 differentiated 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 vimentin-only immunophenotype or phenotypes/patterns of limited specificity, eg, pleomorphic rhabdomyoblastic, smooth muscle–like, myofibroblast-like, osteocartilaginous, primitive small cell or multiple patterns
Lack of histologic patterns that are known to be associated with specific or defining genetic alterations/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
Exclude undifferentiated neoplasms with 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.[44] 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 metastatic melanoma of unknown primary.

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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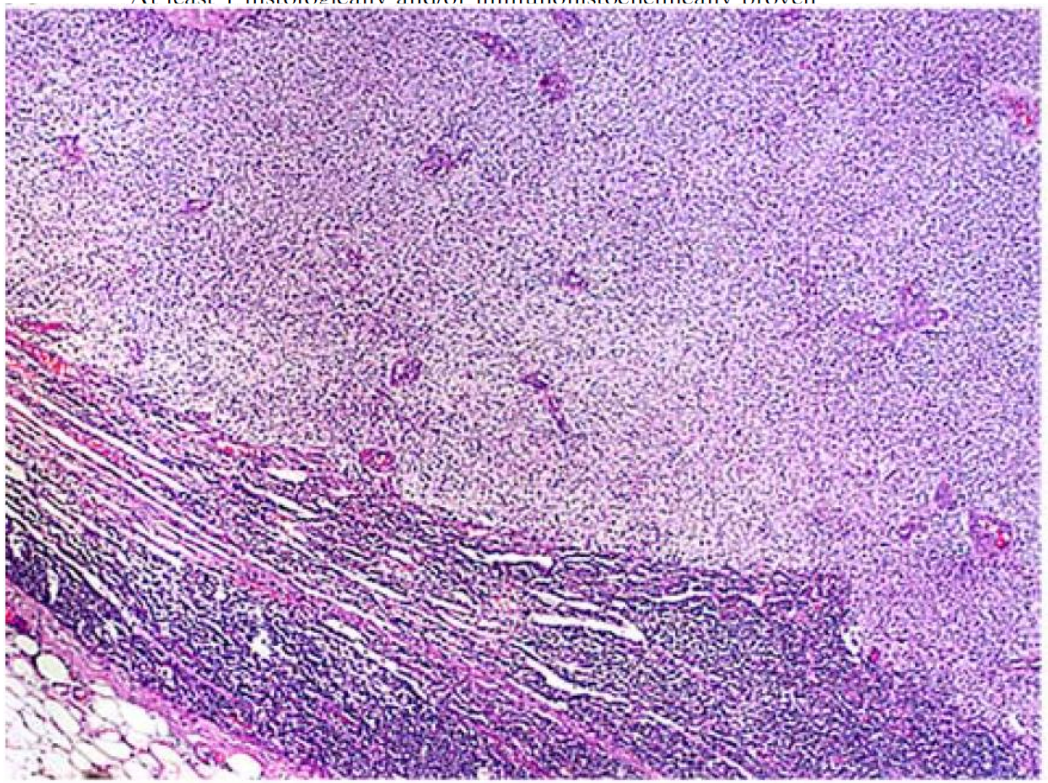

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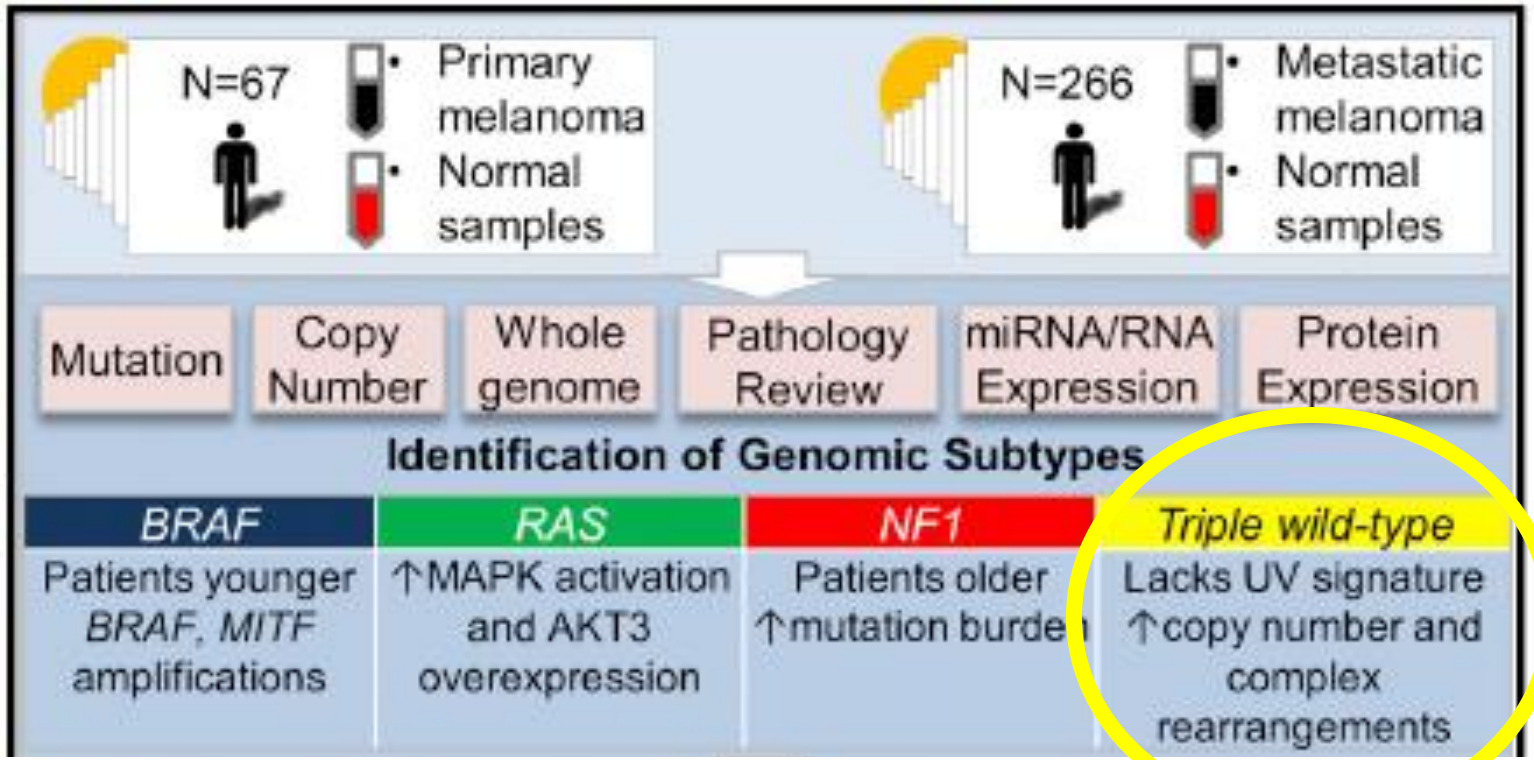
MUP indicates metastatic melanoma of unknown primary.

Resolving diagnostic uncertainty in melanoma

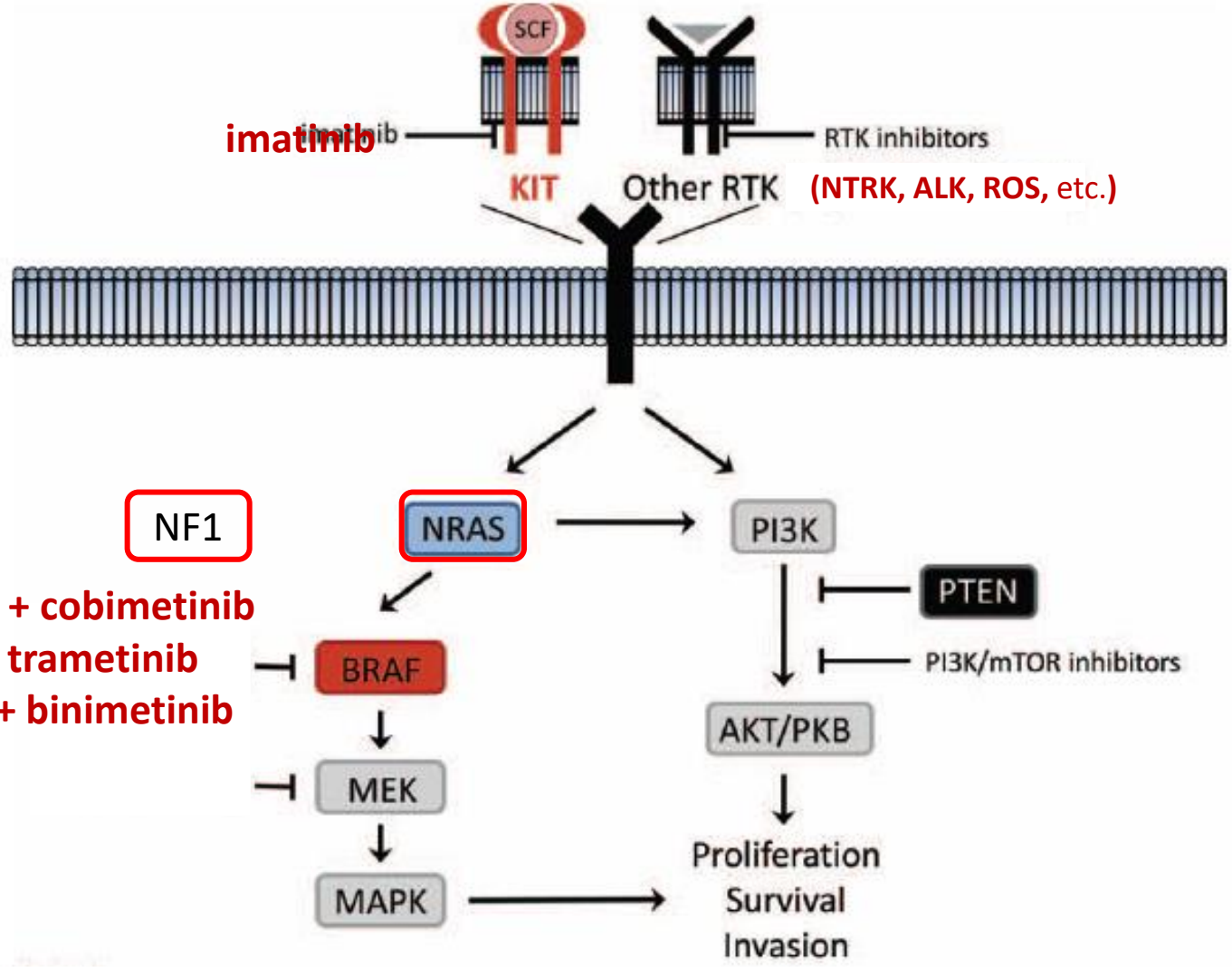
- Detection of ***BRAF*** and ***NRAS*** mutations (≥70% cutaneous melanoma) can help distinguish undifferentiated melanomas, or melanomas mimicking mesenchymal neoplasms, from soft tissue, bone or visceral sarcomas. Exceptionally rare in sarcoma.
  - More challenging to distinguish undifferentiated 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

wild type result does Not exclude melanoma



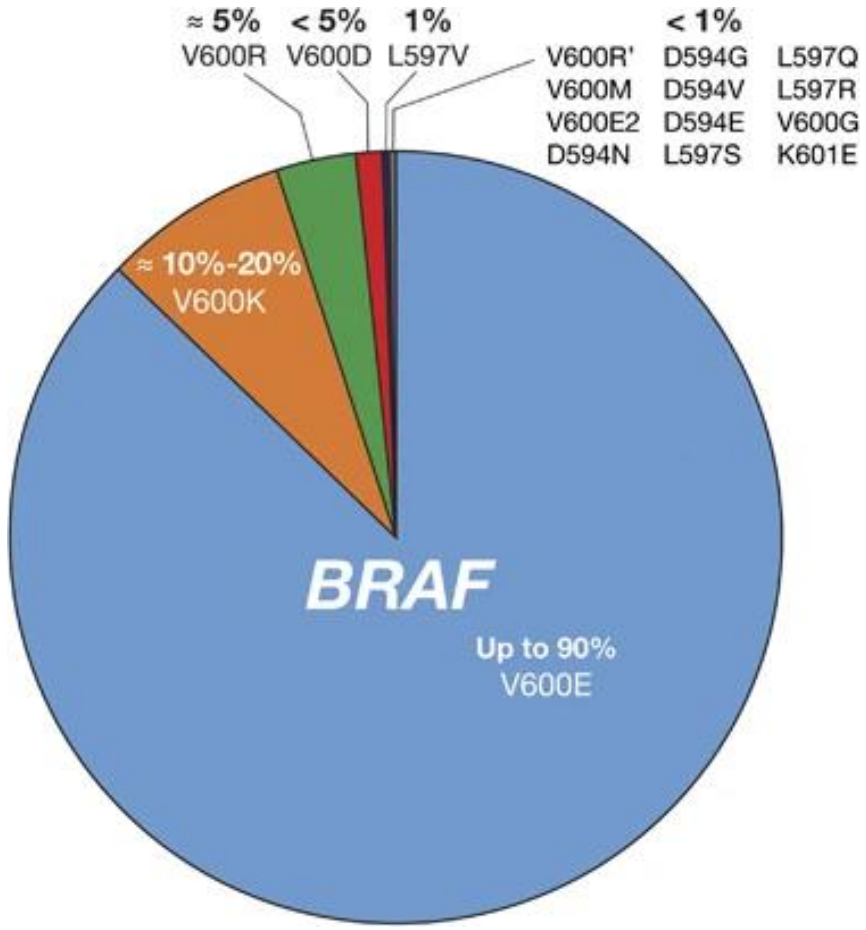
Cell *161*, 1681–1696, June 18, 2015

Testing for Actionable Mutations



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

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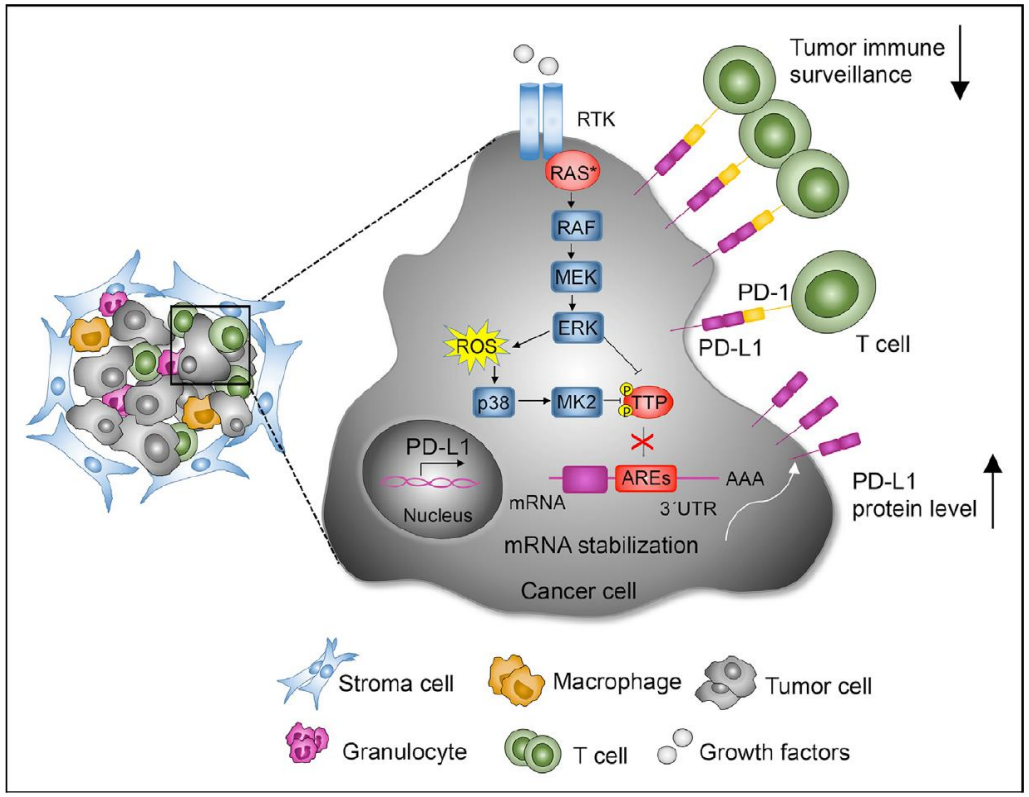
    - ***Contraindicated in BRAF wild type melanoma!!!***
- ***NRAS***$^{Q61R/K/L}$ (15-30%)
  - Major unmet clinical need, ongoing trials
  - Correlates with poor survival
  - Minority respond to targeted MEK inhibition
  - Immune Checkpoint Blockade = First line therapy



Immunity *47*, December 19, 2017

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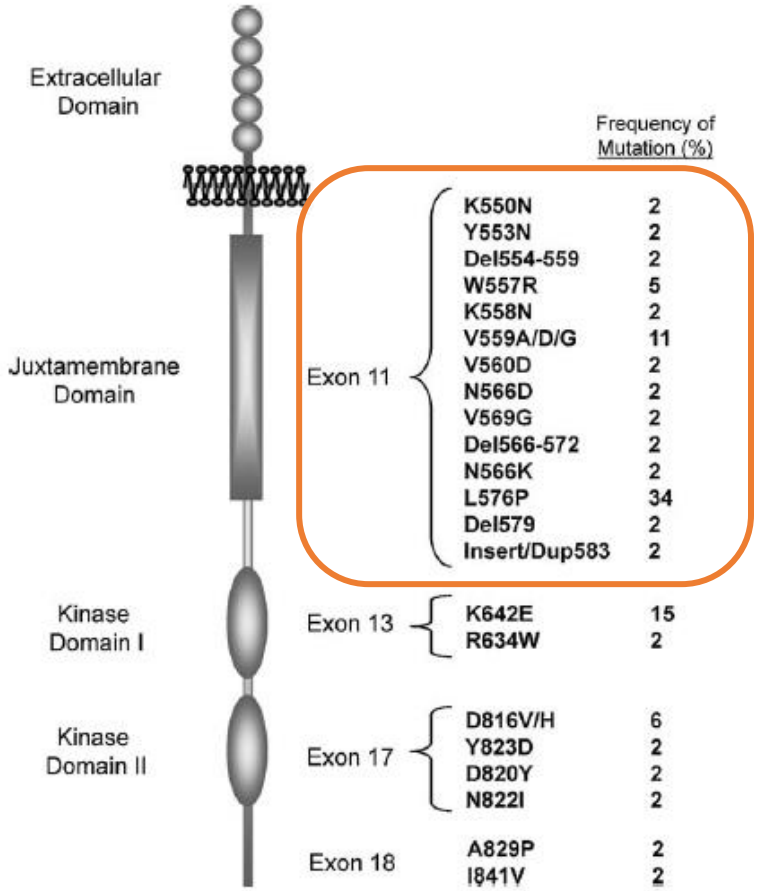
- ***KIT* exon 11** (10-15% of acral, mucosal melanoma)
  - Also enriched in melanoma with chronic sun damage
  - Targeted therapy responses are limited and not durable
- ***NTRK, ALK, ROS*** fusions (<1%)



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

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

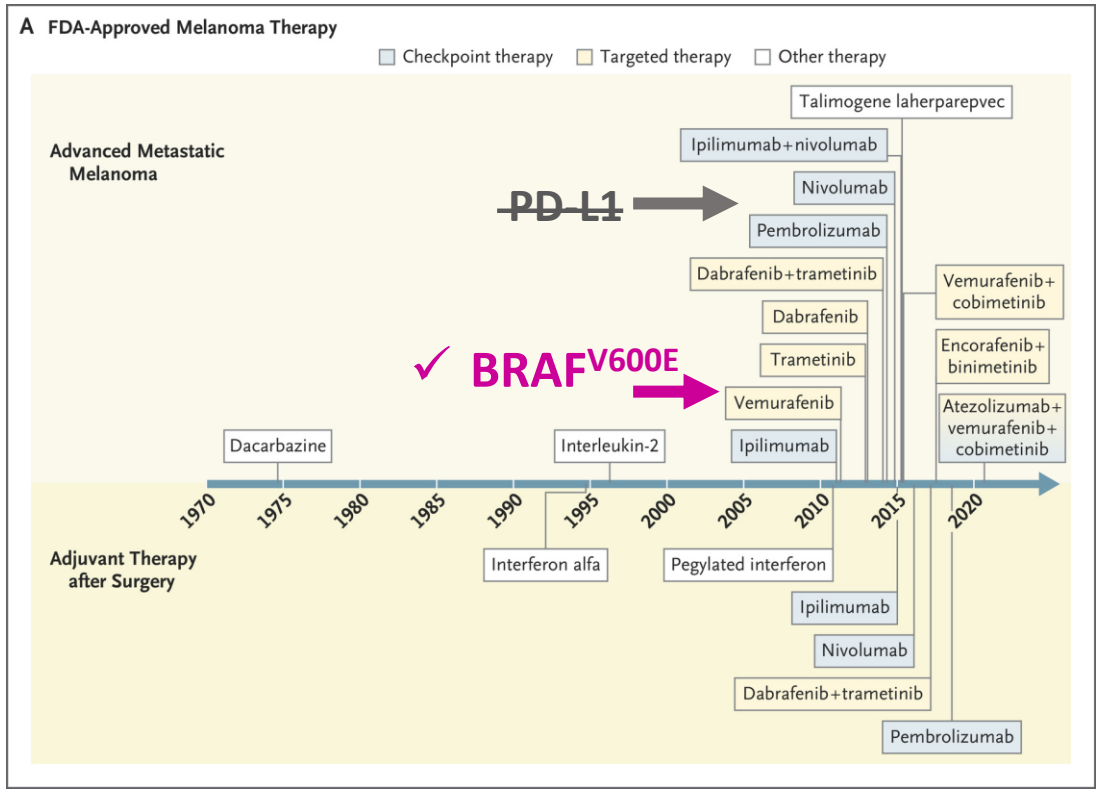
- Stage III
  - Eligibility for RAF + MEK inhibitors as adjuvant therapy (*BRAF*^{V600}-mutant)
  - Ongoing trials for neoadjuvant RAF + MEK inhibition (*BRAF*^{V600}-mutant)

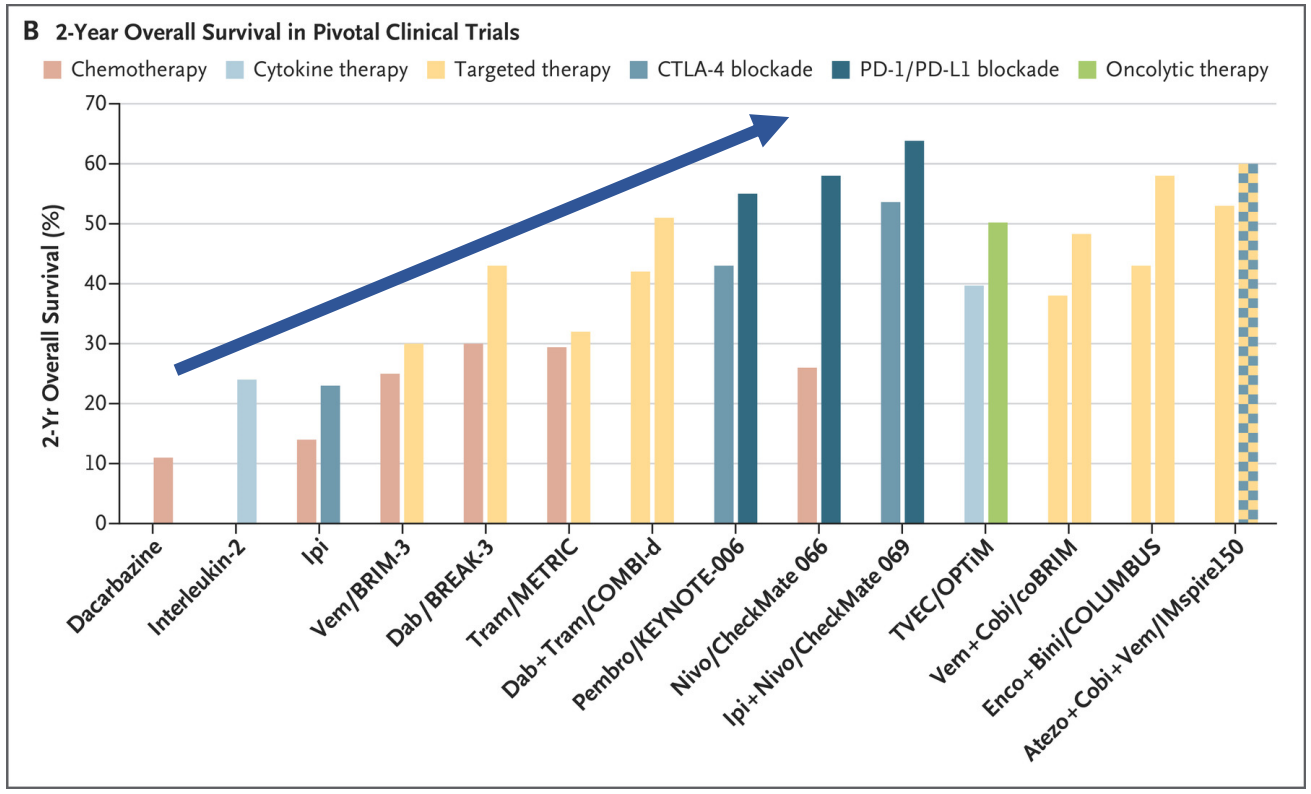
- Stage IV – newly diagnosed and relapsed, eligibility for targeted tx

  (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.

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

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

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

ASCO® Plenary Series

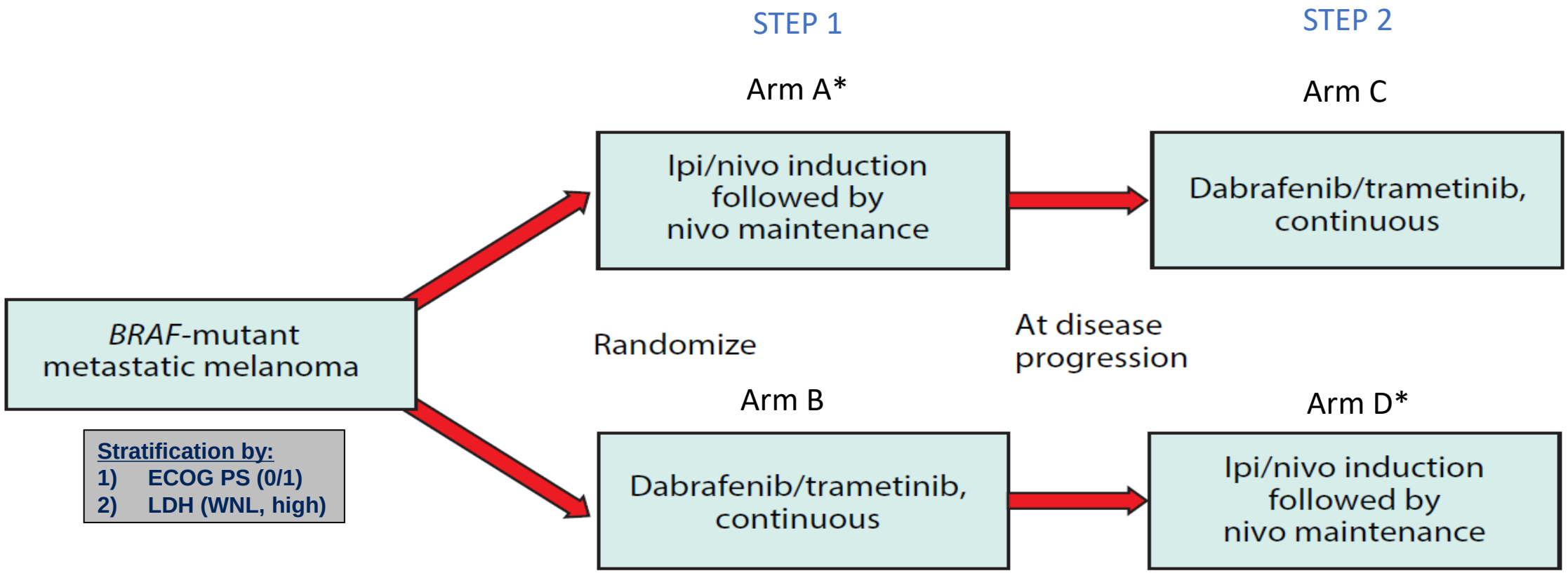
DREAMseq (Doublet, Randomized Evaluation in Advanced Melanoma Sequencing) a Phase III Trial: ECOG-ACRIN EA6134

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

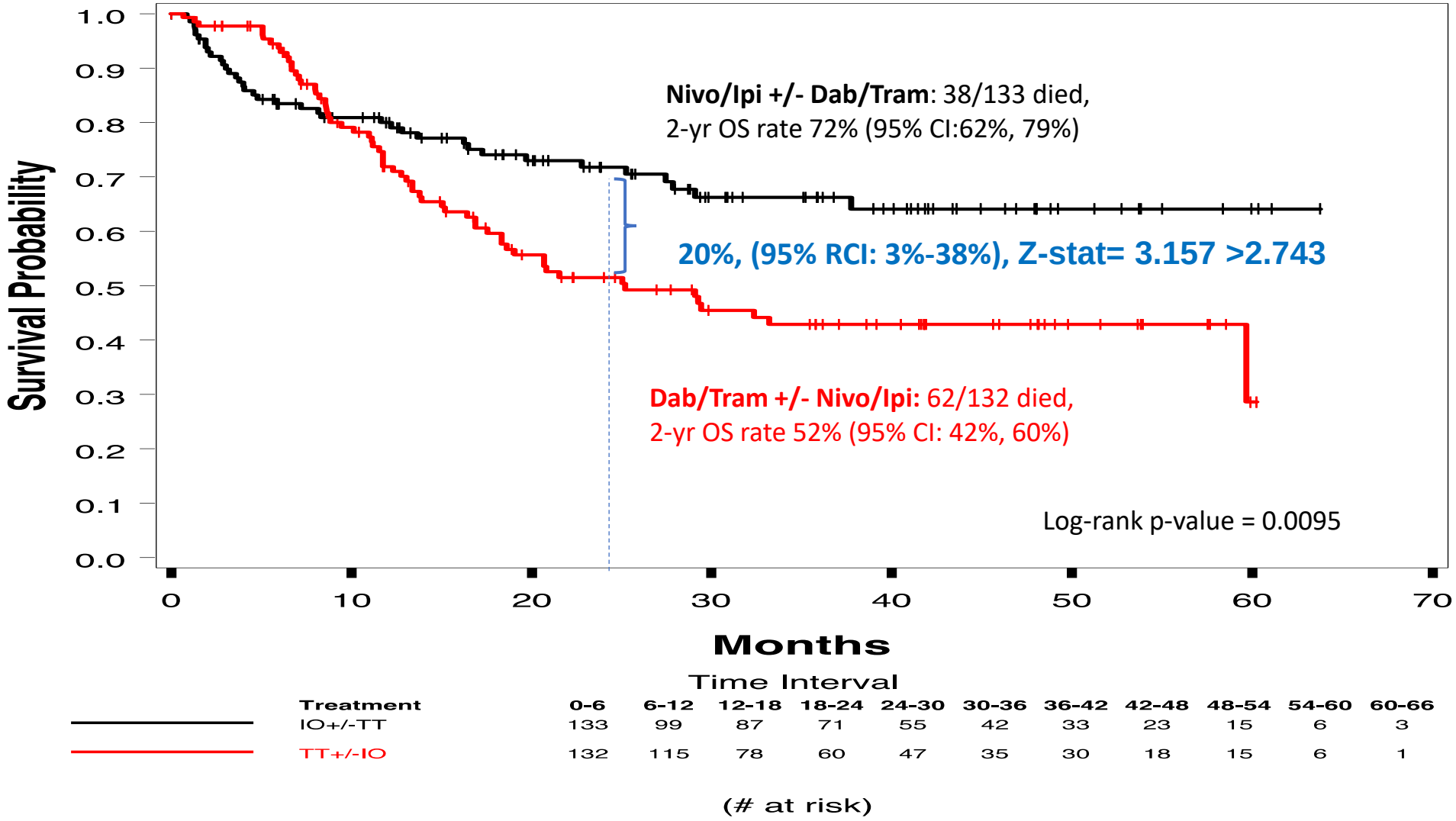
DREAMseq Trial Treatment Schema



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

Slide courtesy Michael B. Atkins, MD

Improved Overall Survival (OS) leading with Nivo/Ipi



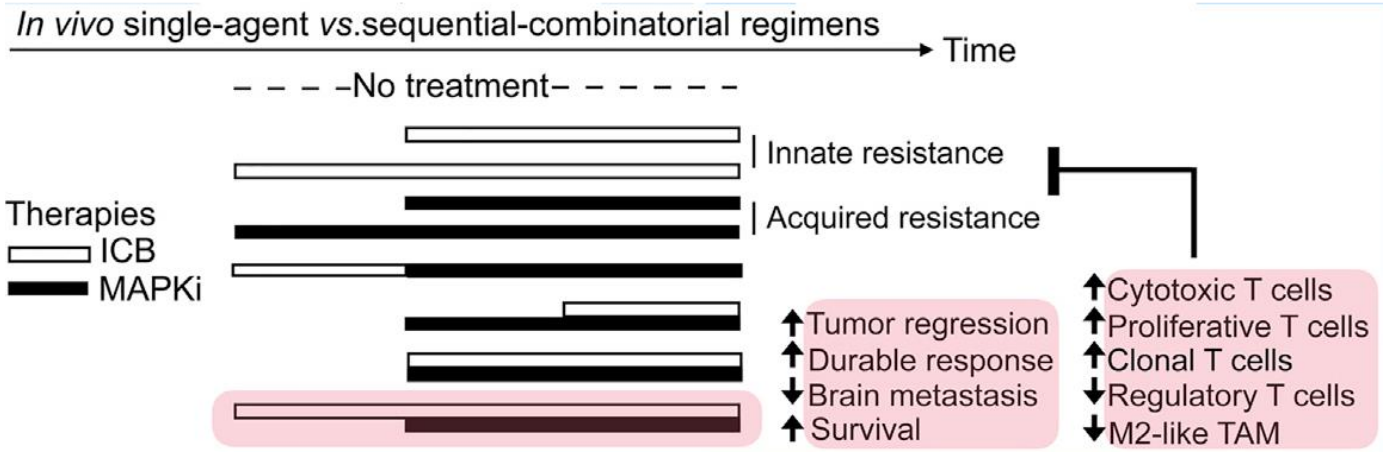
Slide courtesy, Michael B. Atkins, MD,

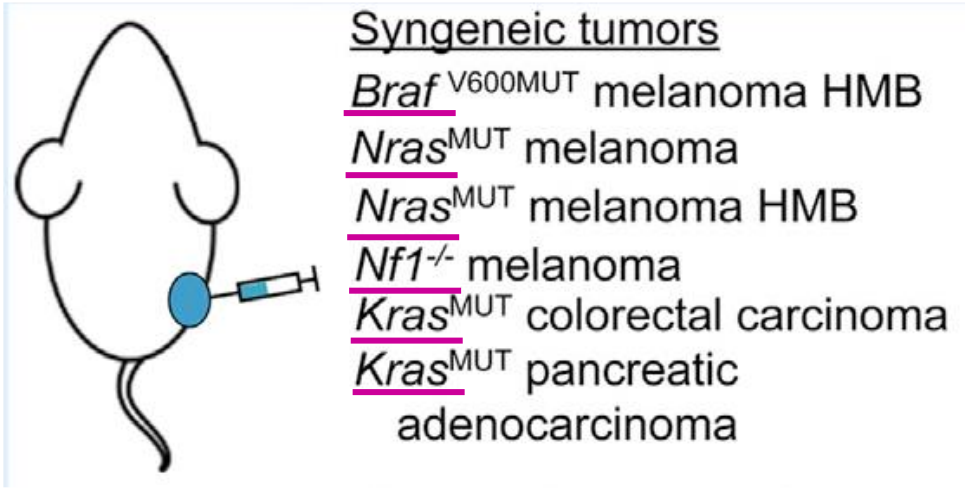
Recent preclinical studies suggest a promising new combo treatment paradigm for multiple cancer types

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

Yujue Wang,[1,16] Sixue Liu,[1,16] Zhentao Yang,[1,16] Alain P. Algazi,[2,16] Shirley H. Lomeli,[1] Yan Wang,[1] Megan Othus,[3] Aayoung Hong,[1] Xiaoyan Wang,[4] Chris E. Randolph,[5] Alexis M. Jones,[6] Marcus W. Bosenberg,[7] Stephanie D. Byrum,[8] Alan J. Tackett,[8] Henry Lopez,[9] Clayton Yates,[10] David B. Solit,[6] Antoni Ribas,[11,12,13,14] Marco Piva,[1,15,17,*] Gatien Moriceau,[1,17,*] and Roger S. Lo[1,13,14,17,18,*]

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





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?

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

Bryan J. Schneider, MD[1]; Jarushka Naidoo, MD[2,3]; Bianca D. Santomasso, MD, PhD[4]; Christina Lacchetti, MHSc[5]; Sherry Adkins, MS[6]; Milan Anadkat, MD[7]; Michael B. Atkins, MD[8]; Kelly J. Brassil, PhD[6]; Jeffrey M. Caterino, MD, MPH[9]; Ian Chau, MD[10]; Marianne J. Davies, DNP[11]; Marc S. Ernstoff, MD[12]; Leslie Fecher, MD[1]; Monalisa Ghosh, MD[13]; Ishmael Jaiyesimi, DO, MS[14]; Jennifer S. Mammen, MD, PhD[15]; Aung Naing, MD[6]; Loretta J. Nastoupil, MD[6]; Tanyanika Phillips, MD[16]; Laura D. Porter, MD[17]; Cristina A. Reichner, MD[18]; Carole Seigel, MBA[19]; Jung-Min Song, MSN, RN, CNS[20]; Alexander Spira, MD, PhD[21]; Maria Suarez-Almazor, MD[6]; Umang Swami, MD[22]; John A. Thompson, MD[23]; Praveen Vikas, MD[24]; Yinghong Wang, MD[6]; Jeffrey S. Weber, MD, PhD[25]; Pauline Funchain, MD[20]; and Kathryn Bollin, MD[26]

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, episcleritis
- 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

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)

Retrospective study

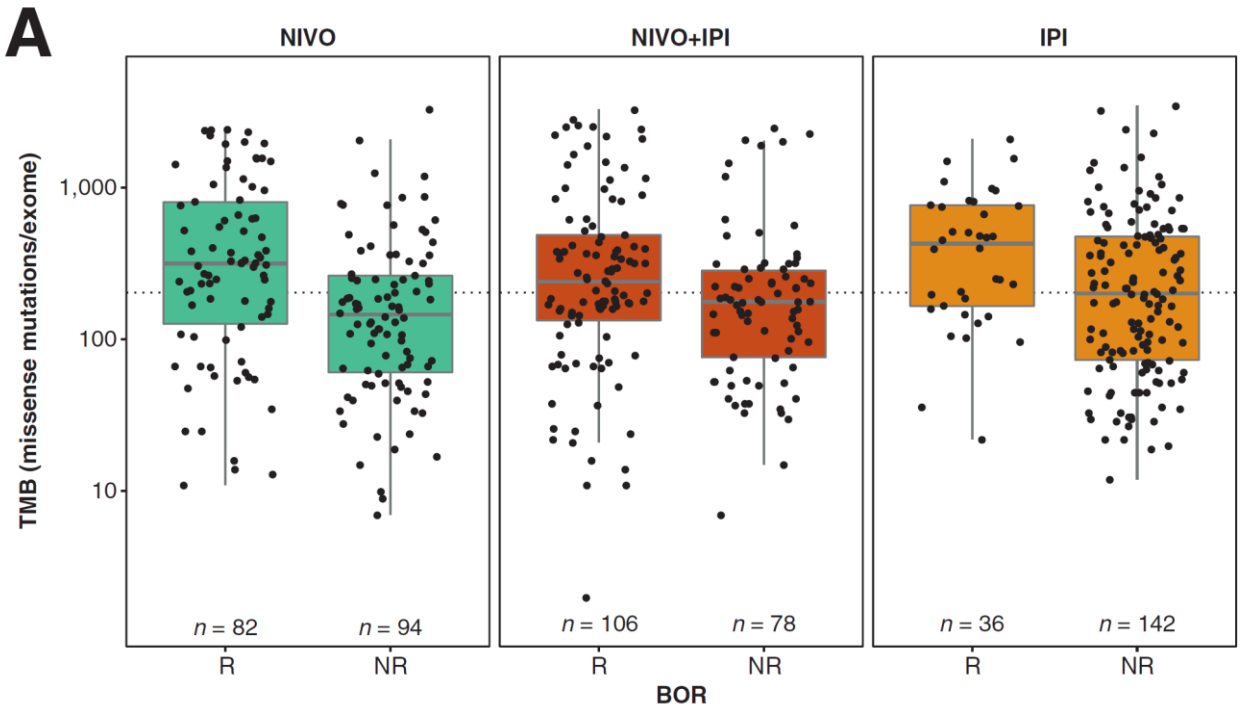
CheckMate 066 (NCT01721772)

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[10], Tina C. Young[11], 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]

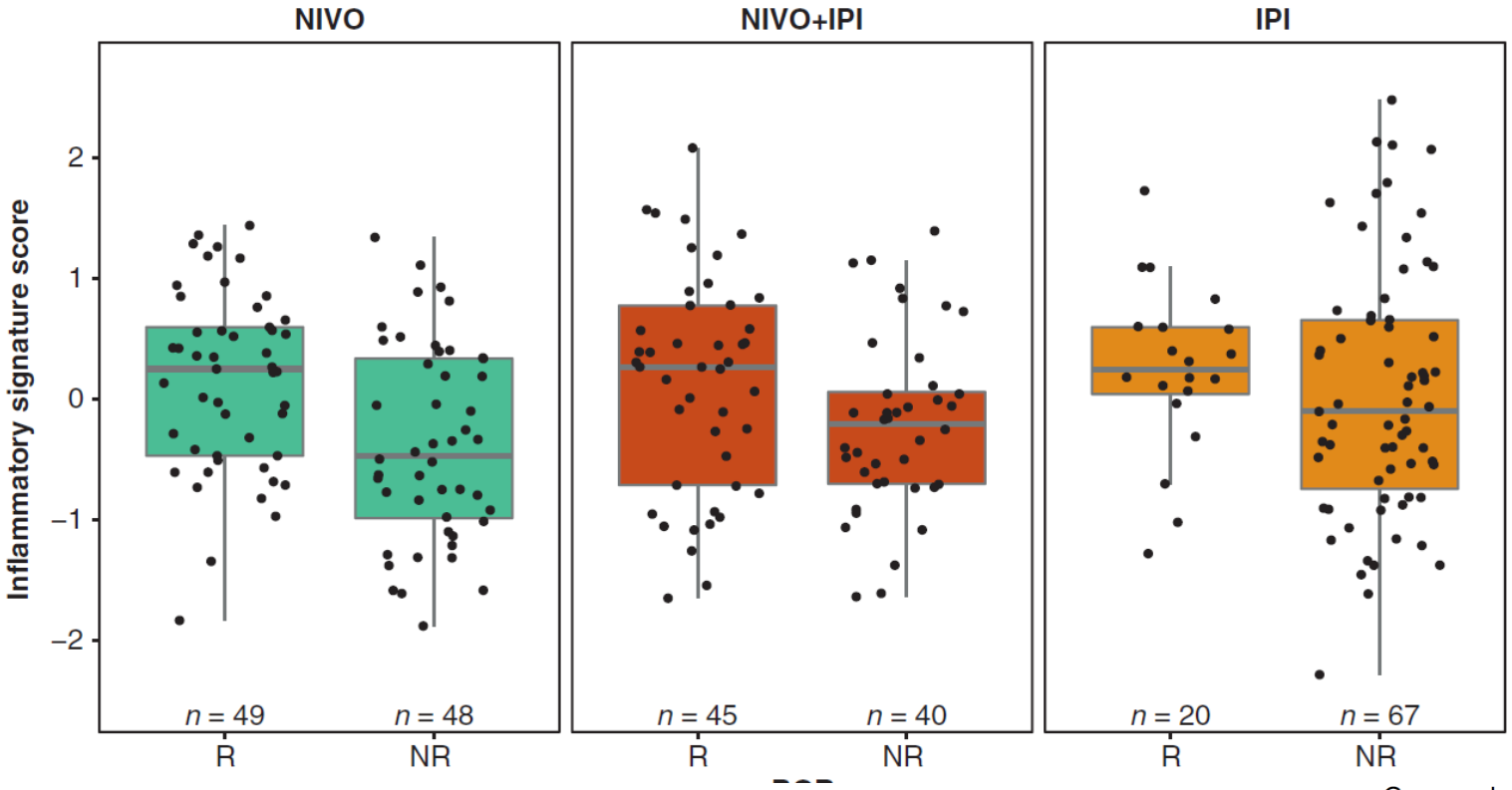


High variance TMB among both Responders vs. Non-responders

Cancer Immunol Res; 9(10) October 2021

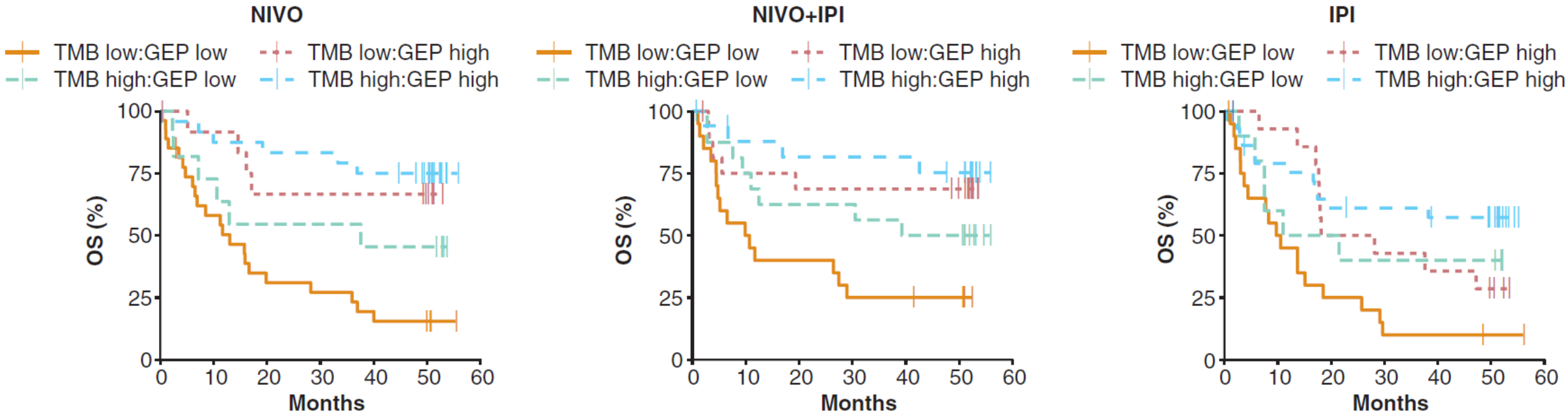
High Variance in Tumor Inflammation Score (TIS) among both responders and nonresponders

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



Cancer Immunol Res; 9(10) October 2021

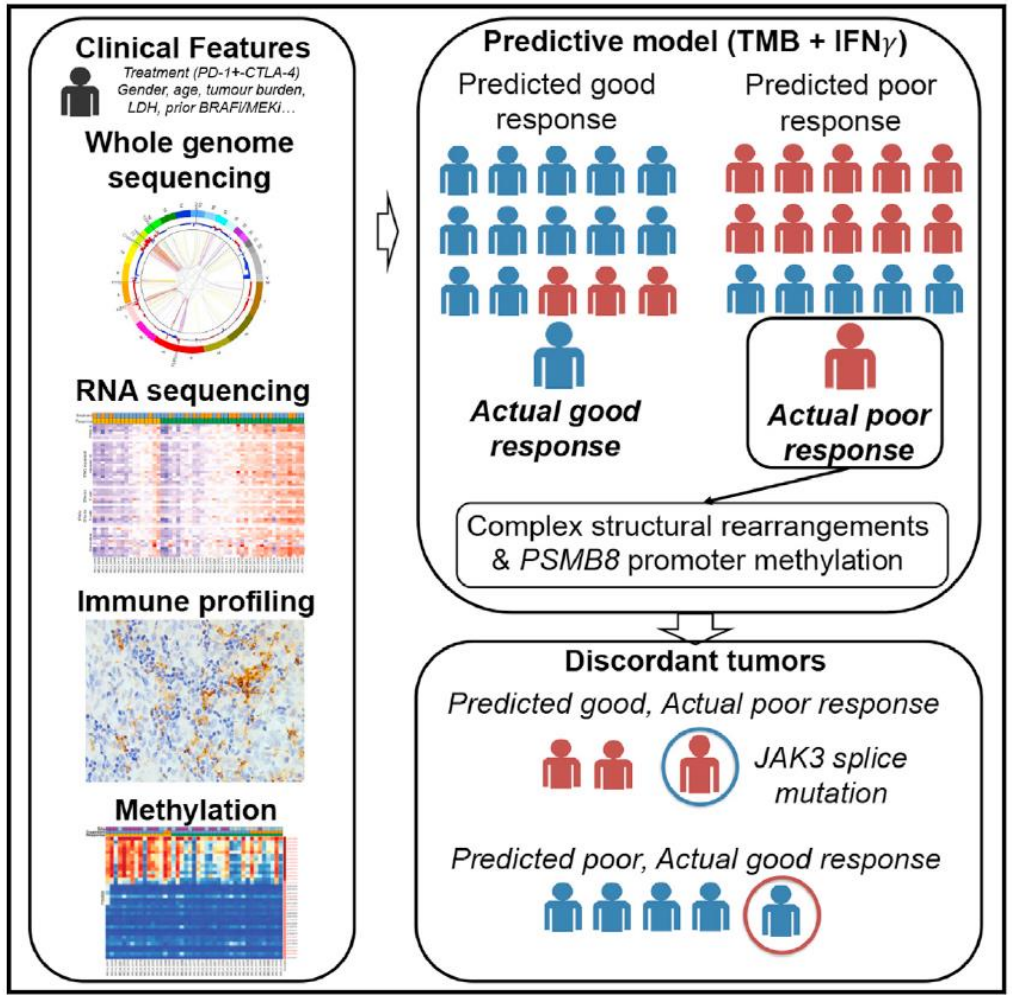
Overall Survival is Stratified by TMB and TIS



Cancer Immunol Res 2021;9:1202–13

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,[2,3,4,5,17] Peter A. Johansson,[1,17] Alexander M. Menzies,[2,4,6,7,17] James S. Wilmott,[2,3,4,17] Venkateswar Addala,[1,8] Matteo S. Carlino,[2,4,9,16] Helen Rizos,[10] 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,[2,4,7,14] J. Spillane,[2,4,7] Robyn P.M. Saw,[2,4,7,14] Kerwin F. Shannon,[2,7,14] John V. Pearson,[1] Graham J. Mann,[2,9,15] K. Hayward,[1,17] Richard A. Scolyer,[2,3,4,11,18] Nicola Waddell,[1,8,18] and Georgina V. Long[2,3,4,6,7,18,19,*]



Restrospective study, Stage IV melanoma

IFNγ gene signature developed with melanoma

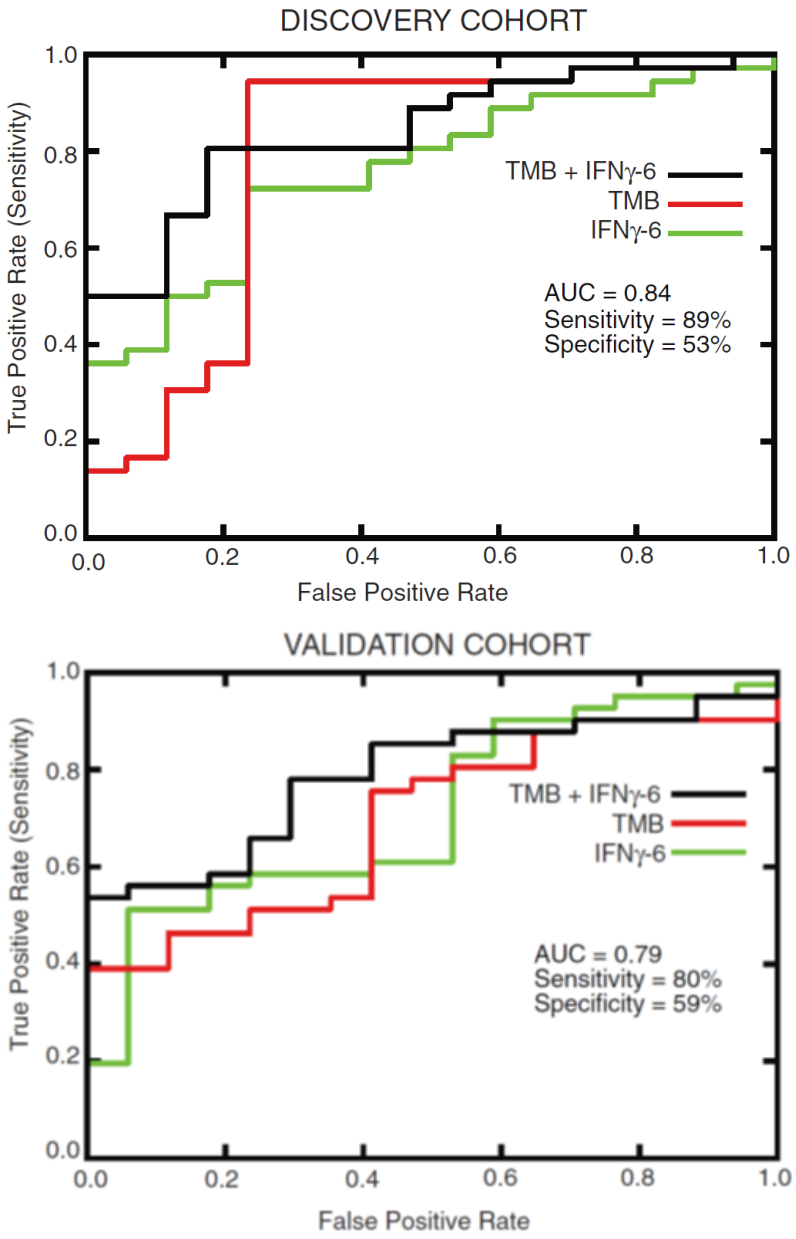
IDO1, CXCL10, CXCL9, HLA-DRA, STAT1, IFNγ

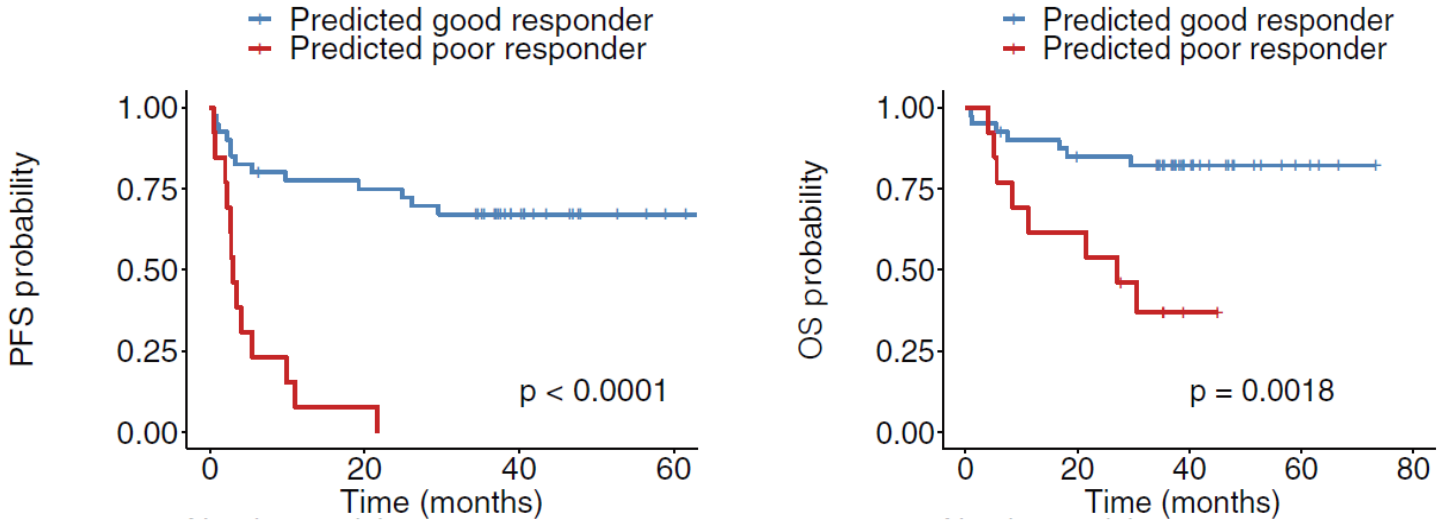
TMB and IFNγ accurately predicted response to ICB (89% sensitivity)

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

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

Performance of combined TMB and IFNγ expression signature





discovery cohort

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

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

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

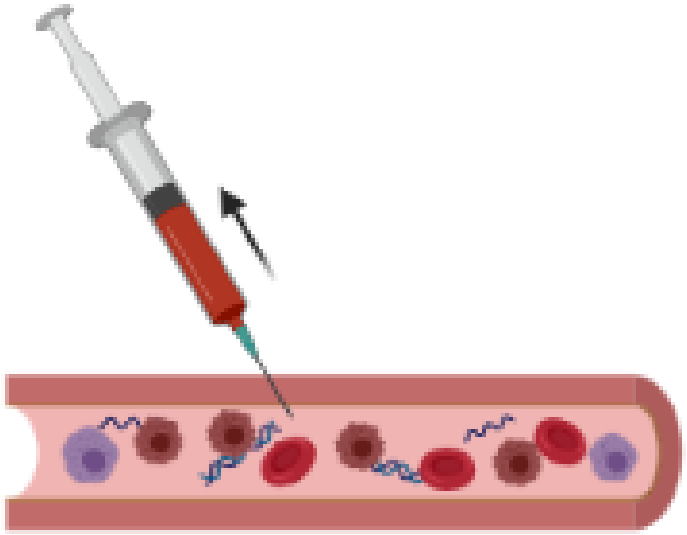
S2101 BiCaZO: A Study Combining Two Immunotherapies (Cabozantinib and Nivolumab) to Treat Patients With Advanced Melanoma or HNSCC, an immunoMATCH Pilot Study

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)



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

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

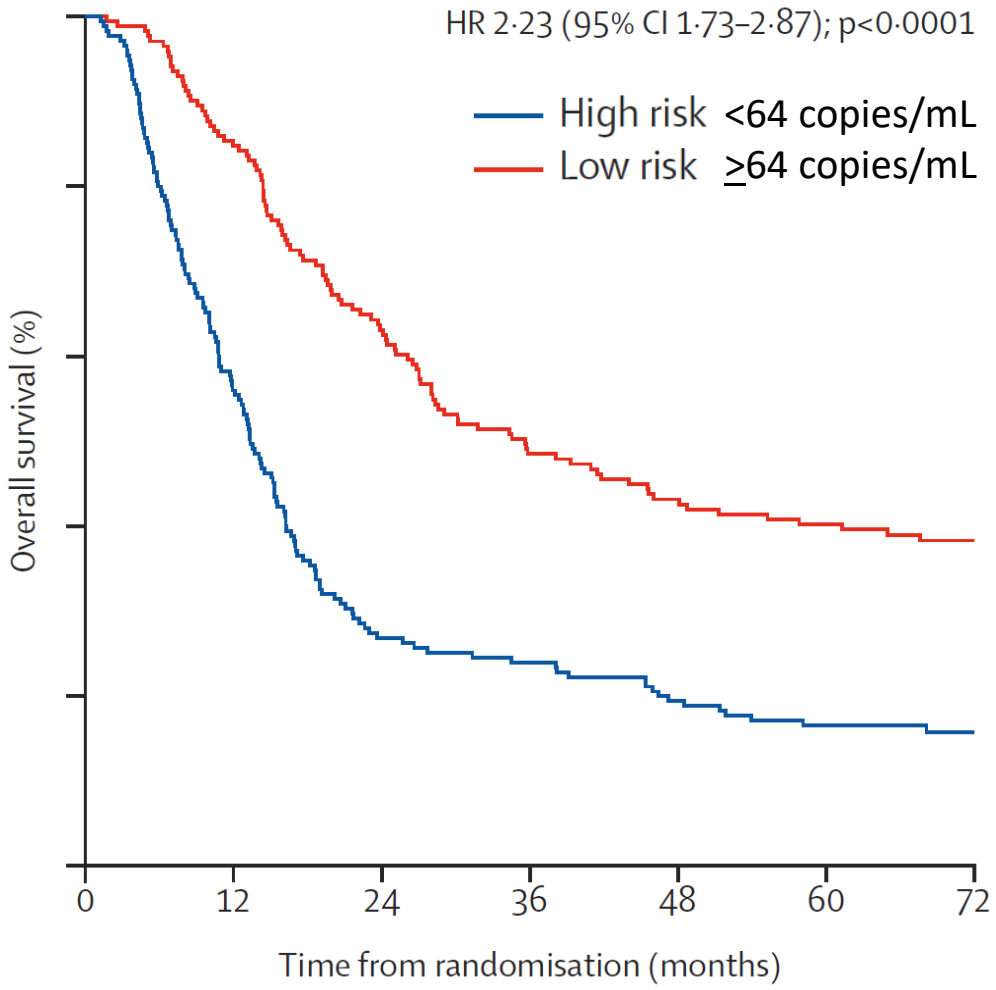
Lancet Oncol 2021; 22: 370–80

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

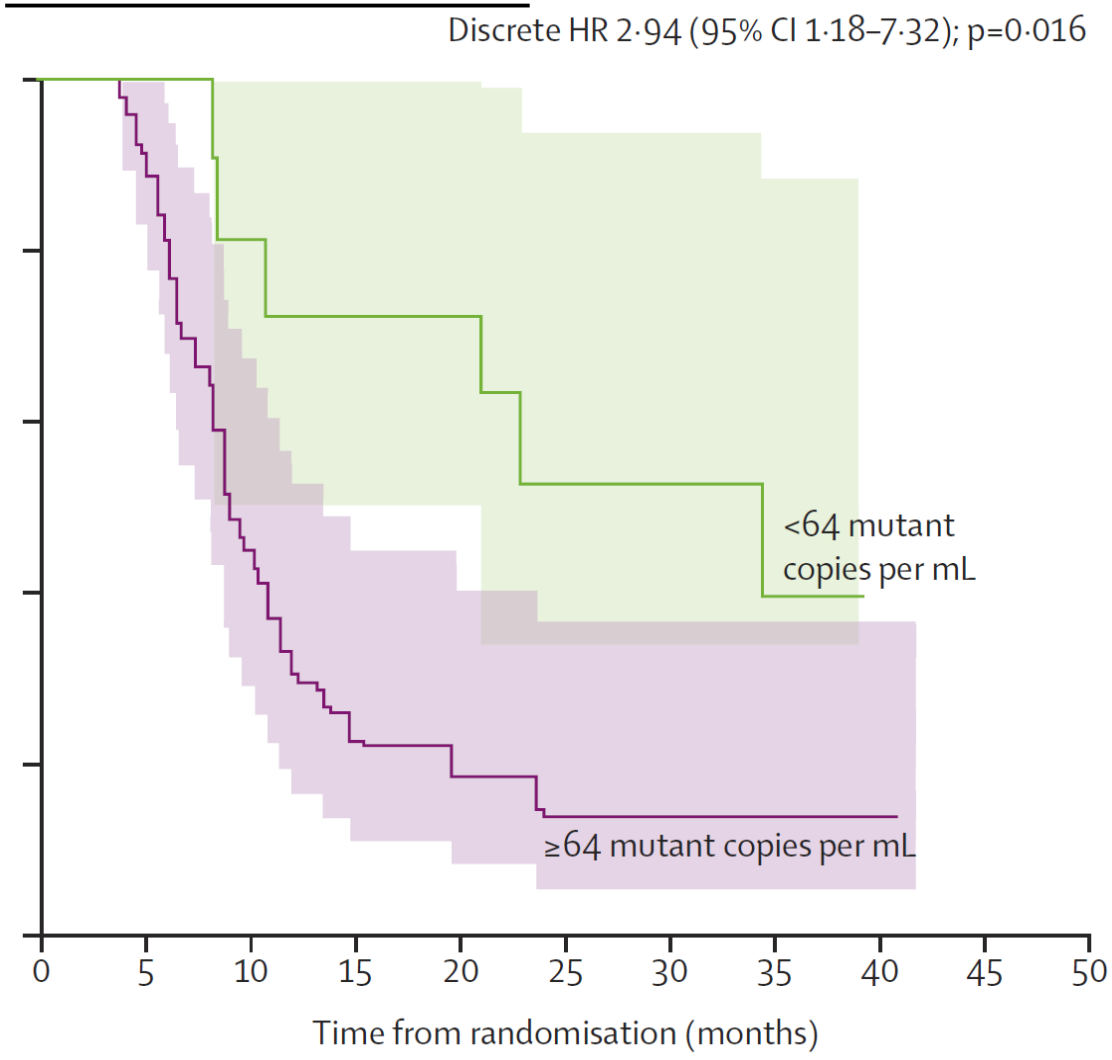
- Retrospective study, unresectable or metastatic *BRAF* -mutant melanoma
  - Advanced stage → expect tumor shedding and detectable ctDNA pre-treatment
- phase 3 COMBI-d and phase 2 COMBI-MB trials
  - dabrafenib ± trametinib
- Measured *BRAF*$^{V600E/K}$ ctDNA by droplet digital PCR , n=345 patients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

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

Pre-treatment



On-treatment 4 weeks



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

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[6], V. Mar[6], D. E. Gyorki[1,7], 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)

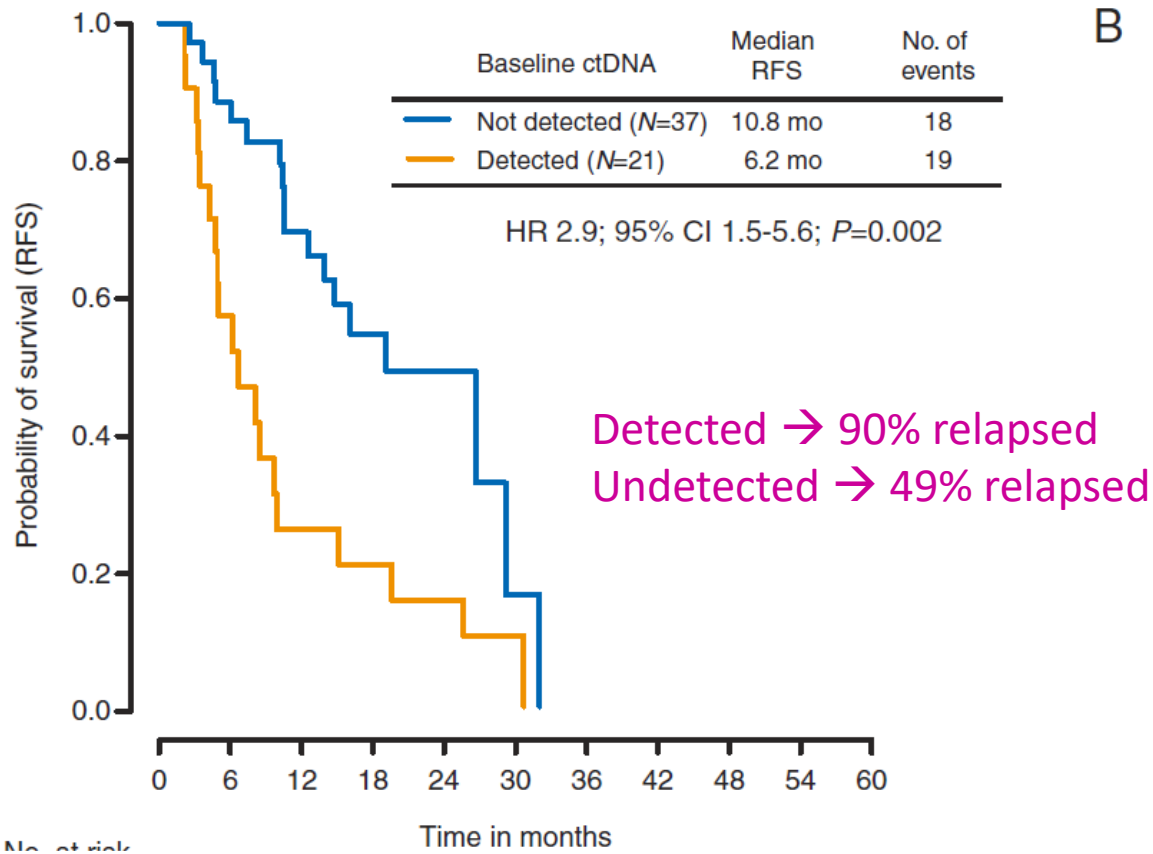
ctDNA detection increases with increasing T Stage
(Breslow/primary tumor thickness, ulceration, lymph node stage)

Table 1. Clinicopathological characteristics of patients in the MRV 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 (62)	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)

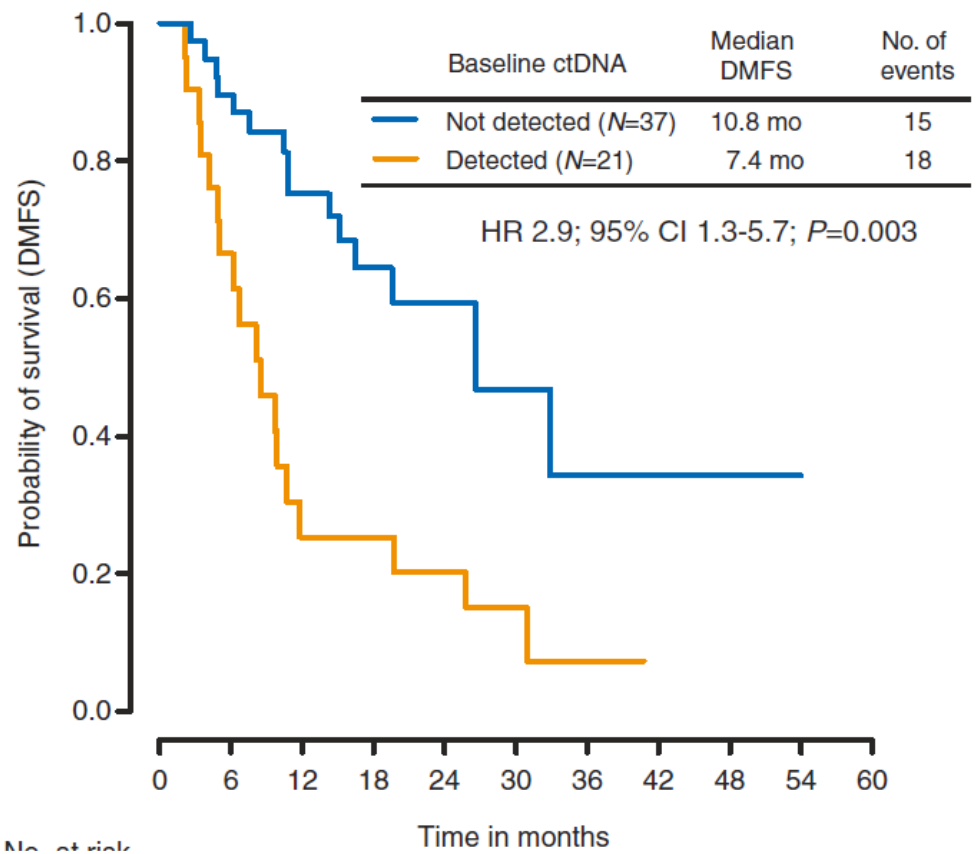
Annals of Oncology 30: 804–814, 2019

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



Detected → 90% relapsed
Undetected → 49% relapsed

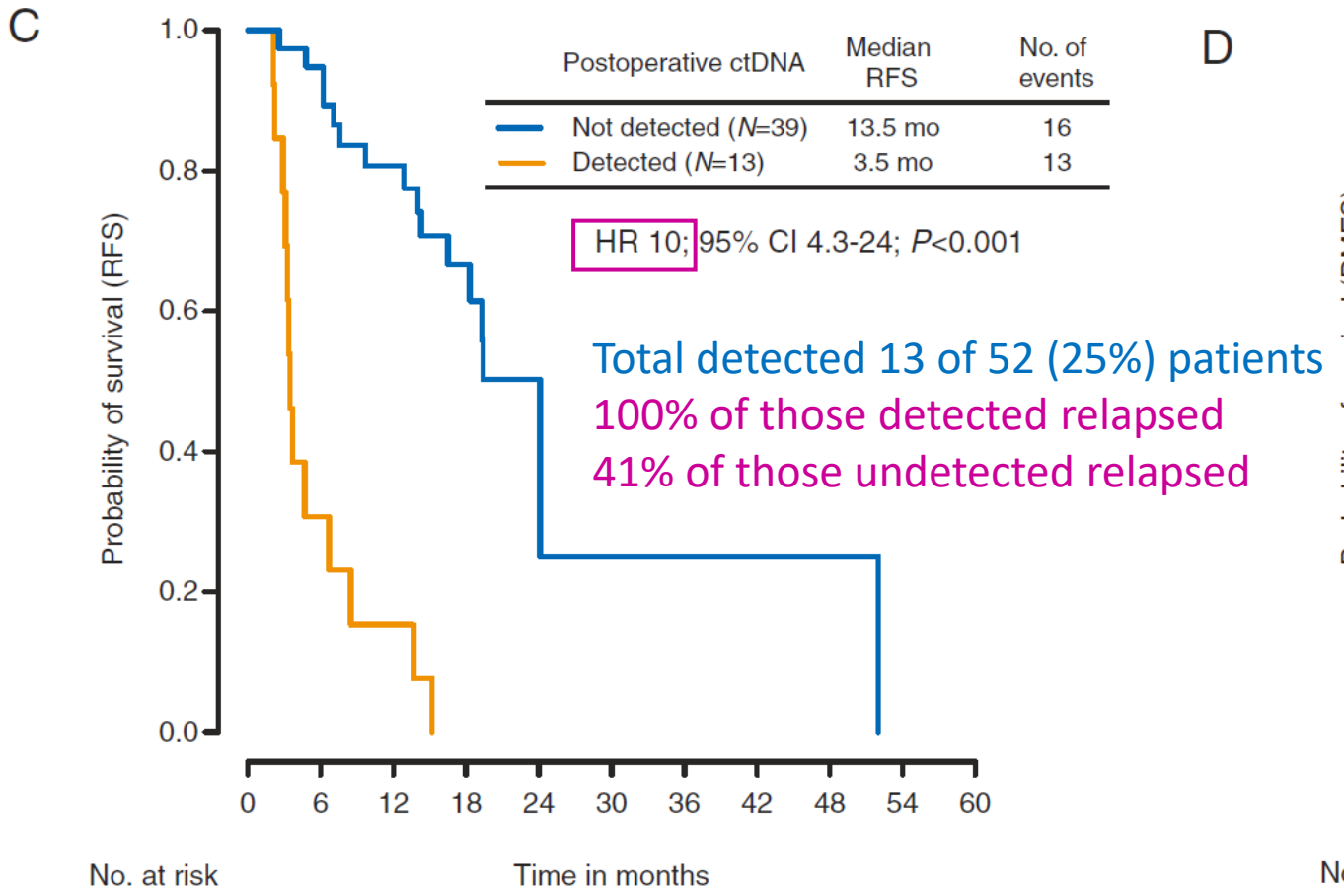
B



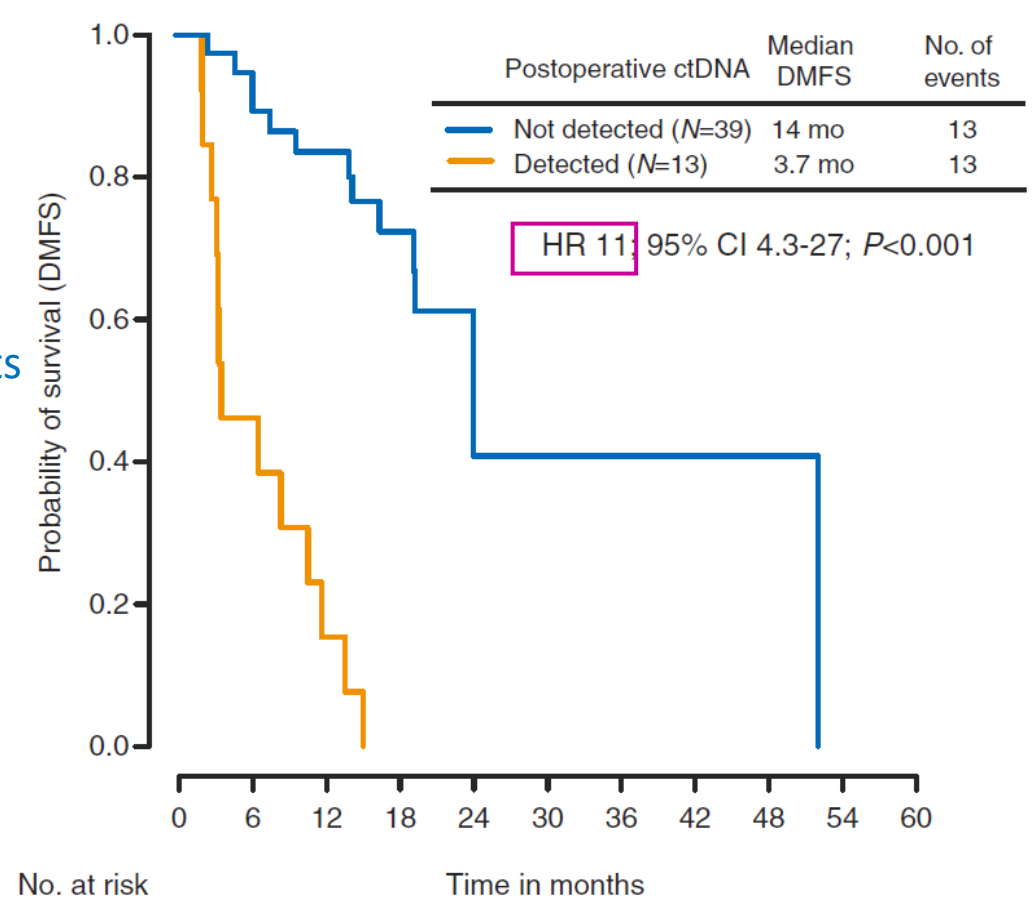
Patients treated with surgery alone - NO systemic therapy

Annals of Oncology 30: 804–814, 2019

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



Total detected 13 of 52 (25%) patients
100% of those detected relapsed
41% of those undetected relapsed

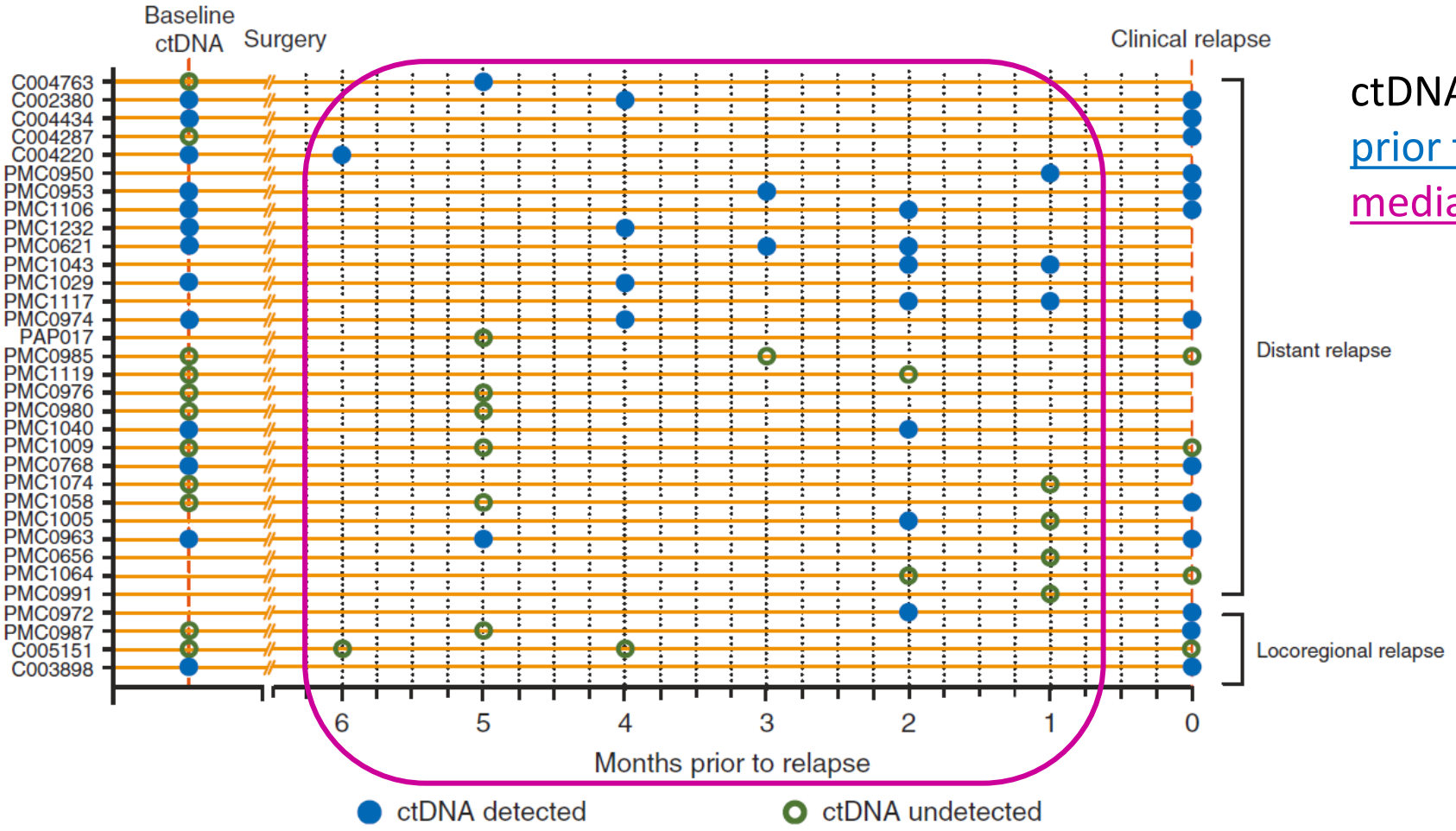


Patients treated with surgery alone - NO systemic therapy

Annals of Oncology 30: 804–814, 2019

Serial postoperative liquid biopsies

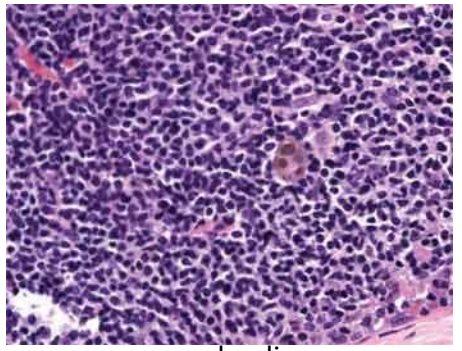
ctDNA was detected *prior* to relapse in half the patients



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

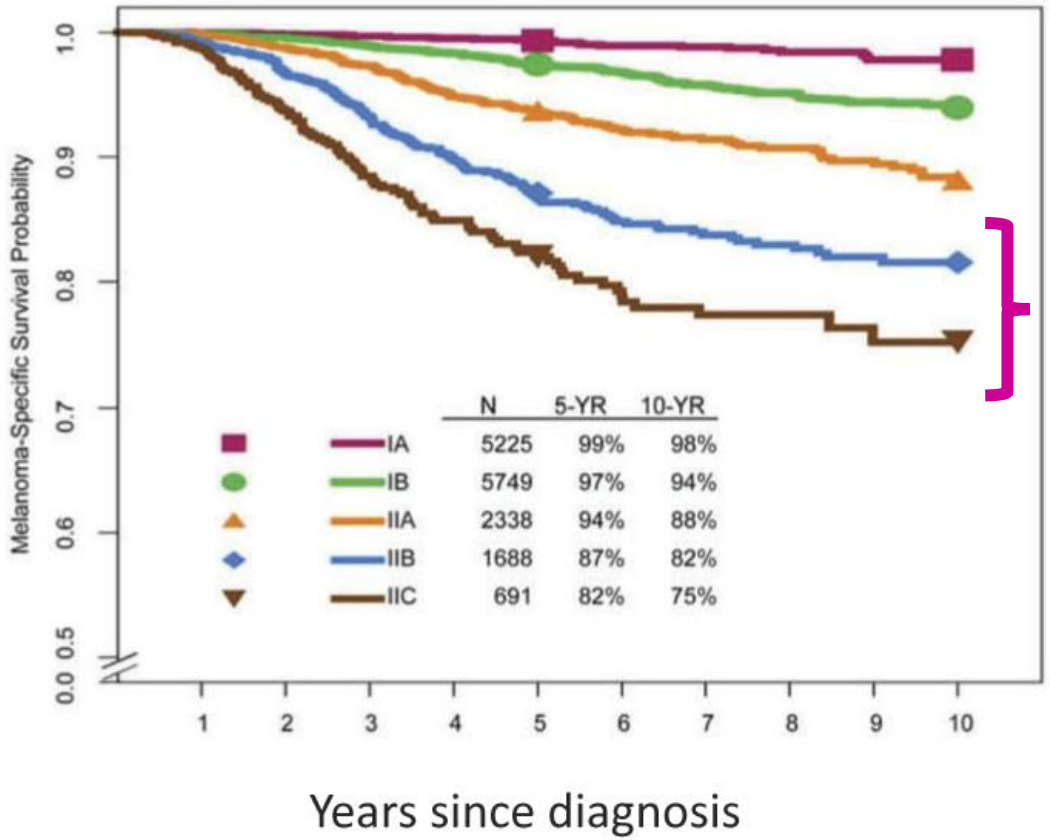
Annals of Oncology 30: 804–814, 2019

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

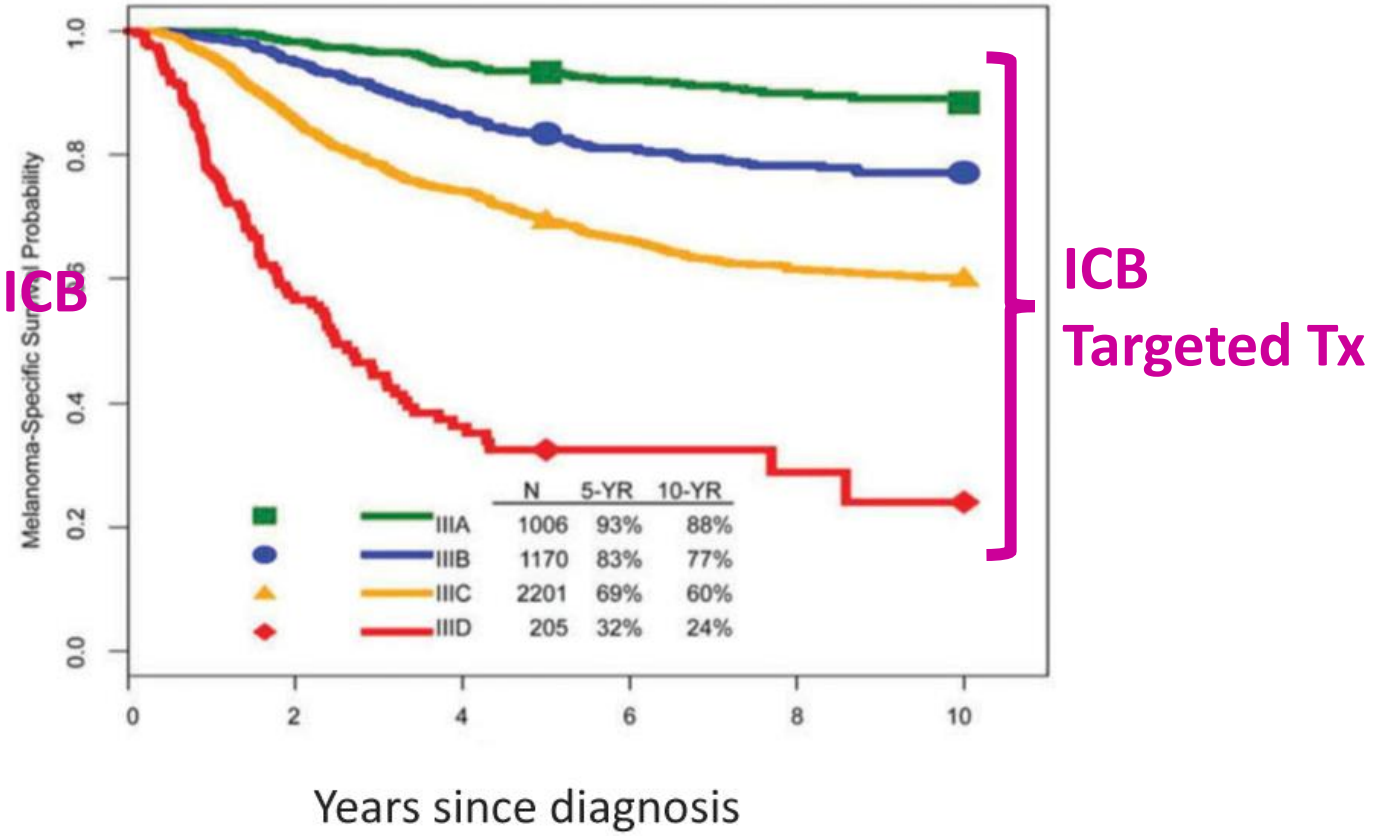


www.healio.com

8th edition AJCC Stage II (non-metastatic)



8th edition AJCC Stage III (lymph node metastasis)



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

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

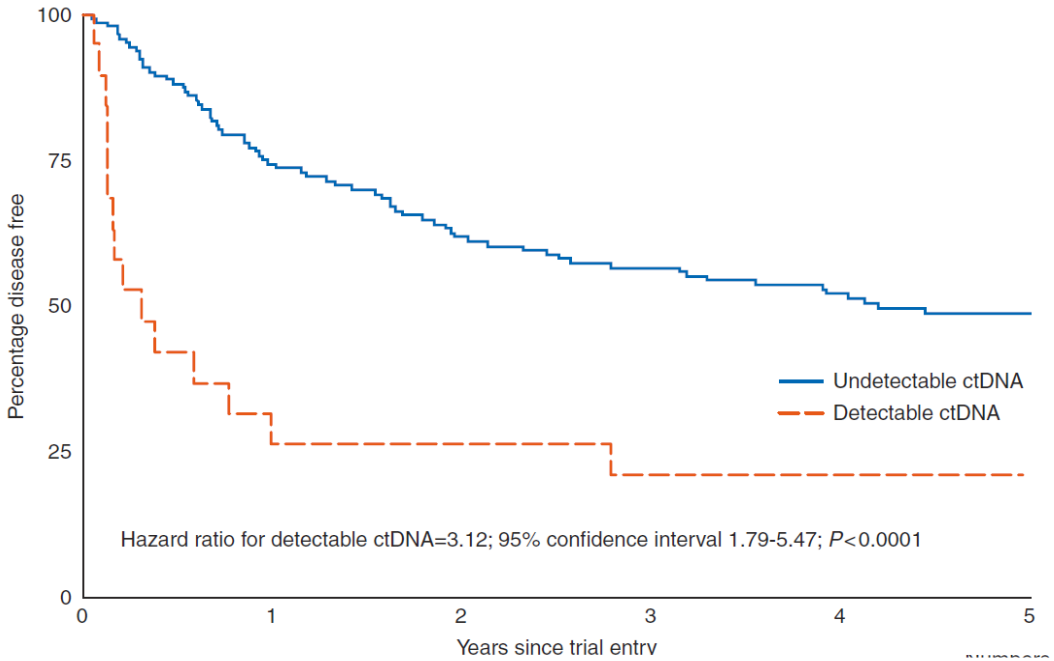
Table 1. Demographics of patients with detectable or undetectable ctDNA

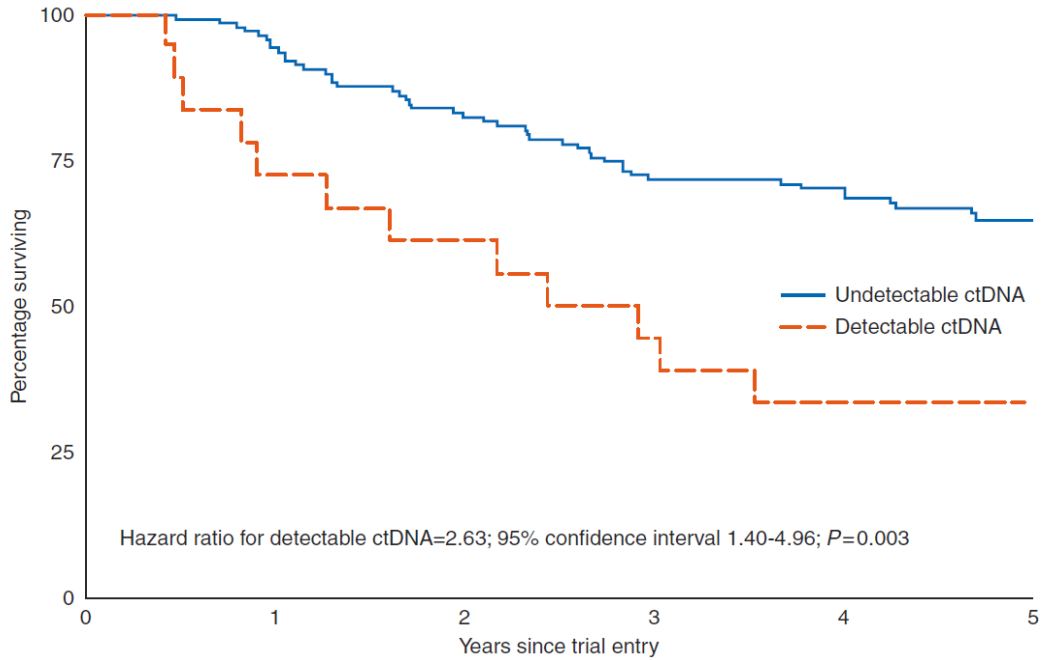
Characteristic	Total *N* (%)	Undetectable ctDNA *N* (%)	Detectable ctDNA *N* (%)
Disease stage			
II	36 (22)	33 (23)	3 (16)
IIIA	29 (18)	27 (19)	2 (11)
IIIB	59 (37)	51 (36)	8 (42)
IIIC	37 (23)	31 (22)	6 (32)
Mutation status			
BRAF V600E	132 (82)	117 (82)	15 (79)
NRAS Q61K/L	29 (18)	25 (18)	4 (21)
Total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

Annals of Oncology 29: 490–496, 2018

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

A

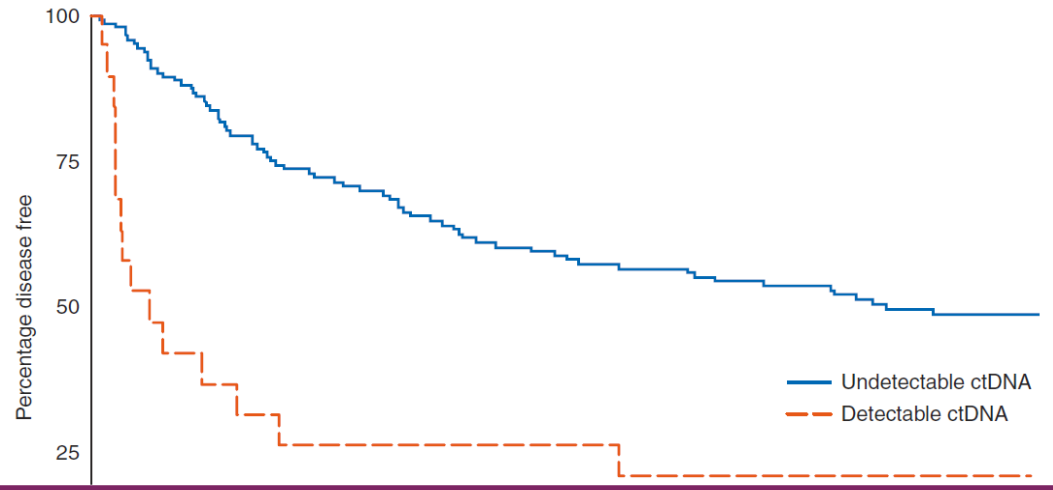




Annals of Oncology 29: 490–496, 2018

Detection of ctDNA improves prognostication of Stage II/III melanoma

A



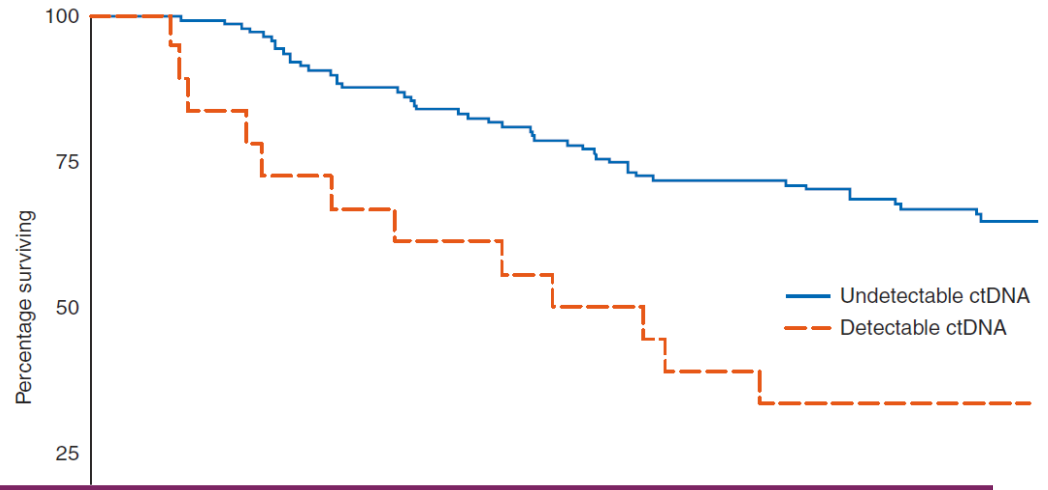


Table 4. Model performance measures for the staging variables associated with AJCC classification (stage, Nodal classification, ulceration and Breslow) and the model adjusted for ctDNA

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

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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

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